Letters to the Editor

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letters to the editor

The following is an abstract of the article discussed in the subsequent letter:

Finegood, Diane T., and Dan Tzur. Reduced glucose effectiveness associated with reduced insulin release: an artifact of the minimal-model method. *Am. J. Physiol.* 271 (*Endocrinol. Metab.* 34): E485–E495, 1996.—We previously demonstrated that minimal model-derived estimates of glucose effectiveness (S_G), based on the frequently sampled intravenous glucose tolerance test (S_{GFSIGT}), were reduced in islet-transplanted or streptozotocin-treated dogs and in patients with insulin-dependent diabetes mellitus. To ascertain the validity of our observations, we compared S_{GFSIGT} with estimates based on a basal hormone replacement glucose clamp ($S_{GBRCLAMP}$) and a basal hormone replacement glucose tolerance test (S_{GBRGTT}) in normal control (CNTL, $n = 12$) and streptozotocin-treated dogs with normal fasting plasma glucose (STZ-Rx, $n = 9$). S_{GFSIGT} was reduced in STZ-Rx compared with CNTL ($P < 0.05$). However, neither $S_{GBRCLAMP}$ nor S_{GBRGTT} was reduced in the STZ-Rx group ($P > 0.05$). Comparison of protocols for each subject indicated that S_{GFSIGT} was greater than either $S_{GBRCLAMP}$ or S_{GBRGTT} in control ($P < 0.002$) but not in STZ-Rx dogs ($P > 0.1$). The relationship of S_{GFSIGT} to insulin secretory function suggests that our previous conclusion that S_{GFSIGT} was reduced in subjects with limited insulin release may be an artifact of the minimal-model method. Our results suggest that caution must be exercised in the interpretation of differences in minimal-model estimates of S_G between subject groups with significantly different levels of insulin secretory function.

Minimal Model Estimate of Glucose Effectiveness: Role of the Minimal Model Volume and of the Second Hidden Compartment

To the Editor: The paper by Finegood and Tzur (9) on a potential artifact of the minimal model (3) in assessing glucose effectiveness addresses a relevant issue given the important role of this index (1, 2, 5). In reading their arguments, we think that some clarification of some of their methodological aspects of data analysis and some of their conclusions would be helpful for other readers. We think that our observations and insights are critical, especially in light of the increasing demand for a better definition of the domain of validity of the minimal model estimate of glucose effectiveness called for by recent experimental (10) and theoretical (6–8) results.

The volume issue. Comparison of the minimal model index of glucose effectiveness, $S_{GFSIVGTT}$, measured from a frequently sampled intravenous glucose tolerance test, FSIVGTT, with the analogous clamp-based index, $S_{GBRCLAMP}$, measured from a basal hormone replacement glucose clamp, BRCLAMP, requires coping with the fact that the two indexes are not expressed in the same units. Whereas $S_{GFSIVGTT}$, a fractional index, is expressed in minutes, $S_{GBRCLAMP}$ is expressed in milliliters per kilogram per minute. To convert the two indexes to common units, one must either multiply $S_{GFSIVGTT}$ or divide $S_{GBRCLAMP}$ by some volume factor. Various approaches have been taken. Ader et al. (1) divided the mean value of $S_{GBRCLAMP}$ by Steele's volume (169 ml/kg) (12). In contrast, Finegood and Tzur (9) chose to divide $S_{GBRCLAMP}$ by the mean total volume of glucose distribution taken from the literature (250 ml/kg). However, as Finegood and Tzur state in their paper, "the approach taken and the (volume) estimate used will affect the magnitude of $S_{GBRCLAMP}$ and could impact on the conclusion that S_G is overestimated by the minimal model method in normal subjects." Moreover, it is important to recognize that the chosen approach and volume are likely to affect their correlation plots (Figs. 4 and 5 in Ref. 9) between $S_{GFSIVGTT}$, $S_{GBRCLAMP}$, and the third index of glucose effectiveness they measured, S_{GBRGTT}, based on a basal hormone replacement glucose tolerance test, BRGTT.

The resolution of the volume issue is thus of paramount importance to put the comparison between minimal model and clamp indexes of glucose effectiveness on firm ground. In a previous paper (6) we suggested that to convert the minimal model and clamp indexes to the same units one should multiply $S_{GFSIVGTT}$ by the minimal model volume of glucose distribution, V, because the information leading to an individualized estimate of the volume is available in each FSIVGTT data set. We will show formally that this is the correct approach. To do so we need to return to the definition of glucose effectiveness. Glucose effectiveness measures the effect of glucose at basal insulin to enhance its own disappearance from plasma (R_d) and inhibit its own endogenous production (EGP) (5)

$$\text{Glucose effectiveness} = \left.\frac{\delta[R_d(t) - EGP(t)]}{\delta G(t)}\right|_{I = I_b} \qquad (1)$$

where I_b denotes basal insulin concentration. Applying the above definition to a glucose clamp in which one attains a steady state for plasma glucose concentration, R_d, and EGP, one has

$$\text{Clamp glucose effectiveness} = \frac{\Delta[R_d - EGP]}{\Delta G} = \frac{GINF}{\Delta G} = S_{GBRCLAMP}(\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \qquad (2)$$

where GINF is the exogenous glucose infusion rate needed to compensate for the increase in R_d and the decrease in EGP.

The minimal model describes glucose dynamics during an intravenous glucose tolerance test with the well-known equations

$$\frac{dG(t)}{dt} = -[S_G + X(t)]G(t) + S_G G_b \qquad G(0) = G_b + \frac{D}{V}$$
$$\frac{dX(t)}{dt} = -p_2[X(t) + S_I(I(t) - I_b)] \qquad X(0) = 0 \qquad (3)$$

where D is the injected glucose dose and V is the minimal model volume of glucose distribution. The index of glucose effectiveness, S_G, will denote $S_{GFSIVGTT}$ or S_{GBRGTT}, depending on whether a FSIVGTT or a BRGTT is carried out. Of note is that during a BRGTT insulin action X in *Eq. 3* is identically equal to zero. If we express the glucose equation in terms of glucose mass instead of concentration, its right member describes the net balance between R_d and EGP

$$R_d(t) - EGP(t) = [S_G + X(t)]Q(t) - S_G Q_b$$
$$Q(t) = G(t)V \quad (4)$$

where Q is the glucose mass in the system. Applying the definition of glucose effectiveness (*Eq. 1*) to the expression of R_d-EGP of the minimal model given by *Eq. 4*, one obtains

$$\text{Minimal model glucose effectiveness} = S_G V \quad (\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \quad (5)$$

Comparing *Eqs. 2* and *5*, one can see that no conversion is necessary because the minimal model method provides an index of glucose effectiveness that has the same units as the clamp-based index. Of note is that V can be estimated from the same data that provide $S_{GFSIVGTT}$ or S_{GBRGTT} and can therefore be individualized in each subject. Because the volume information is contained in the data, the use instead of Steele's volume [as in Ader et al. (1)] or of a mean total glucose distribution volume [as in Finegood and Tzur (9)] is hardly justifiable. Incidentally, this individualized volume has been already used in the validation studies of the minimal model insulin sensitivity index, where $S_I V$ has been evaluated against the insulin sensitivity index measured by the clamp technique (3, 11).

In light of the above considerations, the question arises as to whether the conclusions drawn in Ref. 9 are confirmed if an individualized volume is used instead of the mean total distribution volume. Specifically, two points need to be readdressed.

$S_{GFSIVGTT}$ vs. S_{GBRGTT}. Finegood and Tzur found that, in normal subjects, the fractional glucose effectiveness index measured during a traditional FSIVGTT, $S_{GFSIVGTT}$, is higher than the one estimated during a glucose tolerance test at basal insulin, S_{GBRGTT}. Does this relationship still hold when each fractional index is multiplied by the companion volume V? The question arises because in all likelihood the minimal model volume V estimated from a FSIGT is lower than that estimated from a BRGTT.

Noncorrelation between S_{GBRGTT} and $S_{GBRCLAMP}$. The noncorrelation ($r = 0.05$) between S_{GBRGTT} and $S_{GBRCLAMP}$ of Fig. 5 in Ref. 9 is quite a surprise and would have deserved more discussion in the paper. This result implies that, even at basal insulin, i.e. under optimized experimental conditions, the minimal model does not provide a valid measure of glucose effectiveness. Of the two measures of glucose effectiveness $S_{GBRCLAMP}$ has more history, so let's assume for the sake of reasoning that $S_{GBRCLAMP}$ is not a problem. In calculating glucose effectiveness from the BRGTT, what's still missing is the volume V estimated in each individual. Thus the correlation between the BRGTT- and BRCLAMP-based indexes of glucose effectiveness needs to be reevaluated by comparing $S_{GBRCLAMP}$ to $S_{GBRGTT}V$.

Role of the hidden compartment. Finegood and Tzur found that $S_{GFSIVGTT}$ is higher than S_{GBRGTT} in normal dogs but not in dogs with reduced insulin secretory function. The result obtained in normal dogs is in keeping with the results reported by Quon et al. (10), who found in insulin-dependent diabetic patients a discrepancy between the minimal model prediction and the experimentally observed profile of glucose concentration during a BRGTT. Finegood and Tzur formulated the hypothesis that, when the minimal model is applied to individuals with a normal insulin secretory function, it is unable to correctly segregate glucose and insulin effects on glucose disappearance. This is an elegant way of putting the issue, but it does not help much in clarifying what's wrong with the minimal model.

The finding that $S_{GFSIVGTT}$ is higher than S_{GBRGTT} in normal dogs is clearly a symptom of model error, because the value that is taken on by S_G should be independent of the insulin profile during the glucose tolerance test. So where is the error in the minimal model? Finegood and Tzur did not answer this question. Here we would like to offer our interpretation of their findings by building on a recent paper in which we theoretically analyzed the effect of the single-compartment approximation of the minimal model on S_G estimation by using a two-compartment model as a reference (7). First, Finegood and Tzur showed that glucose decay during a BRGTT is biexponential and not monoexponential as dictated by the minimal model, thus confirming our theoretical prediction (7). They then concluded that the monocompartmental description of glucose kinetics is sufficiently adequate, since the minimal model was well able to describe the BRGTT glucose data from 10 min onward. Unfortunately, the finding that the single-pool description is reasonably good when the glucose system is studied at basal insulin does not ensure that such an approximation is also adequate when insulin, in addition to glucose, changes during the test. Extrapolating to the FSIVGTT what has been found with the BRGTT is not only methodologically questionable but probably fallacious. During the BRGTT glucose decay is governed only by glucose effectiveness, and S_G is estimated from the whole glucose data set between 10 and 180 min. Because the fast component of glucose disappearance becomes negligible after ~20 min, S_{GBRGTT} is mainly determined by the slow component. In contrast, during the FSIVGTT, glucose decay reflects both glucose effectiveness and insulin sensitivity, and S_G is mainly estimated in the initial portion of the test, when glucose is high and insulin action, albeit increasing, is low. Because this is the moment when the fast component of glucose disappearance plays the major role (7), it is easy to realize that one will obtain higher values of $S_{GFSIVGTT}$ than S_{GBRGTT}. In other words, we speculate that the monocompartmental approximation

is much more critical during the FSIVGTT than during the BRGTT, and the higher values observed for $S_{GFSIVGTT}$ than for S_{GBRGTT} can be explained by the presence of a second, inaccessible compartment. The finding that the gap between $S_{GFSIVGTT}$ and S_{GBRGTT} is reduced in animals with impaired secretory function also fits with the above reasoning: when the early insulin response is absent, the time window crucial for S_G estimation (glucose high and insulin action low) widens, and the relative importance of the fast vs. the slow component of R_d in determining the value of S_G diminishes. As a result, $S_{GFSIVGTT}$ comes close to the value of the slow component and thus to S_{GBRGTT}.

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REPLY

To the Editor: Drs. Caumo and Cobelli have raised several issues with regard to our 1996 publication on artifacts in minimal model-derived glucose effectiveness (3). We appreciate this opportunity to expand on explanations given in our 1996 paper. Caumo and Cobelli raise two issues and consider them independently: *1*) the means by which we corrected for the volume of distribution and *2*) the two-compartment nature of glucose kinetics. These two issues are not independent. If, as they argue in the second part of their letter, a second compartment is responsible for the observed artifact, then it is not consistent to assume that a single time-invariant volume of distribution, estimated from the initial slope of glucose fall during an intravenous glucose tolerance test, is the best way to put clamp and frequently sampled intravenous glucose tolerance (FSIGT) data on the same unit basis.

The correspondents argue that it is more correct to multiply S_{GFSIGT} estimates by the initial distribution volume than to divide the clamp-based estimate, $S_{GBRCLAMP}$, by a steady-state glucose distribution volume. Their argument is based on the fact that an individualized volume of distribution can be obtained from each FSIGT experiment and a tautological manipulation of the one-compartment glucose kinetics equation. Although we agree that it would be helpful to be able to use a volume of distribution that is based on each individual subject's data, we reject the notion that this is theoretically more correct. Furthermore, we believe this approach is not optimal because of the so-called "hidden compartment."

Caumo and Cobelli perform some algebraic manipulations, which they provide as proof that minimal-model glucose effectiveness must be calculated as S_GV. In their manipulations, the distribution volume comes up on the right side of the equation because their starting definition of glucose effectiveness lacks consideration of the distribution volume and is, in fact, different from the original definition put forth by Bergman et al. (1), including Dr. Cobelli. In the original minimal-model paper, S_G was defined as

$$S_{GFSIGT} = \left.\frac{\delta \dot{G}}{\delta G}\right|_{I=I_b} \qquad (1)$$

If we start with the basic mass balance equation governing both the clamp and the FSIGT situation, we have

$$EGP(t) - R_d(t) + GINF(t) = \frac{d[VG(t)]}{dt} \qquad (2)$$

Because it has been assumed both by ourselves and by Caumo and Cobelli that, in this instance, the hidden compartment is not important and that the volume of distribution is time invariant, this equation is equiva-

lent to

$$EGP(t) - R_d(t) + GINF(t) = V\frac{dG(t)}{dt} \quad (3)$$

In an FSIGT, $GINF(t) = 0$, so

$$\frac{\delta[EGP(t) - R_d(t)]}{\delta G} = V\frac{\delta \dot{G}}{\delta G} \quad (4)$$

Combining *Eqs. 1* and *4*

$$S_{GFSIGT} = \frac{1}{V}\left.\frac{\delta[R_d(t) - EGP(t)]}{\delta G}\right|_{I=I_b} \quad (5)$$

As Caumo and Cobelli have stated, clamp glucose effectiveness is defined as

$$S_{GBRCLAMP} = \frac{\Delta(R_d - EGP)}{\Delta G} \quad (6)$$

Combining *Eqs. 5* and *6*, we have

$$S_{GFSIGT} = \frac{1}{V} S_{GBRCLAMP} \quad (7)$$

or the equivalent expression

$$S_{GBRCLAMP} = S_{GFSIGT} \times V \quad (8)$$

Clearly the result of this algebraic manipulation depends on your starting point, and from a theoretical point of view both equations are correct. The form of the equation that first emerges is determined by whether you start with the original definition of glucose effectiveness or with a definition that lacks consideration of the distribution volume.

Given that true glucose kinetics are approximated only by a single compartment and that a second so-called hidden compartment may be important, we must also consider the effect of the hidden compartment on estimates of the distribution volume. Wolfe (5) clearly demonstrated that, in the nonsteady state, a single-compartment volume of distribution varies with time, with the greatest time dependence occurring at the beginning of a perturbation such as glucose administration. In contrast, as the system approaches steady state, the distribution volume varies less in time and approximates the original value determined by Steele at 25% of body weight (4). For this reason, we believe the nonindividualized steady-state estimate of distribution volume used to correct the clamp calculation may be more accurate than the individualized, but highly time-dependent estimate of the initial distribution volume obtained during the FSIGT. Because the assumption that the distribution volume does not vary is incorporated in the definition of glucose effectiveness, we believe use of the steady-state estimate to correct the steady-state experiment is less subject to error.

The correspondents suggest that, if we correct S_{GFSIGT} and S_{GBRGTT} by the individual estimates of the distribution volume, these two parameters will become equivalent, because they believe that the volume estimated from an FSIGT will be lower than that estimated from a BRGTT. The reason why they believe that the volume estimated from the FSIGT would be lower than that from the BRGTT was not explained. Contrary to their expectations, the distribution volume estimates from these two types of experiments are identical (1.32 ± 0.13 vs. 1.31 ± 0.13 dl/kg, $P = 0.89$ by paired *t*-test), and the relationship between $S_{GBRGTT} \times V$ and $S_{GFSIGT} \times V$ is the same as in Fig. 4*B* of our original manuscript (3).

Drs. Caumo and Cobelli also speculated that if we correct S_{GBRGTT} rather than $S_{GBRCLAMP}$, the correlation in Fig. 5 might improve. In fact, $S_{GBRGTT} \times V$ is also not correlated with $S_{GBRCLAMP}$ ($r = 0.28$, $P = 0.43$), and the previously equivalent estimates of glucose effectiveness are no longer equivalent ($S_{GBRGTT} = 1.9 \pm 0.2$ vs. $S_{GBRCLAMP} = 4.3 \pm 0.5$ ml·min^{-1}·kg^{-1}, $P < 0.001$). We take this as further proof that correction of $S_{GBRCLAMP}$ is more appropriate than that of S_{GBRGTT} or S_{GFSIGT} with individualized values of the distribution volume. The lack of correlation between $S_{GBRCLAMP}$ and S_{GBRGTT} is more likely due to the rather small (~3-fold) range of normal values and the fact that the estimate of $S_{GBRCLAMP}$ is not very precise. As we indicated in our paper (3), the coefficient of variation for $S_{GBRCLAMP}$ was $60 \pm 15\%$. Although the coefficient of variation for S_{GBRGTT} was not determined, we expect that it would be similar to that of S_{GFSIGT}, which was found to be $23 \pm 4\%$.

Finally, we agree with the correspondents that their subsequent model-based analysis (2) of the role of the hidden compartment helps to explain the reason for the artifact identified in our paper (3). Their arguments provide a theoretical basis for our contention that the inadequacy of the one-compartment representation of the BRGTT experiments in control animals but not in streptozotocin-treated animals might provide at least a partial explanation for the observed artifact. The correspondents' demonstration that the hidden compartment is important (2) was beyond the scope of our original paper (3).

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