Evaluation of a 'True' Fractional Removal Rate of Glucose in Man by Bolus and Simulated-ramp Increase of Glucose

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SUMMARY

An infusion method designed to produce a gradual ramplike rise in plasma glucose levels in man showed that the *observed* slope of the ramp was not significantly different from the *calculated* slope only when both of the following parameters were used:

1. When the glucose disappearance rate coefficient (k) was calculated by an incremental method in which the fasting plasma glucose level was subtracted from each plasma glucose value obtained during a standard rapid intravenous glucose tolerance test. This proved to be superior to a contrasting method in which *absolute* glucose values are used for the semilogarithmic plot of glucose concentration versus time.

2. When the dilution technic was used to estimate directly the volume distribution of glucose rather than relying on standard reference tables that predict glucose distribution space in man.

By using these two parameters obtained during standard intravenous glucose tolerance testing, we have shown that it is possible to calculate the rate of glucose infusion required to achieve successfully in man a ramp of plasma glucose of any desired steepness and to characterize the consequent insulin secretion. This simulated ramp increase of glucose provides a potentially useful tool for investigation of the dynamics of islet-cell function in man. DIABETES *25*:580-85, July, 1976.

The disappearance rate of glucose in man is normally measured as the fractional removal rate (k), but the methods for its estimation vary. After a bolus injection of glucose, some investigators simply use the value of k obtained directly from the semilogarithmic plot of the concentration of glucose versus time,[1,2] while others subtract the fasting concentration of glucose before the determination of k is made.[3,4] For a more complete understanding of the dynamics of glucose, one would need to know the "true" value of k that would be consistent with data obtained under different experimental conditions. Successful achievement of a gradual rise in plasma glucose levels to provide a ramplike stimulation of insulin release offers a potentially useful means to determine the "true" k and to study the dynamics of insulin release under conditions that approximate postprandial hyperglycemia more closely than do rapid bolus injections of glucose. In the following we present the description of the method for obtaining a simulated ramp and suggest a method for estimating the "true" k value as well.

SUBJECTS AND METHODS

Informed consent was obtained from five healthy men (aged 22 to 27 years) with normal body weights and no previous history of diabetes. They were first studied by a standard intravenous glucose tolerance test (IVGTT) and, three to four days later, a simulated ramp. Blood was collected for the determination of plasma glucose (g)[5] and serum insulin concentrations[6] through an indwelling catheter that was kept open by a slow infusion of saline solution.

IVGTT study. Glucose (0.33 gm. per kilogram of body weight) was administered over a two-minute period. Samples were obtained before the injection and then at five-minute intervals for 60 minutes. The glucose curve was analyzed by the two different ways, i.e., by fitting the data to

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Accepted for publication March 19, 1976.

$$g = g_0{}'e^{-k't} \quad (1)$$

where the $g_0{}'$ stands for the glucose concentration extrapolated to zero time when the fasting level is not subtracted and k′ is the corresponding glucose fractional removal rate; and

$$g = g_{\infty} + g_0e^{-kt}. \quad (2)$$

Here, g_0 is the glucose concentration extrapolated to zero time when g_{∞}, the fasting level, is subtracted and k is the fractional removal rate corrected for the fasting level.

All these five parameters were obtained by a nonlinear least-squares fit (BMD07R).[7] The measured glucose fasting levels were utilized in the estimation of the value of g_{∞} by setting them at a distant time after the beginning of the experiment (minute 1,000 was chosen) as the values to which the glucose concentration has to return.

The distribution volume of glucose was determined simultaneously (by the dilution technic) as

$$V_g = \frac{D}{g_0} \quad \text{(in deciliters)} \quad (3)$$

where D is the dose of glucose in milligrams and the estimate of the excess glucose concentration in plasma (g_0) is in mg. per 100 ml. The fractional distribution volume of glucose was obtained by dividing the distribution volume by the body weight (W), viz.:

$$v_g = \frac{V_g}{W}. \quad (4)$$

Ramp study. The handling of glucose by human subjects is complex. In addition to distribution throughout the body, glucose is metabolized or excreted through a threshold mechanism or both. Hence, a constant infusion of glucose would not produce a ramp increase of glucose in plasma. We therefore tried by a simple experimental procedure to achieve an approximate ramp. Using computer simulations of the glucose curve under simplified linear disappearance process, we expressed the rate of the change of the *amount* of glucose in plasma as the difference between a constant infusion rate (R) and the removal rate, proportional to the *amount* of glucose (G) present in plasma, viz.:

$$\dot{G} = R - kG. \quad (5)$$

Here, k is the fractional removal rate of glucose. By a number of successively increased constant-infusion rates (R_1, R_2, etc.), we attempted to obtain an approximated ramp (see figure 1).

This approach is purely operational—i.e., intended to achieve a ramp via simple steps of infusion considering the value of k constant during a step. In the experiment, it might be necessary to change the value of k for each step to correct for the effects of the variety of processes coming into play throughout the ramp increase, such as excretion when a certain plasma concentration is reached or the effects of insulin on metabolism, since the level of this hormone will not remain constant during the ramp stimulation.

In order to obtain a fairly linear plasma glucose curve, the infusion would have to be stepped up

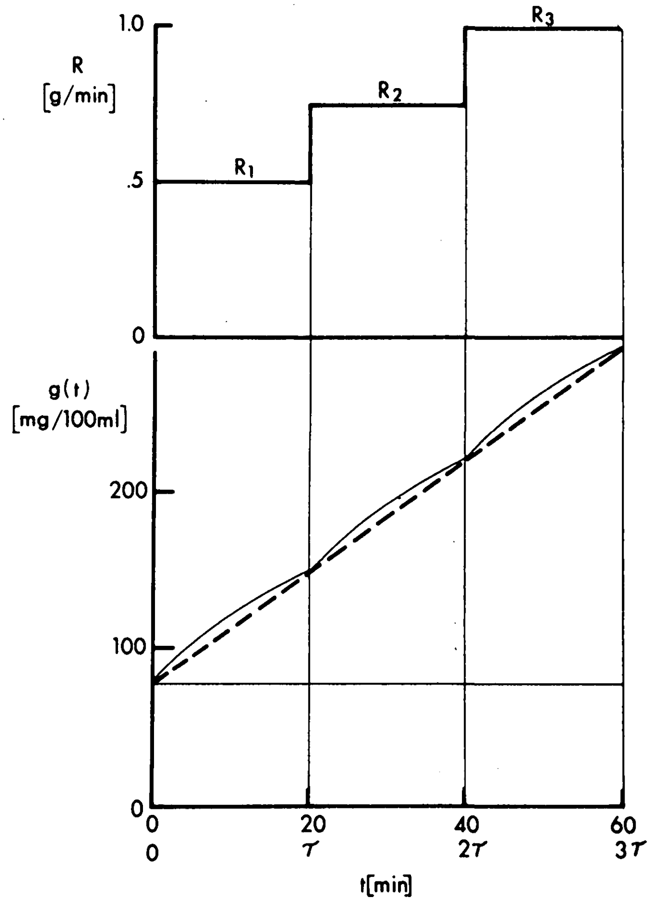

FIG. 1. Schematic diagram representing the generation of a glucose ramp in plasma of human subjects. At zero time, an infusion (rate R_1) is started (the first step at the top) and carried out for a time interval τ (20 minutes). The resulting glucose rise in the plasma is shown in the lower portion of the diagram by a slightly convex curve. At times τ and 2τ the infusion rate is stepped up to R_2 and R_3, respectively (top), resulting in similar convex curves that approximate a dotted line—the sought ramp (linear) increase of the glucose concentration in plasma.

when, or before, the time equal to the time constant of the system (1/k) has elapsed. For an average estimate of $k = 0.035$ minute $^{-1}$, the time constant has a value of about 30 minutes, and, thus, we have a *maximum* time for the duration of the step, τ. Then, solving equation **5**:

$$g(t) = \frac{R_1}{V_g k} (1 - e^{-kt}) \qquad \textbf{(6)}$$

where g stands for the excess of glucose *concentration* in plasma over the fasting level at the time t, and R_1 is the infusion rate during the first step, i.e. $0 \leqslant t < \tau$ (cf. the thin arcs above the dashed ramp in figure 1). This, then, determines the infusion rate

$$R_1 = \frac{V_g\, k\, g(\tau)}{1 - e^{-k\tau}} \qquad \textbf{(7)}$$

necessary to obtain the concentration $g(\tau)$ at the end of the step infusion.

We require that $g(\tau)$ be the concentration of glucose that at time τ is on the ramp whose slope is c (in [milligrams per 100 ml.]/minute). Hence,

$$g(\tau) = c\tau \qquad \textbf{(8)}$$

and, therefore, according to **6**:

$$c = \frac{R_1 (1 - e^{-k\tau})}{V_g\, k\tau} . \qquad \textbf{(9)}$$

During the second step, the infusion rate R_2 would have to be adjusted in order to attain a glucose concentration at the end of the second infusion double that at the end of the first, namely:

$$g\,(2\tau) = 2\,c\tau . \qquad \textbf{(10)}$$

Although this, as well as $g(n\tau)$ at the end of the "n"th step, can be computed directly (see Appendix), computer simulations were carried out instead, because in the actual experiment R_i would have to be adjusted also to account for the possibility of a changing value of k. For a typical value of $k = 0.035$ minute $^{-1}$ and $\tau = 20$ minutes, the three consecutive steps of the infusion rate ought to be in the ratio

$$R_1 : R_2 : R_3 = 1 : 1.5 : 2 \qquad \textbf{(11)}$$

in order to obtain concentrations of glucose in plasma that, by the end of each infusion period, would match the sought ramp.

In the digital simulations (PDP-12, FOCAL) we found that, at $\tau = 20$ minutes, a near-ramp is produced if the above schedule of constant-infusion rates is used; the deviation from the straight line was found to be 10 per cent at most.

The rate of glucose infusion was estimated from either k′ (uncorrected) or k (corrected) by using the distribution volume of 15 per cent of the body weight, which represents the total extracellular water,[8] and the rate of the ramp increase c = 5 (milligrams per 100 ml.)/minute. The steps of infusion were three, i.e. $\tau = 20$ minutes. Two different step increases were tested. In one, the ratios of the successive rates of infusion were as computed above (**11**) for constant fractional rate of removal, and in the second,

$$R_1 : R_2 : R_3 = 1 : 1.5 : 2.25, \qquad \textbf{(12)}$$

which allows a correction to the ramp taking into consideration the change of the value of k during the last 20-minute period.

A 20 per cent solution of glucose was administered by a Harvard infusion pump, which was always calibrated before use. Samples for plasma glucose and serum insulin were collected every two minutes from a scalp-vein needle placed in the contralateral antecubital vein.

RESULTS

The IVGTT results are summarized in table 1. The estimated fractional removal rates of glucose were essentially normal in all subjects tested.

The results of the "ramp infusion" in each subject are shown in figure 2. As is seen, the increase of plasma glucose was satisfactorily linear during the first 40 minutes. The last 20 minutes of the "ramp" curves, using the infusion ratio of 1:1.5:2, did not follow a linear rise (upper panels of figure 2). Therefore, an empirical schedule of infusion rates 1:1.5:2.25 was tested in two subjects and proved to be satisfactory for obtaining a near-ramp in plasma glucose curve in normal human subjects during the first 60 minutes (lower panels of figure 2).

The release of insulin in response to the ramp stimulation of glucose is also shown in figure 2 (open circles).

Table 2 contains the results of the observed rate of increase of the glucose ramp (c_{obs}). This table also shows the computed values of c by formula **9** with use of the individual infusion rates during the first period, R_1, employed in different subjects and the corre-

TABLE 1

Parameters estimated from standard intravenous glucose tolerance testing

Patient	Age (yr.)	Weight (kg.)	Glucose fractional removal rate Uncorrected k' (± S.D.) (minute^{-1})	Corrected* k (± S.D.) (minute^{-1})	Glucose distribution volume Absolute† V_g (dl.)	Fractional‡ v_g
MP	22	69	0.0180 ± 0.0009	0.0375 ± 0.0028	100.4	0.146
RF	25	63	0.0128 ± 0.0006	0.0249 ± 0.0009	108.6	0.172
PZ	24	83	0.0157 ± 0.0005	0.0340 ± 0.0026	146.9	0.177
DW	27	70	0.0099 ± 0.0008	0.0202 ± 0.0016	145.1	0.207
DM	24	89	0.0109 ± 0.0009	0.0293 ± 0.0029	173.2	0.194

*Corrected by subtracting fasting plasma glucose level from each measurement.
†See 3.
‡See 4.

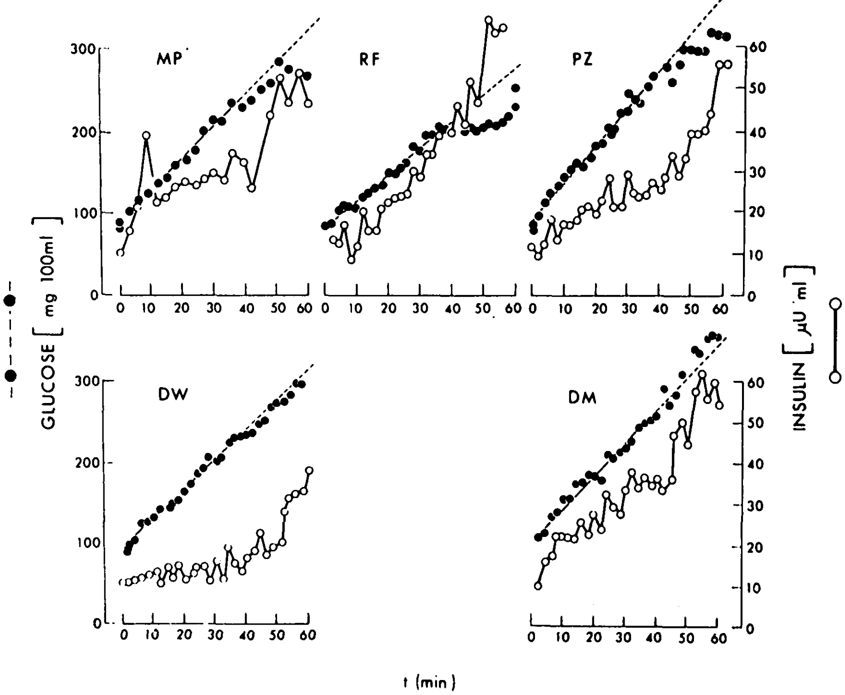

FIGURE 2

The ramp plasma glucose (closed circles) and the corresponding insulin response (open circles) in five subjects. In the upper three, the ramp extends only through the first 40 minutes. When an adjustment is made in the third infusion rate, R_3 (see figure 1)—allowing for an increased loss of glucose from plasma—a satisfactory ramp covering the period of 60 minutes is obtained in the lower two subjects (D.W. and D.M.).

TABLE 2

Slopes of plasma glucose ramp as obtained either by direct measurement or by calculation using two estimates of fractional removal rate and two estimates of glucose distribution volume

Patient	Rate of glucose infusion R_1 [mg./min.]	Observed slope of plasma glucose ramp c_{obs} [(mg./100 ml.)/min.]	Calculated slope of plasma glucose ramp c_{cal} [(mg./100 ml.)/min.] Uncorrected k' Dilution Volume* k', V_g	Volume fraction of body weight k', 0.15 W	Corrected k Dilution volume* k, V_g	Volume fraction of body weight k, 0.15 W
MP	608	3.91	5.09	4.93	4.26	4.13
RF	534	3.17	4.34	4.99	3.87	4.45
PZ	820	4.46	4.79	5.63	4.05	4.76
DW	640	3.71	4.00	5.53	3.63	5.01
DM	860	4.07	4.46	5.77	3.99	5.15

*See 3.

sponding values of k and k′ and two kinds of estimates of the distribution volumes (i.e., V_g from **3** and 15 per cent of the body weight).

In table 3 we present the fractional differences between the calculated and observed rates of ramp increase c (i.e., $\frac{c_{cal} - c_{obs}}{c_{obs}}$).

When the fractional difference is closer to zero, the corresponding values for k and V_g are better estimates of their actual values than those for which the fractional difference deviates further from zero. In table 3, probability values for fractional differences show that only those calculated from corrected values for k and the volume distribution for glucose estimated by dilution technic (V_g) are not significantly different from zero. The other three methods are significantly different, at least at a 5 per cent level or more, suggesting that they do not provide estimates as accurate for fractional removal rate and actual distribution volume of glucose.

DISCUSSION

The present study demonstrates that it is possible to produce a ramp increase of plasma glucose in man by a simple scheme for glucose infusions. During this work it became apparent that the method for calculating the glucose disappearance rate coefficient or fractional removal rate (k) was important. The statistical analysis in table 3 shows that, when k is computed from data corrected for the fasting levels of glucose, it can best predict the ramp increase. In addition we found that V_g, as computed by the dilution technic from the IVGTT, was a better estimate than using 15 per cent of the body weight (table 3).

It was apparent (figure 2) that the disappearance rate of glucose changed during the last 20 minutes, since the calculated steps (1:1.5:2) did not produce a ramp during this part of the experiment. We found that by a 12.5 per cent increase of the last step a satisfactory ramp could be obtained. The enhanced rate of glucose disappearance could be due to an increased urinary glucose excretion or the action of insulin released during the experiment or both. As seen in figure 2, the glucose ramp produced a marked release of insulin during the latter part of the ramp experiments, which could account for this effect.

Achievement of a slow rise in plasma glucose will more closely reproduce the effects from a postprandial rise in glucose without involving the variety of associated neurogenic and humoral effects on islet secretion accompanying meal consumption. Such a plasma glucose pattern may be a valuable means of characterizing glucose-induced insulin release in man under conditions simulating the rise in glucose after a meal.

APPENDIX

Using the balance equation for total glucose in plasma (cf. **5**)

$$\dot{G} = R - kG,$$

the concentration of glucose in plasma at the end of the first infusion ($t = \tau$) is (cf. **6**)

$$g(\tau) = \frac{R_1}{V_g k} (1 - e^{-k\tau})$$

TABLE 3

Comparison of calculated and observed values for the slope of plasma glucose ramp

	Fractional difference of slopes $\left[\frac{c_{cal} - c_{obs}}{c_{obs}}\right]$			
	Uncorrected k′		Corrected k	
Patient	Dilution volume k′, V_g	Volume fraction of body weight k′, 0.15 W	Dilution volume k, V_g	Volume fraction of body weight k, 0.15 W
MP	0.30	0.26	0.09	0.06
RF	0.37	0.57	0.22	0.40
PZ	0.07	0.26	−0.09	0.07
DW	0.08	0.49	−0.02	0.35
DM	0.10	0.42	−0.02	0.27
t	2.9291	6.4634	0.6633	3.2625
p	<0.05	<0.005	>0.5	<0.025

When the second infusion is ended ($t = 2\tau$), the concentration of glucose in plasma is

$$g(2\tau) = \frac{R_2}{V_g k}(1 - e^{-k\tau}) + g(\tau)\, e^{-k\tau}.$$

Since we require that

$$g(2\tau) = 2\, g(\tau),$$

one obtains an expression for the rate of the two infusion rates, i.e.

$$\frac{R_2}{R_1} = 2 - e^{-k\tau}.$$

Analogously, because $g(3\tau) = 3\, g(\tau)$,

$$\frac{R_3}{R_1} = 3 - 2e^{-k\tau}.$$

For the typical value of $k = 0.035$ minute^{-1} and for the duration of one infusion step $\tau = 20$ minutes, one finds

$$\frac{R_2}{R_1} = 1.5 \text{ and } \frac{R_3}{R_1} = 2.$$

If the product of the fractional removal rate k and the duration of a step infusion τ is kept constant, then another rate of the ramp increase c is achieved by finding out the corresponding first-step infusion rate

$$R_1 = \frac{V_g\, ck\tau}{1 - e^{-k\tau}}.$$

ACKNOWLEDGMENTS

This investigation was supported in part by grants AM-13659 (U.S.P.H.S.), HL 06285, HL 5251, and AM-12763(05) from the National Institutes of Health and by a grant from the Levi J. and Mary Skaggs Foundation of Oakland, California.

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