Computer Analysis of Intravenous Glucose Tolerance Tests

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Summary. A computer program which simultaneously calculates glucose disappearance rates after intravenous injection using three different mathematical equations is described. This program has been applied to the results obtained from several groups of pregnant women. The reasons for suggesting that the "absolute K value" may be preferable on practical grounds are discussed. This program, written in Fortran, is available on application to D. W. Neill.

Analyse sur ordinateur des tests de tolérance au glucose intraveineux

Résumé. Les auteurs décrivent un programme sur ordinateur qui calcule simultanément les vitesses de disparition du glucose après injection intraveineuse, à l'aide de trois équations mathématiques différentes. Ce programme a été appliqué aux résultats obtenus dans plusieurs groupes de femmes enceintes. Les raisons suggérant que la "valeur K absolue" peut être préférable pour des

raisons pratiques, sont discutées. Ce programme, écrit en Fortran, est disponible sur demande à D. W. Neill.

Analyse intravenöser Glucosetoleranzteste mit Hilfe einer elektronischen Rechenmaschine

Zusammenfassung. Es wird ein Programm für die elektronische Rechenmaschine angegeben, welches die Glucoseabbaurate nach intravenöser Verabfolgung gleichzeitig nach drei verschiedenen mathematischen Gleichungen errechnet. Dieses Programm wurde bei den Befunden von verschiedenen Gruppen schwangerer Frauen angewandt. Die Gründe dafür, daß im praktischen Gebrauch wahrscheinlich die „absoluten K-Werte“ vorzuziehen sind, werden diskutiert. Das von Fortran geschriebene Programm steht allgemein zur Verfügung und kann bei D. W. Neill angefordert werden.

Keywords: Intravenous glucose tolerance; computer analysis; glucose disappearance; pre-diabetes; pregnancy.

The intravenous glucose tolerance test (I/V GTT) has not been widely used in clinical practice, largely because of difficulties of interpretation of the result. These stem from two sources, the need to plot an actual graph of the results and the more theoretical arguments as to which equation to use to best describe this graph.

The primary advantage of a computer in this situation is to eliminate the tedious problems of draughtsmanship which are subject to human error, but this approach will also allow a more rigorous comparison between the different methods of data analysis which are available.

The aim of the present study was to derive a series of computer programs applicable to this problem. Our chief use of the intravenous GTT has recently been in the assessment of minor differences in glucose tolerance during pregnancy in the potentially diabetic mother [1] and we have used the computer to facilitate the analysis from the clinical standpoint.

We have applied the programs to our own results in two separate groups of mothers, and also for purposes of comparison to the published data [2] which have been accepted as the best available normal control series at present.

Theory of various methods of data analysis

1. Symbols used are defined as follows:

C Glucose concentration at any time, measured in mg/100 ml.

t time in min. All times are measured from the end of the glucose injection.

C₀ Glucose concentration in the first specimen taken after injection.

C_f fasting glucose concentration.

C_{eq} theoretical 'equilibrium' glucose concentration.

K rate constant for glucose disappearance.

2. Hamilton and Stein [4] proposed the equation:

$$C = C_0 e^{-Kt} \quad [A]$$

to describe the disappearance of glucose from the blood after injection.

Since $\log C = \log C_0 - Kt$

the value of K is obtained as the regression coefficient of $\log C$ on t.

We have performed this calculation in two cases:

a) Using all the results, except the fasting value: i.e. all the results after about ten minutes. The rate constant obtained is designated $_{10}K_g$.

b) Using all the results except the fasting value and the first value after injection: i.e. all the results after about twenty minutes. The rate constant obtained is designated $_{20}K_g$.

3. Amatuzio *et al.* [5] proposed the equation:

$$C = (C_0 - C_f)e^{-Kt} + C_f \quad [B]$$

assuming that eventually the glucose concentration returns to the fasting value. In this case:

$$\log (C - C_f) = \log (C_0 - C_f) - Kt$$

and the value of K is obtained as the regression coefficient of $\log (C - C_f)$ on t. Two calculations are again performed, yielding rate constants designated as $_{10}K_f$ and $_{20}K_f$.

4. Greville [6] proposed the equation:

$$C = (C_0 - C_{eq})e^{-Kt} + C_{eq} \quad [C]$$

assuming that the glucose concentration eventually falls to an equilibrium value C_{eq} not necessarily equal to the fasting value. In this case no easy transformation to linear form is available, since C_{eq} is unknown. A curve of this form is fitted to the data using the Taylor series modification of the classical least squares method, and the equilibrium constant C_{eq} , and K_{eq} the rate constant, are estimated from the curve.

Methods and Patients

The I/V GTT in the Belfast patients was carried out as previously published. A problem has been to determine how many blood sugar samples are necessary to obtain an acceptable degree of accuracy, and at what times to take them after the intravenous glucose injection. For this reason the results from two sets of patients are presented, and are compared with two sets of standard data.

1. R.M.H. Group 1. 39 mothers attending the antenatal clinic of the Royal Maternity Hospital in whom an I/V GTT was requested for one of the standard criteria (see program documentation). These tests were standardised to obtain at least 6 to 8 specimens for blood glucose over a period of 60 min after the injection.

2. R.M.H. Group 2. 74 mothers, similarly selected. The timing of these tests was more variable, rarely extending beyond 30 min and there were not more than 5 specimens.

3. The data obtained by Silverstone and his colleagues for 'normal' (not potentially diabetic) mothers [2] during the third trimester of pregnancy has been utilised for purposes of comparison.

4. The data for 20 normal newborns was provided by Dr. Bengt Persson, Stockholm. N. Par. The values for $_{10}K_g$ had been initially obtained by graphical means for groups 1 to 3, and these are included for comparison with the computer results.

Outline Program Documentation

1. The computer system in use consists of an I.B.M. 1130 with an 8K memory, and associated peripheral equipment includes a card reader, lineprinter, and random-access disk storage.

2. The suite of programme was written in the subset of Fortran IV available on the 1130, and use was made of the Fortran extended precision capabilities to ensure accuracy.

3. The input medium used is punched cards. For each patient the following data is provided:

- patient's name, initials and hospital number;
- patient's ward and/or department;
- date of test, coded as day of year;
- a maximum of ten results for time (recorded on a 24-h clock scale) and glucose concentration (as mg/100 ml);
- in the case of pregnant women, the age and weight of the mother and the maturity and parity of the pregnancy, and
- the reason for the test, coded as follows:
 - not stated
 - glycosuria
 - big baby
 - abnormal random blood sugar
 - previous abnormal GTT
 - family history of diabetes
 - abnormal baby
 - unexplained stillbirth or neonatal death
 - maternal weight over 200 lb
 - unclassified.

4. The programs perform two main functions:

a) short-term: for each patient a report giving the values of $_{10}K_g$, $_{20}K_g$, $_{10}K_f$, $_{20}K_f$, K_{eq} , C_{eq} , and C_f is printed.

b) long-term: a card containing the above details is punched, which will be used in follow-up statistical surveys.

5. The programs operate as follows:

a) Program 1 reads the input data from punched cards and writes it into a disk file, where all results are stored prior to computations. It also checks for the presence of certain (predefined) preoperative error conditions such as data missing, incorrectly punched, or out of sequence. If an error condition exists, all data from the patient concerned is ignored, and an appropriate diagnostic message is printed on the console typewriter. The computer operator may then correct the error and resubmit the data for the patient concerned. After all the data has been read, edited and stored, control passes to Program 2.

b) Program 2 reads the data for each patient in turn from the disk file, and performs appropriate calculations to find the rate constants $_{10}K_g$, $_{20}K_g$, $_{10}K_f$, $_{20}K_f$, K_{eq} and the equilibrium concentration C_{eq} . It then prints a report for the patient, and also punches a card containing the results in a form suitable for use as data for Program 3.

c) Program 3 provides follow-up statistical analysis of I/V GTT results. It is designed to use as data the cards punched by Program 2, and prints details of the mean, standard deviation, and extreme values of the variables, and also the correlation coefficients and regression constants for the relationship between any given pair of variables. Some results are shown below.

Results

The means (and standard errors of these means) for the calculated functions in the different groups are shown in Table 1.

For Group 1, there is little difference between the K_g values calculated with or without the first blood glucose value after the I/V injection ($_{10}K_g$ and $_{20}K_g$).

Fig. 1. Theoretical basis for the equations used to describe the glucose disappearance rate K

The values obtained for $_{10}K_f$ and $_{20}K_f$ are also similar, but are markedly different from the K_g values. The K_{eq} value is intermediate. The equilibrium blood glucose value C_{eq} is considerably less than the fasting C_f . The $_{10}K_g$ value obtained by hand graphically (K_g graph) is very similar to the computer value for $_{10}K_g$.

Groups 2, 3 and 4 show similar characteristics. The pattern of changes for all these rate constants in normal pregnancy also conforms well to that originally shown by Silverstone *et al.* by K_{graph} values only, for non-pregnant, first and second trimester and post-partum patients.

The variability of the results of the different methods of deriving the glucose disappearance rate is shown by the coefficient of variation in Table 1. There is least variability of the $_{10}K_g$ value in all instances except the figure derived from the data published for normal pregnancy (group 3) where the variability of K_{eq} is less: however this finding is not supported by

between the calculated equilibrium and fasting values for blood glucose. There is again very close correlation between the computer value for $_{10}K_g$ and that obtained by graphical means (K_{graph}). These patterns of correlation are again repeated in groups 2 to 4. The means and normal ranges (mean $\pm$ 2 S.D.) throughout normal pregnancy for 108 of the glucose tolerance tests published [2] have been calculated:

$_{10}K_g$	1.72 (0.79 to 2.65)
$_{10}K_f$	4.28 (0.00 to 8.75)
K_{eq}	2.81 (0.47 to 5.16)
C_{eq}	33.8 (—47 to 114)
C_f	60.9 (43 to 78)

Table 1. Means, standard errors and coefficients of variation

Group	No. of patients	$_{10}K_g$	$_{20}K_g$	$_{10}K_f$	$_{20}K_f$	K_{eq}	C_{eq}	C_f	$_{10}K_{\text{graph}}$
1. R.M.H. [1]	39	1.88	1.75	5.33	6.17	3.36	—0.13	76.2	1.94
		0.12	0.11	0.57	0.87	0.37	26.8	2.22	0.12
		37.2	37.2	68.2	86.8	68.2			
2. R.M.H. [2]	74	2.11	2.00	4.44	4.66	4.21	10.7	75.4	2.11
		0.09	0.09	0.25	0.32	0.41	20.6	0.98	0.09
		34.4	38.7	48.2	60.2	81.7			
3. Silverstone (3rd Trimester)	20	1.78	1.73	4.66	5.22	2.43	26.5	59.6	1.91
		0.09	0.10	0.45	0.60	0.09	5.62	1.85	0.10
		22.5	25.7	43.2	49.5	15.7			
4. Persson (newborns)	20	1.36	1.32	2.30	2.36	2.07	—15.1	51.3	—
		0.09	0.10	0.29	0.34	0.28	39.0	4.15	—
		37.0	34.1	56.7	64.8	60.8			

In all cases the upper figure is the mean, the middle is the standard error of the mean, and the bottom the coefficient of variation.

Table 2. Correlation coefficients

Group	No. of patients	$_{10}K_g$ and $_{20}K_g$	$_{10}K_g$ and $_{10}K_f$	$_{10}K_f$ and $_{20}K_f$	$_{10}K_g$ and K_{eq}	$_{10}K_f$ and K_{eq}	K_{eq} and C_{eq}	C_{eq} and C_f	$_{10}K_g$ and $_{10}K_{\text{graph}}$
1. R.M.H. [1]	39	0.96 ***	0.85 ***	0.94 ***	0.41 *	0.55 ***	0.45 **	0.00	0.98 ***
2. R.M.H. [2]	74	0.96 ***	0.79 ***	0.97 ***	0.12	—0.03	0.50 ***	0.20	0.86 ***
3. Silverstone (3rd Trimester)	20	1.00 ***	0.78 ***	1.00 ***	—0.16	0.40	0.63 **	—0.07	0.88 ***
4. Persson (newborns)	20	0.99 ***	0.80 ***	1.00 ***	0.08	0.08	0.70 ***	—0.13	—

*** $P < 0.001$

** $P < 0.01$

* $P < 0.05$

computer analysis of other data of Silverstone and his colleagues, and for his total group of 108 patients, the $_{10}K_g$ value is also the least variable.

Table 2 shows the correlation coefficients and associated significance values for these results. For Group 1, there is very close correlation between $_{10}K_g$ and $_{20}K_g$, $_{10}K_g$ and $_{10}K_f$, and $_{10}K_f$ and $_{20}K_f$. There is less useful correlation between $_{10}K_g$ and K_{eq} or $_{10}K_f$ and K_{eq} , although these still achieve significance. There is some correlation between the two equilibrium constants derived from the equation [C], but none

Discussion

The "K values" derived from the same blood glucose data using the three available equations [A], [B] and [C] show very considerable differences chiefly of magnitude but also of variability. These differences are entirely due to different methods of data handling, and have little or no bearing on the biochemical processes which are taking place. Our primary aim would be to find an equation which best represents these biochemical processes in the clinical situation,

and this would require a more detailed examination of the glucose assimilation process from the theoretical viewpoint [3]. Nevertheless using a mathematical approach, this investigation suggests that the value calculated as $_{10}K_g$, being the least variable, is preferable on purely practical grounds despite some reservations about the theoretical basis of equation [A]. Butterfield and his colleagues [8] in a critical appraisal of the I/V GTT using a computer program to derive K_g and glucose disappearance rates, also showed that the 'absolute index' K_g gave the highest correlation coefficients with various other parameters of glucose disposal.

There is virtually no difference in rate constants calculated with or without the 10-min value: this suggests that the appropriate biochemical processes are already established by this time and that this is not a point of major inaccuracy in the interpretation of the I/V GTT. There is a wide numerical difference between the rate constants calculated by equations [A] and [B], although the results are closely correlated. This is not surprising as the first case represents the rate of decrease of absolute blood glucose value while the second represents the rate of decrease of the increment above fasting: since the latter function falls equally fast towards a 'higher' zero the rate constant is consequently greater.

Another aspect of the clinical use of glucose tolerance tests is the physical discomfort involved to the patient: any technique which reduces this, consistent with the required degree of statistical accuracy, would be desirable. There has been a tendency, as long as a graphical display of the I/V GTT has been necessary, to ask for at least 8 specimens during the hour following the injection of glucose: even with an indwelling cannula this can be tedious to both patient and the person performing the test. For this reason we were interested in the comparison of groups 1 and 2, the latter representing a shortened (30 min) procedure with only five specimens. The standard errors for group 2 are very comparable to group 1 in both cases at about 5% of the mean. For routine clinical purposes, it would appear that acceptable data can be obtained with less rigorous procedures than those often advocated, when a computer program is available to improve the data handling facility.

Equation [C] has theoretical attractions, particularly because it includes [A] and [B] as special cases when $C_{eq}=0$ and $C_{eq}=C_t$ respectively. However, in practical terms K_{eq} is slightly less reproducible than $_{10}K_g$. Also the small degree of positive correlation between the two constants K_{eq} and C_{eq} means that they cannot be considered as entirely independent

functions in considering the underlying biochemical processes. A further disadvantage of equation [C] is the very wide variability of C_{eq} , which can only be considered a most theoretical function as it is frequently negative. There is no correlation between C_{eq} and C_t .

Finally, it is salutary that there was such good correlation between the computer analysis of $_{10}K_g$ and the laborious graphical determination of this function: although all these graphs for the R.M.H. data were drawn and analysed by the same person, there appears to be a slight but consistent tendency to overestimate by hand.

We have added the analysis of the data of Silverstone and of Persson to our own results in order to emphasise the usefulness of the computer program, which would enable comparisons between different workers who have utilised alternative methods of analysis to be more easily made.

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