Upsala J Med Sci 89: 244–253, 1984

# Reevaluation of Home Urine Glucose Measurements in Diabetic Children

## *A computerized study of short-term control variables in the prediction of long-term diabetic control*

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ABSTRACT

The relationship between Clinitest[R] home control results and $HbA_I$ and triglycerides was analyzed by a computerized optimizing procedure.

The morning urine glucose excretions in the 10 preceding weeks were strongly correlated with $HbA_I$ as were the evening excretions, while the afternoon records showed only weak correlations with $HbA_I$. Maximal correlations between home glucose records and $HbA_I$ were achieved when mornings and evenings were added, with higher weights given to the mornings. The practical consequences are that home urine test results in the mornings and evenings are of most value when adding home scores for assessment of long-term diabetic control, whereas afternoon records should be ignored for this purpose.

Serum triglycerides were correlated with glucose excretion during the week of triglyceride determination, but not with glucose excretion measured in the preceding weeks. Serum cholesterol did not reflect diabetic control at all.

## INTRODUCTION

During the last few years a number of reports have indicated that good control of the blood glucose level reduces the risk of long-term complications of diabetes mellitus (2). To reach this goal, reliable methods for testing diabetic control are necessary. In diabetic children height and weight measurements, home urine tests of glucose and ketone body excretion and sometimes plasma glucose, serum triglyceride (TG) and serum cholesterol assays have been included in the routine check-ups.

Since 1976, when Koenig et al. (8) demonstrated that the degree of glucose control in diabetics over a long period of time was reflected in the relative concentration of glycosylated hemoglobin ($HbA_{Ic}$) in the blood, a number of studies have been undertaken on the relationship between $HbA_I$ and diabetic control. The correlations found between $HbA_I$ (or $HbA_{Ic}$) and the results of conventional test methods vary considerably between different studies. In older adults with non-insulin-dependent diabetes, most authors have found good correlations between fasting plasma glucose in a single specimen and $HbA_I$, and between 24 hour urinary glucose excretion and $HbA_I$ (6,15,16). In insulin dependent diabetics, especially in young ones, these correlations

seem to be generally weak (1,9,12,13,16). The correlation between $HbA_I$ and the clinical evaluation of the patient is good, however, even in diabetic children (12,14). In 11, probably adult patients Lanoe et al. (9) found a strong correlation between Clinitest home records and $HbA_{Ic}$. Gabbay et al. (5) measured the glucose in 24 hour urinary specimens in diabetic children and adolescents and found that it correlated better to $HbA_I$ (r=0.57) when the urine was collected 43-70 days before the $HbA_I$ determination than when it was collected earlier or later. In a study by Dunn (3), the correlation between $HbA_I$ and the mean glucose value in multiple plasma samples taken during the preceding eight weeks was very strong (r=0.78).

The aims of the present investigation were to examine the correlation between $HbA_I$ levels and data obtained by conventional methods of diabetic control in a group of diabetic children and to try to define the temporal representativeness of a $HbA_I$ value and to ascertain the relative weights of Clinitest results from different times of the day in predictions of $HbA_I$ and thus reevaluate older data based on Clinitest records.

## SUBJECTS AND METHODS

The study comprised 28 children aged 3-16 years, 17 girls and 11 boys, with Type I diabetes mellitus. All children were being treated with two daily injections of intermediate monocomponent porcine insulin (Monotard$^R$) daily. In some of them a rapid monocomponent insulin (Actrapid$^R$) was being added in the morning and/or in the afternoon. The mean total dosage was 0.85 IU of insulin per kg body weight (range 0.27-1.23). All these children were being treated according to the same general principles and were seen by the same pediatrician (TT) every two to three months. They all had had a normal height and weight increase according to Swedish standards (7). None of them had albuminuria, hypertension, retinopathy or any other clinical signs of vascular complications. Fourteen of the diabetic children had peers of the same age and sex, who were willing to participate as matched controls.

The diabetic children recorded their urine glucose excretion by the Clinitest two drop method three times daily (in the morning before breakfast, before dinner at 4.30 p.m. and at bed-time) on three fixed days a week for at least one year. $HbA_I$ and 24-hour urine glucose excretion (day and night specimens separated) were determined at each visit to the hospital. Measurements of serum cholesterol, serum TG and fasting plasma glucose were added sixmonthly. At the first visit the same analyses were performed in the accompanying control children. All blood specimens were taken in the fasting state at 08.00-08.15 in the morning before breakfast and insulin injection.

$HbA_I$ was measured by a commercial microchromatographic technique from Bio-Rad Laboratories, Richmond, California (16). The temperature of the eluting buffer was kept constant at 22.0°C and therefore no temperature corrections were necessary. Two $HbA_I$ standards (level 7 % and 10 %) were run simultaneously with the patient samples. Standards were prepared from hemolysates of washed erythrocytes and stored at -70°C. Under these conditions they were stable for at least one year. The method

displayed an intra- and inter-assay coefficient of variation of 3 % and 6 %, respectively.

Plasma glucose, serum TG and cholesterol concentrations were determined as described earlier (4).

This work is part of a prospective study on a population of diabetic children, started in 1978, approved by the Ethics Committee of Uppsala University. All patients and peers and their parents gave their informed consent to participation in the study.

## STATISTICAL ANALYSIS

The statistical analysis was mainly performed by the SAS program package on an IBM 158-4341 at Uppsala University. A schematic description of the procedure is given in Fig. 1.

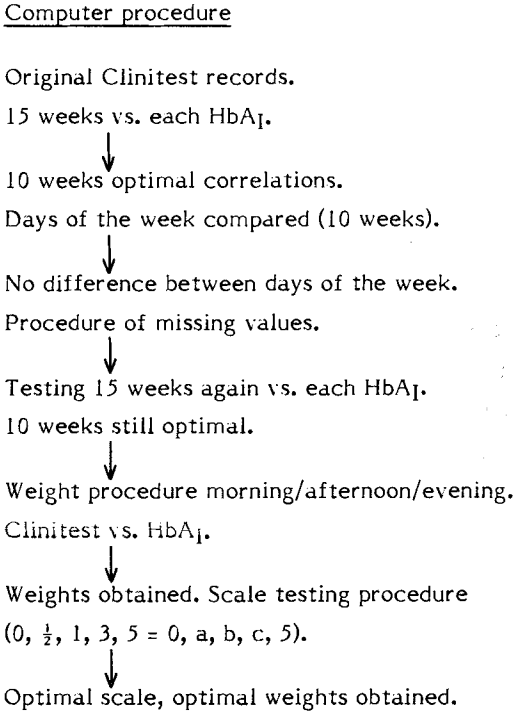


Fig.1 Schematic description of the computer procedure.

The computer procedure included five main steps, the first four of which are within the SAS program:

1. The original Clinitest records (0-5 %) (n >10.000) were used in the first step, in which the records for the week of a $HbA_1$ determination and the 14 preceding weeks were correlated against this $HbA_1$ value. As a few home test results were lacking for most of the patients for the period in question, a procedure for

interpolation of missing values was used. The Clinitest records from the different days of the week during the weeks of highest correlation were then tested against $HbA_I$. No significant differences were found between the different days. This was considered to justify giving the missing values the mean of the preceding and the subsequent value obtained at the same time of day.

2. The correlation between $HbA_I$ and the Clinitest results for each week during a four-month period prior to each $HbA_I$ sampling was calculated.
3. The Clinitest results from the 10 weeks of highest correlations with $HbA_I$ were used in a procedure to weigh the relative importance of morning, afternoon and evening Clinitest records for prediction of $HbA_I$. The procedures for general linear models, canonical correlation, and systems regression in the SAS program package were used. Coefficients for the relative weights of Clinitest results from the different times of day were obtained. These weighted data were used for estimating the total coefficient of determination ($R^2$) between home test results and $HbA_I$.
4. Non-linear transformed scales were constructed in an attempt to reduce the non-linearity of the relationship between Clinitest results (0-5 %) and blood glucose levels. In a semi-quantitative scale, the crude Clinitest values of 0, 0.5, 1, 3, and 5 % were transformed to 0, 0.5, 1, 1.5 and 2 respectively. A clinically often used scale of qualitative scoring, 0-0.5 % = 0 and 1-5 % = 1, was also used (Fig.2).

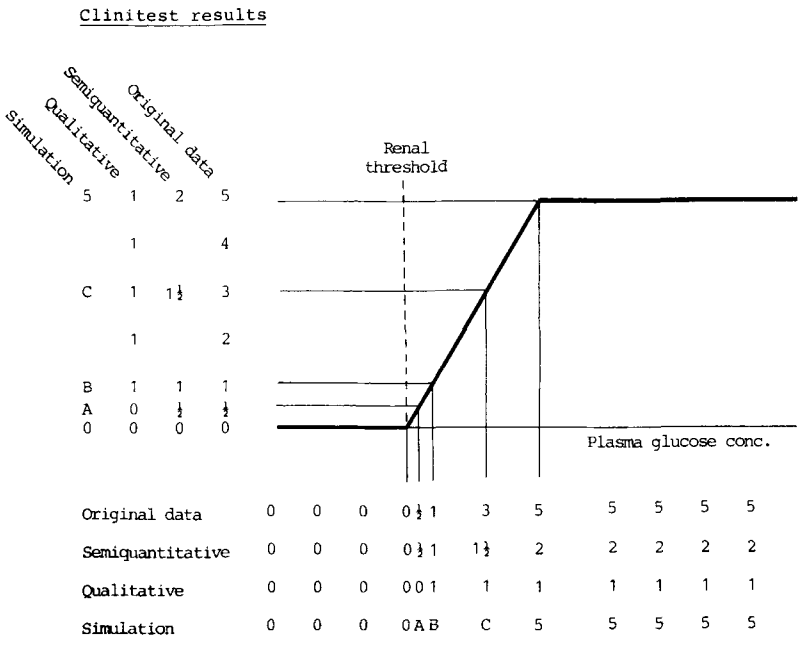


Fig.2 Relationship between plasma glucose concentration and urine glucose concentration as expressed by the different scales tested.

5. In an attempt to construct an optimal scale, a separate program, including a procedure for optimization of the correlation coefficients, was written. 0 % was fixed as ▫ zero, 0.5 % = A, 1 % = B, 3 % = C, 5 % = 5. The values for A to C giving the best fit of the scale were requested.

## RESULTS

The $HbA_I$ values for the healthy matched control children were all within the range 5-8 % (6.5±0.4, M±SD). The values for the diabetic children varied between 7 and 16 % (11.0±2.0, M±SD).

The absolute Clinitest results did not differ between the different days of the week, neither was there any difference in their correlation to $HbA_I$.

The correlation coefficients (r) for the relations between each $HbA_I$ value and the summed Clinitest scores (0-5 %) for the preceding 10 weeks (using all three daily values) all lay within the range 0.55-0.72. The correlation with $HbA_I$ was generally lower for the preceding weeks 11-17 ($r < 0.50$) (Fig.3).

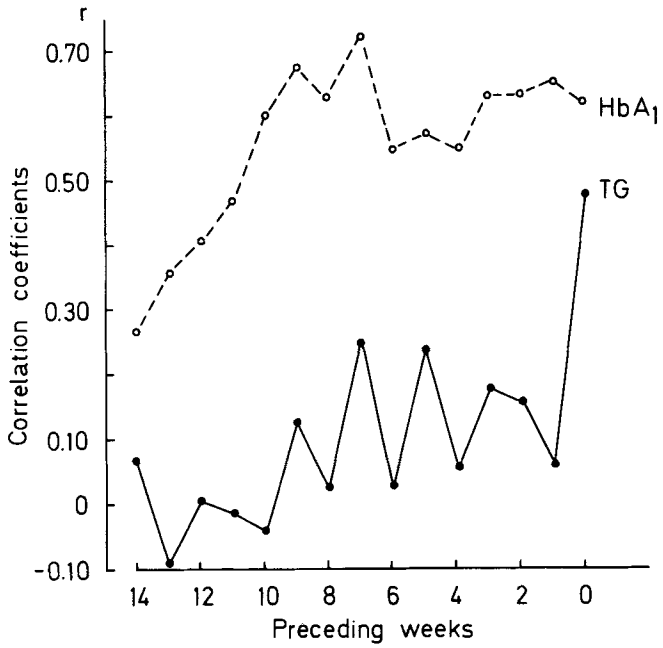


Fig.3 Coefficients of correlation between Clinitest home test results and $HbA_I$ and between home test results and serum triglycerides. Correlations for home tests performed in the week of the blood test and in the 14 preceding weeks are given.

Clinitest scores transformed according to the non-linear scale (0-2) showed similar correlation coefficients as the original scores for the 10 weeks preceding each $HbA_I$ determination when mornings, afternoons, and evenings were added with the same weights. The qualitative scoring (0/1) showed slightly lower correlation coefficients versus $HbA_I$ under these circumstances.

Using the Clinitest results from the 10 weeks immediately preceding each $HbA_I$ determination, the relative weights of morning, afternoon, and evening Clinitest records in the prediction of $HbA_I$ were estimated (Table I).

Table I. Relative weights for Clinitest records from different times of day giving the maximal correlation between Clinitest results during ten weeks and the $HbA_I$ value.

| | Morning | Afternoon | Evening |
|---|---|---|---|
| Optimal relative weights | 0.103 | -0.041 | 0.079 |

The morning records tested alone against $HbA_I$ showed highly significant correlations and a high coefficient of determination ($R^2$ = 0.46). The evening records were also strongly correlated to $HbA_I$ ($R^2$ = 0.42). The afternoon records tested alone showed only weak correlations to $HbA_I$ (Table II).

Table II. Relationship between Clinitest results from each time of day versus $HbA_I$ as expressed by coefficient of determination ($R^2$) and the statistical significance (p) for the correlation between the two variables.

| | p | $R^2$ |
|---|---|---|
| Morning/$HbA_I$ | <0.001 | 0.46 |
| Evening/$HbA_I$ | <0.01 | 0.42 |
| Afternoon/$HbA_I$ | <0.05 | 0.23 |

When the morning and evening records were added (and their relative weights optimized), the total coefficient of determination ($R^2$) for the 10 weeks immediately preceding the $HbA_I$ sampling was 0.50. The afternoon records added almost no further information. Using an optimum weighting procedure, giving the afternoon value a negative weight, $R^2$ could be increased to 0.51.

The number of missing Clinitest values showed a slight positive correlation with high Clinitest scores and high $HbA_I$. The number of missing values was very low (<10%) during the first five months of the study, but increased later. The correlations found during these first five months exceeded those found later. During this optimal period, the coefficient of determination ($R^2$) was as high as 0.60 (morning and evening records, relative weights 2.2:1, scale 0-5 %). This high $R^2$ corresponds to a correlation coefficient

of 0.78.

Using the relative weights producing the best fit, various attempts were made to optimize the Clinitest scale. Besides the 0/1 , 0-2 and 0-5 scales, different more or less complicated scales were tried. Even complicated transformations of the scale did not significantly increase the coefficient of determination, despite the use of automatic optimization procedures.

There was a correlation between serum TG concentrations and $HbA_I$ when the samples were taken simultaneously ($r=0.58$; $p<0.01$). The TG concentration was tested against home urine test results for each week during the 14 weeks immediately preceding the TG sampling. The home records for the week of the TG sampling were significantly correlated to TG concentration ($r=0.47$; $p<0.05$). The morning Clinitest results were not significantly correlated to TG ($p>0.1$) while the afternoon ($r=0.41$; $p<0.05$) and evening values ($r=0.51$; $p<0.01$) showed significant correlations to TG. A similar difference was found for the relation between TG and 24 hour urine glucose excretion. The correlation coefficient for TG and day-time glucose excretion was 0.55 ($p<0.01$), but TG did not correlate at all to night-time excretion ($r=0.17$; $p>0.4$). The home test results for 1-10 weeks preceding the TG value were not significantly correlated to TG (correlation coefficients for the different weeks varying between -0.09 and +0.25; $p>0.1$) (Fig. 3).

Serum cholesterol showed no significant correlation to $HbA_I$ in simultaneously taken samples ($r=0.1$; $p>0.5$). $HbA_I$ was correlated to 24-hour urinary glucose excretion when these specimens were obtained at the same visit ($r=0.56$; $p<0.01$).

The correlation between fasting plasma glucose and $HbA_I$ varied from insignificant to slightly significant during the study ($r=0.16$-$0.49$). $HbA_I$ did not correlate significantly to sex, age, stage of puberty, duration of diabetes, relative body weight, or insulin dosage per kilogram body weight.

## DISCUSSION

The high coefficients of determination ($R^2$) found here for Clinitest home records in prediction of $HbA_I$ show that home urine tests, properly carried out, give important information about the degree of long-term diabetic control. The high correlations found between home test results and $HbA_I$ for the 9 to 10 weeks preceding the $HbA_I$ determination indicate that in this population $HbA_I$ measured every 10th week will give a covering description of carbohydrate control. The temporal relationship fits with the corresponding finding of Gabbay (5) of 43 to 70 days (6-10 weeks) and with data from insulin infusion pump trials, demonstrating complete $HbA_I$ normalization after about 8 weeks (11). The correlation method has limitations, of course, in studies of the temporal relationship between $HbA_I$ and glucose values, as the method does not take into account the difference in $HbA_I$ kinetics between periods of increasing and decreasing values. The high correlations found for about 10 weeks do not merely indicate a general stability of the diabetic state. This is shown from the comparisons made here with TG. When the same correlation method was used for TG versus Clinitest home records, a significant

correlation was only found for home results obtained during the week of the TG sampling, while home results in the 10 preceding weeks showed no correlation to TG. It is interesting to note that TG correlated highly with day-time glucose excretion but not at all with the excretion during the night. Similar findings were made for the home tests. Thus, the Clinitest values for the afternoons and evenings showed a positive correlation to TG, but not the morning Clinitest values, which reflected the night excretion. The findings strongly indicate that TG is not related to the degree of basic diabetic control, but reflects the amplitude of the postprandial glucose peaks in the last few days. This result is not unexpected considering the influence of rapid carbohydrates on TG formation. TG is thus not a good indicator of long-term diabetic control. Serum cholesterol, in this population not different from normal controls, did not seem to measure diabetic regulation at all, a finding consistent with the results of at least some earlier studies, where no significant correlations were found between serum cholesterol concentration and diabetic control (10).

With the $HbA_I$ method used, total $HbA_I$ was determined and no correction was made for the labile $HbA_I$ fraction. The lack of strong correlations with simultaneously measured plasma glucose indicates that the $HbA_I$ was not significantly influenced by the glucose content in the plasma. Such influence seems to occur mainly at high plasma glucose concentrations and can be avoided (16).

The changes in plasma glucose from 0 to 8-10 mmol/l produce no parallel changes in the Clinitest results, while within the range 10-20 mmol/l the Clinitest values rapidly rise to 5 % and still remain at 5 % at the most extreme plasma glucose concentrations. Thus it is obvious that the best relation between $HbA_I$ and Clinitest is probably a non-linear one, with two breaking points (Fig.2). The Clinitest scale transformation was an attempt to correct for this non-linearity. The simple 0/1 scale, often used in clinical practice, was almost as efficient as the other more complicated scales when morning, afternoon, and evening values were given the same weight. In the diabetic child the morning Clinitest result represents about 10 stable hours, uninfluenced by any meal. The relative weight of the morning value was 1.3-2.2 times higher than that of the evening value when tested on data from different parts of the study period. These figures should not be considered as exact, but are dependent on the population under study. This emphasizes, however, the importance of the morning urine glucose estimations. In contrast, the afternoon Clinitest values gave little or no further information for prediction of $HbA_I$ when morning and evening values had been taken into account. In fact, the afternoon Clinitest records were given negative weights in most of our optimization studies. From a practical point of view this makes it quite clear that afternoon Clinitest values should be neglected when adding the scores for assessment of long-term diabetic control. The morning values should be weighted somewhat more (about 50 %) than the evening values.

The coefficient of determination found here for the first five months, weighting mornings and evenings in this way, namely $R^2 = 0.60$, means that as much as 60 % of a

$HbA_I$ value can be predicted by a simple semi-quantitative analysis of urine glucose in the morning and at bed-time on three fixed days a week. This figure is unexpectedly high, as it corresponds almost exactly to the correlation coefficient of 0.78 found by Dunn using multiple plasma glucose measurements (3).

The sophisticated attempts made here to optimize the scales by giving the glucose percentage values different coefficients did not improve $R^2$ significantly. For practical purposes it is thus not justifiable to complicate the scales further. On the other hand, the use of different weights for the tests performed at different times of the day increased the prediction of long-term control as measured by $HbA_I$.

$HbA_I$ (or $HbA_{Ic}$) is presently accepted as the reference method of choice for long-term evaluation of carbohydrate control in diabetics. The availability of this method makes it possible to assess earlier reference methods and might thus be of value for retrospective reconsideration of earlier long-term studies. The present findings indicate that the results of home tests of glucose excretion in the morning and in the evening are of value for judging long-term diabetic control, while afternoon urine test results, serum TG and serum cholesterol are of very little use for this purpose. These data strongly suggest that the results of earlier studies based on Clinitest home controls and evaluated according to empirical tradition should be re-assessed.

ACKNOWLEDGEMENTS

Anna Gunsjö, B.Sc. and Hans Falk, M.Sc. Uppsala University Data Center, are acknowledged for their help with the computer analyses. This study was financed by a grant from AB Novo, Malmö, Sweden, to Torsten Tuvemo.

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