# Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT

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COBELLI, CLAUDIO, GIOVANNI PACINI, GIANNA TOFFOLO, AND LUIGI SACCÀ. *Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT.* Am. J. Physiol. 250 (Endocrinol. Metab. 13): E591–E598, 1986.—The "minimal model" of glucose disappearance provides noninvasive estimates of insulin sensitivity and glucose effectiveness from an intravenous glucose tolerance test (IVGTT). However, this model does not allow the separation of glucose production from utilization. To overcome this limitation, labeled glucose was injected along with cold glucose in six normal dogs, and both cold and labeled glucose time courses were monitored along with insulin concentration. A revised minimal model was fitted to tracer data to obtain new measures of insulin sensitivity ($S_I^* = 6.41 \pm 0.91\ 10^{-4}\ min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$) and fractional glucose clearance ($S_G^* = 0.0092 \pm 0.0009\ min^{-1}$). $S_G^*$ was compared with a direct measure obtained by a hepatic arterial-venous difference technique, which yielded a value of $0.0097 \pm 0.0002$, virtually identical to $S_G^*$, thereby validating the model estimate. When the original minimal model was identified from cold data, we obtained $S_I = 4.52 \pm 1.39$ and $S_G = 0.042 \pm 0.009$. $S_I^*$ and $S_G^*$ were different from $S_I$ and $S_G$, respectively. In particular $S_G$ overestimates fractional glucose clearance by approximately five times. The revised minimal model yields glucose disposal parameters $S_I^*$ and $S_G^*$ that are not affected by the confounding effect of insulin and glucose inhibition of glucose production. Limitations inherent in cold IVGTT and original minimal model are overcome by labeled IVGTT and the revised minimal model, while test simplicity remains.

glucose tolerance; glucose kinetics; insulin action; mathematical modeling; system identification; parameter estimation; tracer kinetics

PHYSIOLOGICAL PARAMETERS are usually not accessible to direct measurement, especially in studies performed in vivo. To overcome this difficulty, mathematical models in conjunction with dynamic data offer an important noninvasive approach, as parameter evaluation can often be posed as an estimation problem. This line of thought has been used in developing two models of the glucose-insulin system: one of glucose disappearance (3, 5) and one of insulin kinetics (24). These models are models of system rather than models of data, and are called minimal because they are physiologically based and have the smallest number of precisely estimated parameters for describing a particular data set. These two models allow the estimation of several metabolic parameters, like peripheral tissue sensitivity to insulin and $\beta$-cell responsivity, from glucose and insulin concentration discrete time courses measured after a frequently sampled intravenous glucose tolerance test (IVGTT). They have been widely applied in dogs (5, 24) and in humans in different conditions, such as glucose intolerance (6) and aging (8).

The minimal model of glucose disappearance has proven particularly robust in quantifying glucose disposal processes, by providing a measure of insulin sensitivity and glucose effectiveness. The model-based measure of insulin sensitivity has been validated against an independent measure both in dogs (14) and in humans (2), and its routine potential compared with the other available techniques, which are rather invasive and require sophisticated expertise, has been reviewed (4).

However, with unlabeled non-steady-state data like the glucose and insulin concentration time courses measured during an IVGTT, it is difficult to separate the production and utilization processes. This forces a parametric description of the system that must compromise for simplicity to have a fully identifiable model (5) and may result in an error in the model structure, which thus provides a biased parametric description of the system. Moreover, in this simplified structure, processes occurring both at the production and utilization side may need to be lumped in the same model parameter. In this case, physiological interpretation of the obtained numerical estimates is quite difficult.

To minimize model errors and avoid ambiguities that may arise when estimating parameters describing glucose disposal, based on unlabeled data only, it is essential to

 

separate the individual contribution of production and utilization. Because tracers allow the monitoring of disappearance processes only, an experiment with labeled glucose, along with a cold glucose perturbation, was designed to quantitate utilization. By use of these data and a revised minimal model, a new measure of the glucose disposal processes and of insulin's effect on them was determined.

In this study, IVGTTs with cold and labeled glucose have been performed in dogs. Two models of glucose disappearance have been identified: the original minimal model from unlabeled glucose data and the revised model from the labeled glucose data. Two parametric metabolic portraits are derived. The revised model with labeled data provides a parametric measure of glucose utilization processes alone, in particular of tissue insulin sensitivity and fractional glucose clearance. This last parameter has been validated against an independent measurement obtained by an arteriovenous difference technique. In addition, by comparing this tracer-determined parameter set with the cold-determined set, both model errors and undesired confounding effects on single estimated parameters can be quantified.

## MINIMAL MODEL OF GLUCOSE DISAPPEARANCE: METABOLIC PARAMETERS FROM AN UNLABELED GLUCOSE INJECTION

*Model.* Basic physiology and assumptions underlying the structure of the original minimal model of glucose disappearance after an IVGTT have been described in detail (5). Here only the model equations are briefly restated.

The model (Fig. 1) is described by two dynamic equations, one for glucose kinetics and one for the action of insulin upon them. Insulin's effect is exerted by insulin concentration, $I'(t)$, in a compartment remote from plasma, the kinetics of which is linear and described by

$$\frac{dI'(t)}{dt} = k_2[I(t) - I_b] - k_3 I'(t) \qquad I'(0) = 0 \quad (1)$$

where $I(t)$ is plasma insulin concentration, $I_b$ its basal value and $k_2$ and $k_3$ are constant nonnegative parameters.

Glucose kinetics are described by a one-compartment mass balance equation

$$\frac{dG(t)}{dt} = R_a(t) - R_d(t) \qquad G(0) = G_0 \quad (2)$$

where $G(t)$ is plasma glucose concentration, $R_a(t)$ and $R_d(t)$ are, respectively, hepatic glucose production and utilization normalized to glucose distribution volume, and $G_0$ is the extrapolated value of G at *time 0* and incorporates the effect of the injection.

Cold IVGTT data did not allow the explicit description of $R_a(t)$ (5). However, it was possible to describe the net hepatic glucose balance [NHGB($t$)], i.e., the difference between liver glucose production $R_a(t)$ and uptake $R_{dL}(t)$. As $R_{dL}(t)$ is a component of $R_d(t)$, *Eq. 2* becomes

$$\frac{dG(t)}{dt} = R_a(t) - R_{dL}(t) - R_{dP}(t) = NHGB(t) - R_{dP}(t) \qquad G(0) = G_0 \quad (3)$$

where $R_{dP}(t)$ is extrahepatic glucose utilization, i.e., $R_d(t) = R_{dL}(t) + R_{dP}(t)$.

The processes occurring both in the liver and periphery have been described as functions of glucose and remote insulin. In particular the NHGB is given by

$$NHGB(t) = B_0 - k_6 I'(t)G(t) - k_5 G(t) \quad (4)$$

where $B_0$ is extrapolated NHGB at zero glucose and $k_5$ and $k_6$ are constant nonnegative parameters.

Extrahepatic glucose utilization is given by

$$R_{dP}(t) = k_1^P G(t) + k_4^P G(t) I'(t) \quad (5)$$

where $k_1^P$, $k_4^P$ are constant nonnegative parameters. By substituting *Eqs. 4* and *5* in *Eq. 3*, we obtain

$$\frac{dG(t)}{dt} = -(k_1^P + k_5)G(t) - (k_4^P + k_6)I'(t)G(t) + B_0 \quad (6)$$

*Equations 1* and *6* along with $G(0) = G_0$ describe the original minimal model of glucose kinetics, where $I(t)$, the measured plasma insulin concentration, is assumed to be the input. The model is a priori unidentifiable from the input/output experiment, and it is necessary to aggregate some of the parameters to reach an identifiable parametrization. The following reparametrization has been shown to be uniquely a priori identifiable (5, 7) from IVGTT data

$$\frac{dG(t)}{dt} = -[p_1 + X(t)]G(t) + p_4 \qquad G(0) = G_0 \quad (7)$$

$$\frac{dX(t)}{dt} = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \quad (8)$$

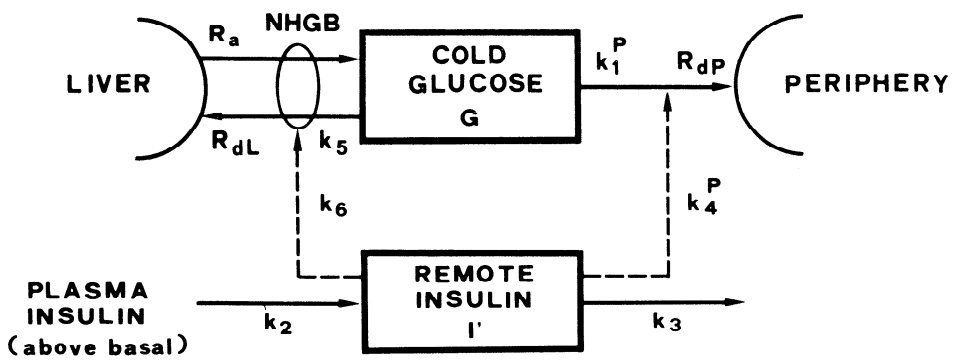


FIG. 1. Block diagram of minimal model used with cold data. Insulin acts via a compartment remote from plasma (I'). Glucose (G) is utilized by periphery ($R_{dP}$) and taken up by liver ($R_{dL}$). Glucose production is described by $R_a$. *Dashed lines* represent controls of remote insulin on peripheral utilization and on liver processes, which are lumped together in net hepatic glucose balance (NHGB). $k$ Parameters are rate parameters characterizing either material fluxes (*solid lines*) or control actions (*dashed lines*).

where

$$X(t) = (k_4^P + k_6)I'(t) \quad (9)$$

$$p_1 = k_1^P + k_5 \quad (10)$$

$$p_2 = k_3 \quad (11)$$

$$p_3 = k_2(k_4^P + k_6) \quad (12)$$

$$p_4 = B_0 \quad (13)$$

In the basal steady state

$$\frac{dG(t)}{dt} = 0 \quad (14)$$

$$G(t) = G_b \quad (15)$$

$$\frac{dX(t)}{dt} = 0 \quad (16)$$

$$X(t) = 0_b \quad (17)$$

and *Eq. 7* gives the following expression for $p_4$

$$p_4 = p_1 G_b \quad (18)$$

*Metabolic parameters derived from model.* From this model it is possible to estimate characteristic metabolic parameters that describe, in quantitative terms, qualitative clinical and physiological concepts. In particular, two of the processes that are responsible for the tissue ability to dispose of administered glucose can be easily estimated from the minimal model. These processes are the sensitivity to insulin of tissue glucose utilization and the effect of glucose levels per se on its disappearance. Insulin sensitivity is described by the insulin sensitivity index (5), which has been shown to be the ratio of two estimated parameters

$$S_I = -\frac{\partial^2[dG(t)/dt]}{\partial G(t)\partial I(t)}\bigg|_{ss} = \frac{p_3}{p_2} = \frac{k_2(k_4^P + k_6)}{k_3} \quad (19)$$

where *ss* denotes that the second derivative of $dG(t)/dt$ is computed at steady state. This parameter describes the ability of insulin to enhance plasma glucose disappearance.

The effect of glucose per se on its own disposition can be quantified via the glucose effectiveness factor (4), which has been shown to be given by

$$S_G = -\frac{\partial[dG(t)/dt]}{\partial G(t)}\bigg|_{ss} = p_1 = k_1^P + k_5 \quad (20)$$

where in this case, too, the derivative is computed at steady state, i.e., $I_{ss} = I_b$. This parameter describes the fractional increase in glucose disappearance per unit increase in plasma glucose with insulin remaining at its basal steady state.

## MINIMAL MODEL APPLIED TO TRACER DATA: NEW INSIGHTS FROM A SIMULTANEOUS COLD AND LABELED GLUCOSE INJECTION

*The Model.* When tracer is injected along with cold glucose, a new dynamic equation for labeled glucose becomes available (Fig. 2). Assuming the tracer monitors only disappearance processes, the kinetics of labeled glucose is, from *Eq. 2*

$$\frac{dG^*(t)}{dt} = -R_d^*(t) \qquad G^*(0) = G_0^* \quad (21)$$

where the asterisk denotes "tracer."

Paralleling *Eq. 3*, the total disappearance rate of the tracer, $R_d^*(t)$, can be split into two components, the liver, $R_{dL}^*(t)$, and extrahepatic, $R_{dP}^*(t)$, utilization

$$R_d^*(t) = R_{dL}^*(t) + R_{dP}^*(t) \quad (22)$$

A functional description of $R_{dP}^*(t)$ can be derived from *Eq. 5*, applying the basic tracer-tracee relation (23)

$$R_{dP}^*(t) = \frac{R_{dP}(t)}{G(t)} G^*(t) = k_1^P G^*(t) + k_4^P I'(t) G^*(t) \quad (23)$$

As concerns $R_{dL}^*(t)$, the same rationale cannot be applied, because for cold glucose we have defined only the net balance between $R_{dL}(t)$ and liver production, $R_a(t)$. To proceed, let us assume that $R_d^*(t)$ of *Eq. 21* has the same functional dependance on $G^*(t)$ and $I'(t)$ as $R_{dP}^*(t)$ of *Eq. 23*, thus

$$\begin{aligned} R_d^*(t) &= k_1 G^*(t) + k_4 I'(t) G^*(t) \\ &= (k_1^P + k_1^L) G^*(t) + (k_4^P + k_4^L) I'(t) G^*(t) \end{aligned} \quad (24)$$

where $k_1^L$ and $k_4^L$ are constant nonnegative parameters responsible of liver uptake. Thus it is possible to write an expression for $R_{dL}^*(t)$, obtained by difference between *Eqs. 24* and *23*. This expression is

$$R_{dL}^*(t) = k_1^L G^*(t) + k_4^L I'(t) G^*(t) \quad (25)$$

It describes glucose uptake by the liver when it is physiologically insulinized. Recent experimental evidence (9, 11, 20) suggests that, while insulin is necessary for hepatic glucose uptake to be activated, under physiological hepatic insulin levels, the magnitude of glucose uptake is predominantly controlled by glucose levels. Therefore $k_4^L$ can be approximately set equal to zero. However, in the following, we will retain $k_4^L$ as a parameter greater or equal to zero.

Substituting *Eq. 24* into *21*, glucose tracer kinetics is described by

$$\frac{dG^*(t)}{dt} = -(k_1^P + k_1^L) G^*(t) - (k_4^P + k_4^L) I'(t) G^*(t) \quad (26)$$

with $G^*(0) = G_0^*$. The same considerations about identifiability made for the system of *Eqs. 1* and *6* may be extended to the system of *Eqs. 1* and *26*. Also, in this case it is necessary to lump some parameters to achieve a priori identifiability of the model. It can be shown that the following parametrization is uniquely identifiable (7) from insulin and labeled glucose measurement

$$\frac{dG^*(t)}{dt} = -[p_1^* + X^*(t)] G^*(t) \quad G^*(0) = G_0^* \quad (27)$$

$$\frac{dX^*(t)}{dt} = -p_2^* X^*(t) + p_3^*[I(t) - I_b] \qquad X^*(0) = 0 \quad (28)$$

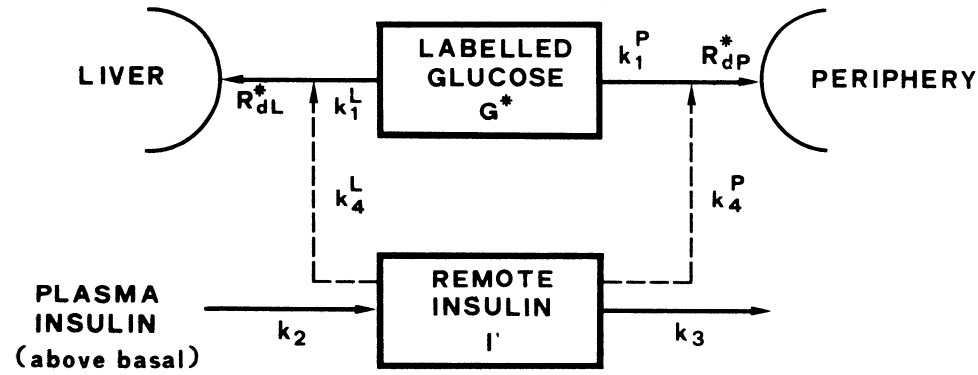


FIG. 2. Block diagram of revised minimal model based on tracer data. Tracer (G*) disappears from plasma into liver ($R^*_{dL}$) and periphery ($R^*_{dP}$). Insulin acts from a compartment remote from plasma.

where

$$X^*(t) = (k_4^L + k_4^P)I'(t) \quad (29)$$

$$p_1^* = k_1^L + k_1^P \quad (30)$$

$$p_2^* = k_3 \quad (31)$$

$$p_3^* = k_2(k_4^L + k_4^P) \quad (32)$$

*Metabolic parameters: estimate from tracer data.* The revised minimal model describing labeled glucose disappearance provides, with a reasoning analogous to that of the previous section, an estimate of insulin sensitivity index

$$S_I^* = \frac{p_3^*}{p_2^*} = \frac{k_2(k_4^L + k_4^P)}{k_3} \quad (33)$$

and of glucose effectiveness factor

$$S_G^* = p_1^* = k_1^L + k_1^P \quad (34)$$

As $S_I^*$ and $S_G^*$ are tracer-determined parameters, they reflect utilization processes only, whereas the analogous parameters estimated from cold data (*Eqs. 19* and *20*) reflect both the utilization and production side of the system. A point to note is that $S_G^*$, but not $S_G$, can be given a clear-cut physiological interpretation: it is (from *Eq. 27*) the fractional clearance of glucose (or fractional turnover rate) when insulin remains at its basal steady-state level. Model-based $S_G^*$ is thus suitable for an independent validation, e.g., by an arteriovenous difference measurement.

*Relationship of parameters estimated from cold and tracer data. Eq. 25* is consistent with a description of cold glucose uptake by the liver in terms of

$$R_{dL}(t) = k_1^L G(t) + k_4^L I'(t)G(t) \quad (35)$$

By subtracting this expression from NHGB (*Eq. 4*), the liver glucose production $R_a$ can be obtained as

$$\begin{aligned} R_a(t) &= NHGB(t) + R_{dL}(t) \\ &= B_0 - (k_5 - k_1^L)G(t) - (k_6 - k_4^L)I'(t)G(t) \end{aligned} \quad (36)$$

where the following constraints must hold to ensure physiological plausibility

$$k_5 \geq k_1^L \geq 0 \quad (37)$$

$$k_6 \geq k_4^L \geq 0 \quad (38)$$

This allows us to derive the following relationships among the parameters of the cold (*Eqs. 7* and *8*) and the tracer (*Eqs. 27* and *28*) minimal models

$$p_1^* = k_1^L + k_1^P \leq p_1 = k_5 + k_1^P \quad (39)$$

$$p_2^* = k_3 = p_2 = k_3 \quad (40)$$

$$p_3^* = k_2(k_4^L + k_4^P) \leq p_3 = k_2(k_6 + k_4^P) \quad (41)$$

From these relationships it follows that

$$S_G^* = p_1^* \leq S_G = p_1 \quad (42)$$

because in $S_G$ parameter $k_5$, besides $k_1^L$, contains the inhibitory component of glucose on its own production. Analogously

$$S_I^* \leq S_I \quad (43)$$

because in $S_I$ the inhibition of insulin on glucose production is explicitly considered via parameter $k_6$.

## EXPERIMENTS

Experiments were performed on six conscious beagles (11 ± 2 kg) of either sex, after a 14- to 16-h overnight fast. A Teflon catheter was inserted percutaneously into a femoral artery for blood sampling. Furthermore, a superficial vein of the hindlimb was cannulated with a radioopaque catheter (Cavafix, Braun, Melsungen, AG) which was advanced under fluoroscopic control until its tip reached the right atrium. This catheter was used for administration of the glucose load.

Twenty minutes after the preparation was completed, two blood samples were taken for basal determination of plasma glucose and insulin. Next, a glucose load, consisting of 0.33 g/kg of unlabeled glucose (as 50% dextrose) to which 8.9 ± 1.1 μCi/kg of [2-$^3$H]glucose (Amersham, Buckinghamshire, England) had been added, was given over a 10-s period. Blood samples of 1 ml each were drawn through the arterial catheter every 1 min from 1 min after injection up to 15 min, every 2.5 min until 60 min, every 10 min until 120 min, and at 150 and 180 min.

The same dogs used in the above protocol were next employed for experiments involving endogenous glucose production measurements based on the hepatic arterial-venous (A-V) difference technique. In this approach, simultaneous portal, hepatic vein, and arterial blood samples are needed together with estimation of hepatic blood flow. For portal blood sampling a catheter, chron-

ically implanted 10 days before the study was used. Under general anesthesia, a laparotomy was performed. A catheter (Central Venous Catheter, Wallace, Colchester, Essex, England) was then inserted into a tributary of the splenic vein and advanced until its tip was 2–3 cm beyond the bifurcation of the portal vein. The catheter was filled with heparin and its free extremity placed in a subcutaneous pocket.

On the day of the study, the subcutaneous end was exteriorized under local anesthesia and the heparin content aspirated. Arterial blood samples were drawn from a femoral artery catheterized percutaneously. Furthermore, an angiographic catheter (Cobra 7 F, Cordis, Miami, FL) was inserted through a hindlimb vein surgically exposed under local anesthesia and advanced under fluoroscopic control to a main hepatic vein. Total hepatic blood flow was estimated using indocyanine green dye (Cardio-Green, Hynson Westcott & Dunning, Baltimore, MD) as previously described (16). In calculating the rate of hepatic glucose production, it was assumed that glucose utilization by the liver is negligible [L. Saccà, unpublished results; Ref. (1)] and that the arterial and portal component of hepatic blood flow represented 28 and 72%, respectively (15).

Unlabeled glucose, [2-$^3$H]glucose, and insulin plasma concentrations were measured as previously described (21).

## DATA ANALYSIS

The two minimal models (*Eqs. 7–8* and *27–28*) were identified from insulin and cold and labeled glucose concentrations, respectively, using nonlinear least squares (7). The measurement error associated with cold glucose was assumed to be additive, white, gaussian with zero mean and a constant coefficient of variation of 1.5%. The error associated with labeled glucose had a similar structure, but with an experimentally determined nonconstant coefficient of variation [as in (10)], and ranged from 2–3%. Precision of parameter estimates were computed from the inverse of the Fisher information matrix and reliability of this measure was tested by Monte Carlo simulation studies (7). Results are given as means ± SE unless otherwise designated.

## RESULTS

Experimental data and model fit for two typical animals are shown in Fig. 3. Insulin time course, $I(t)$, has been used as input to the models; cold, $G(t)$, and labeled, $G^*(t)$, glucose data have been fitted by the respective models. In addition, the models provide the time courses of insulin action [$X(t)$ and $X^*(t)$].

In all the other animals similar patterns were observed, i.e., the match between observed data and models' solutions for both $G(t)$ and $G^*(t)$ was satisfactory, according to well-established criteria (7). The time courses of $X(t)$ and $X^*(t)$ were always different in shape and values. In particular $X(t)$ always exhibited a slower dynamics with a lower maximum.

The two sets of estimated parameters, along with their precision are shown in Tables 1 and 2, respectively, for the two minimal models. The values of $S_G$ ($= p_1$) resulted significantly larger than $S_G^*$ ($= p_1^*$) (0.0420 ± 0.0089 vs. 0.0092 ± 0.0009 $min^{-1}$, $P < 0.02$). Also insulin action parameters were different in the two models; in particular, $p_2$ was 0.053 ± 0.013 vs. $p_2^* = 0.116 \pm 0.022$ $min^{-1}$ and $p_3$ was 0.26 ± 0.13 vs. $p_3^* = 0.68 \pm 0.11$ $10^{-4}$ $min^{-2} \cdot \mu U^{-1} \cdot ml^{-1}$). From the estimates of the extrapolations $G_0$ and $G_0^*$ it is possible to calculate the initial distribution volumes, which, as expected, were similar for the two models (247 ± 14 vs. 286 ± 19 ml/kg).

Finally insulin sensitivity obtained via the model with cold data was lower than the correspondent metabolic parameter obtained with tracer data: $S_I = 4.52 \pm 1.39$ vs. $S_I^* = 6.41 \pm 10^{-4}$ $min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$) ($P < 0.05$).

Results for the A-V difference experiments are shown in Table 3. A mean hepatic glucose production of 2.32 ± 0.12 $mg \cdot min^{-1} \cdot kg^{-1}$ was measured. Because the experiments were carried out on dogs in basal state, glucose clearance was given as the ratio of production to basal glucose concentration. A clearance of 2.38 ± 0.05 $ml \cdot min^{-1} \cdot kg^{-1}$ was calculated. By assuming a steady-state equivalent distribution volume of 25% of body weight (10, 19), a fractional glucose clearance of 0.0097 ± 0.0002 $min^{-1}$ was obtained, which is virtually identical to $S_G^*$.

## DISCUSSION

Since it was first proposed in 1979 (5), the minimal model describing glucose disappearance has been employed for quantifying glucose tolerance in man (6, 8). From cold IVGTT data, parameters describing insulin action and glucose clearance can be estimated in a much less invasive way than other available experimental techniques [e.g., pancreatic suppression test (22), somatostatin infusion procedure (17), or glucose clamp (12, 18)]. Moreover, the model-based measurement of insulin sensitivity, $S_I$, has been shown to correlate well, in dogs (14) and in humans (2), with an independent reference. Despite these encouraging results, the minimal model must still be considered in a developing phase, as pointed out in a recent review (4).

The present study was conceived to gain further insight into the significance of the minimal model parameters, which are estimated from cold IVGTT data. Originally, a simplified structure had to be adopted (5), because it was not possible to segregate production from utilization. This resulted in a biased parametric description of the metabolic system. Model parameters reflected processes occurring both at production and utilization sites, like the insulin sensitivity index ($S_I$), or they measured simultaneously mass action and control phenomena, like the glucose effectiveness factor ($S_G$). This ambiguity made their interpretation not straightforward.

To overcome these limitations, a novel kinetic study was designed: the minimal model structure, designed for cold data, has been revised with a labeled IVGTT experiment, i.e., both cold and labeled glucose concentrations were measured along with insulin after a simultaneous injection of cold and labeled glucose. The [2-$^3$H]glucose had to be used as tracer to monitor disappearance processes only, even if it provides a slight overestimation of

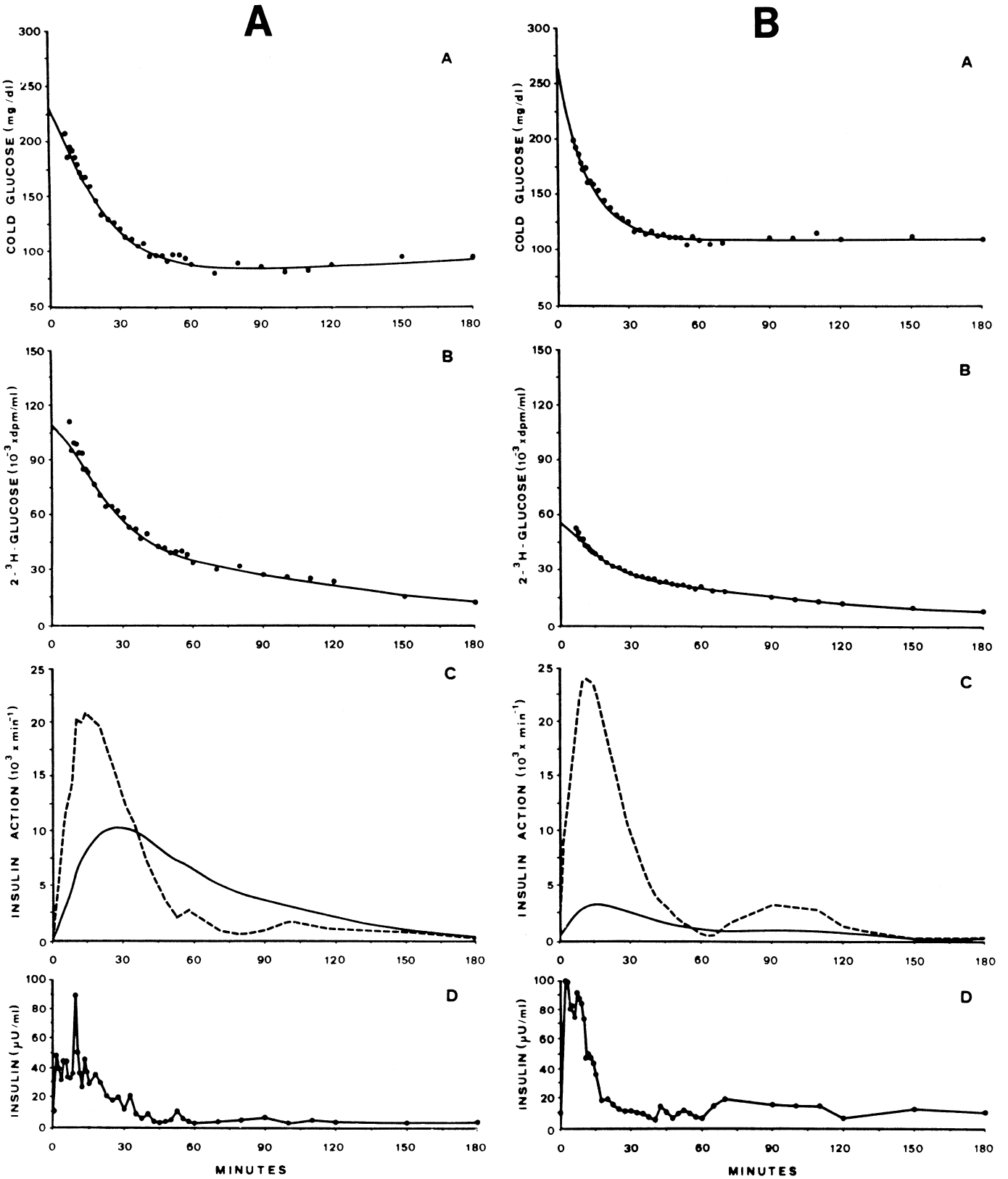


FIG. 3. Cold and labeled intravenous glucose tolerance test (IVGTT) results for *dog 1* (*panel A*) and *dog 3* (*panel B*). Cold glucose (0.33 g/kg) was injected at *time 0* along with 9 $\mu$Ci/kg of [2-$^3$H]glucose. *A: closed circles* represent measured cold glucose plasma concentration, while *solid line* is original minimal model fit. *B*: *closed circles* represent tracer concentration and continous *line* the revised minimal model fit. *C*: *solid line* is insulin action (variable $X$) for original minimal model identified with cold data; *dashed line* is insulin action ($X^*$) for revised minimal model. *D*: plasma insulin concentration time course; data are linearly interpolated.

TABLE 1. *Parameters of minimal model identified from cold IVGTT*

| Dog No. | $p_1 = S_G$, $min^{-1}$ | $p_2$, $min^{-1}$ | $p_3$, $10^{-4}$ $min^{-2}$ / $\mu U/ml$ | $S_I = p_3/p_2$, $10^{-4}$ $min^{-1}$ / $\mu U/ml$ |
|---|---|---|---|---|
| *1* | 0.037 (10) | 0.022 (22) | 0.17 (17) | 7.79 (6) |
| *2* | 0.040 (7) | 0.030 (22) | 0.14 (22) | 4.57 (5) |
| *3* | 0.070 (19) | 0.026 (141) | 0.04 (158) | 1.39 (31) |
| *4* | 0.055 (43) | 0.081 (29) | 0.38 (48) | 4.72 (20) |
| *5* | 0.045 (13) | 0.060 (27) | ≃0 | ≃0 |
| *6* | 0.005 (59) | 0.096 (5) | 0.83 (8) | 8.62 (4) |
| Mean | 0.042 | 0.053 | 0.26 | 4.52 |
| ±SE | ±0.009 | ±0.013 | ±0.13 | ±1.39 |
| mean CV | (25) | (41) | (53) | (23) |

The percent coefficient of variation (CV) is in parentheses as a measure of the precision of the parameter estimate, i.e., CV = 100 × SD of parameter estimate/parameter estimate. Abbreviations are defined in text.

TABLE 2. *Parameters of the minimal model identified from labeled IVGTT*

| Dog No. | $p_1^* = S_G^*$, $min^{-1}$ | $p_2^*$, $min^{-1}$ | $p_3^*$, $10^{-4}$ $min^{-2}$ / $\mu U/ml$ | $S_I^* = p_3^*/p_2^*$, $10^{-4}$ $min^{-1}$ / $\mu U/ml$ |
|---|---|---|---|---|
| *1* | 0.0088 (2) | 0.128 (10) | 0.88 (10) | 6.89 (2) |
| *2* | 0.0082 (2) | 0.164 (14) | 1.12 (14) | 6.87 (2) |
| *3* | 0.0068 (3) | 0.069 (8) | 0.40 (7) | 5.82 (3) |
| *4* | 0.0133 (1) | 0.076 (6) | 0.52 (6) | 6.85 (2) |
| *5* | 0.0089 (2) | 0.193 (20) | 0.50 (21) | 2.60 (3) |
| *6* | 0.0093 (2) | 0.068 (6) | 0.65 (6) | 9.44 (2) |
| Mean | 0.0092 | 0.116 | 0.68 | 6.41 |
| ±SE | ±0.0009 | ±0.022 | ±0.11 | ±0.91 |
| mean CV | (2) | (11) | (11) | (2) |

The percent coefficient of variation (CV) is in parentheses as a measure of the precision of the parameter estimate. Abbreviations are defined in text.

TABLE 3. *Fractional glucose clearance*

| Dog No. | A-V Difference | | | IVGTT |
|---|---|---|---|---|
| | Production, $mg \cdot min^{-1} \cdot kg^{-1}$ | Clearance,[a] $ml \cdot min^{-1} \cdot kg^{-1}$ | Fractional clearance,[b] $min^{-1}$ | $S_G^*$, $min^{-1}$ |
| *1* | 2.24 | 2.49 | 0.010 | 0.009 |
| *2* | 2.08 | 2.21 | 0.009 | 0.008 |
| *3* | 2.03 | 2.41 | 0.010 | 0.007 |
| *4* | 2.22 | 2.39 | 0.010 | 0.013 |
| *5* | 2.78 | 2.51 | 0.010 | 0.009 |
| *6* | 2.54 | 2.27 | 0.009 | 0.009 |
| Mean | 2.32 | 2.38 | 0.0097 | 0.0092 |
| ±SE | ±0.12 | ±0.05 | ±0.0002 | ±0.0009 |

Glucose production has been obtained as a steady-state measurement from arterial-venous (A-V) difference experiments. Fractional clearance is compared with correspondent parameter obtained from the minimal model used with tracer data. Abbreviations are defined in text. [a] Because production was evaluated at basal steady-state, it equals glucose utilization. Glucose clearance is then computed as production/glucose concentration. [b] Steady-state distribution volume has been assumed as 25% of body weight (10, 19).

utilization by measuring the hexose-monophosphate futile cycling (13). Insulin and labeled glucose time courses are used to generate, from a new minimal model, a parametric measure of glucose disposal processes and of insulin's effect on them. Moreover, the reference provided by the new tracer-based minimal model yields a better definition of the parameter significance and an elucidation of the potential bias of the parameters obtained from the minimal model applied to cold IVGTT data.

The minimal model, based on tracer data (*Eqs. 27* and *28*), describes only the uptake processes. In particular, the time course of $X^*(t)$ (*Eq. 29*) represents insulin action on glucose disposal alone, while $S_I^*$ and $S_G^*$ (*Eqs. 33* and *34*), respectively, represent a measure of the effect of insulin and glucose per se on tissue glucose utilization. On the contrary, in the minimal model based on cold data, $X(t)$ (*Eq. 9*) contains the inhibitory effect on net hepatic glucose balance ($k_6 - k_4^L$ or $k_6$ if $k_4^L = 0$), and both $S_I$ and $S_G$ measure also the steady-state inhibitory effect, respectively, of insulin ($k_6 - k_4^L$ or $k_6$ if $k_4^L = 0$) and of glucose ($k_5 - k_1^L$) on hepatic production.

The parameters of the minimal model identified from tracer data (Table 1) are estimated with a very good precision, and also their dispersion is remarkably low, especially in comparison with the corresponding ones from cold data (Table 2). This clearly reveals that a better configuration (i.e., the new model structure relative to the different type of experiment) is realized, because the insulin input of the two models is the same and cold and labeled glucose measurement errors lay in the same range.

The metabolic parameter $S_G^*$ (= $p_1^*$) is, by definition (*Eq. 34*), fractional glucose clearance at basal insulin and the value estimated from the model can be validated against an independent steady-state measure. This has been obtained measuring fractional glucose clearance in the same animals by the A-V difference technique. The results of this study (Table 3) show that the two values of glucose clearance (model dependent and direct measure) are virtually the same, bearing evidence on correctness of $S_G^*$. The dual parameter obtained from cold data, $S_G$, is not fractional glucose clearance at basal insulin, as it also contains (*Eq. 20*) the inhibitory effect (lumped in $k_5 - k_1^L$) of glucose per se on hepatic production. From mathematical reasoning (*Eq. 42*), $S_G$ (= $p_1$) is expected to be greater than $S_G^*$. However, numerical results show that $S_G$ is much greater, by ~4.5-folds, than $S_G^*$. As $S_G$ provides a global measure of two different mechanisms, its numerical value is difficult to interpret physiologically.

As concerns the other parameters of the two models, it is expected from basic mathematical principles that $p_2^* = p_2$ (*Eq. 40*), $p_3^* \leq p_3$ (*Eq. 41*), and $S_I^* \leq S_I$ (*Eq. 43*). However, the numerical picture is not consistent with this expected structural trend. In particular $p_2$ and $p_3$ are half as much as $p_2^*$ and $p_3^*$ (Tables 1 and 2), while $S_I$ is significantly lower by ~30% than $S_I^*$. This is likely to result from some structural error in the minimal model used with cold data. It is suggested that hepatic production is poorly described. This can be seen by rewriting $R_a(t)$ of *Eq. 36*, by using *Eqs. 9, 10, 29*, and *30*

$$R_a(t) = B_0 - (p_1 - p_1^*)G(t) - [X(t) - X^*(t)]G(t) \quad (44)$$

While the trend $p_1^* \leq p_1$ is expected, the estimated value of $p_1$ is much higher than $p_1^*$ (Tables 1 and 2) and

therefore the initial inhibition of liver glucose production, given by $(p_1 - p_1^*)\ G(t) = (k_5 - k_1^L)\ G(t)$, as $X(t)$ and $X^*(t)$ are initially zero (*Eqs. 10* and *30*), results to be greater than $B_0$. Consequently glucose production would become negative. This fact suggests that estimated $p_1$ is unphysiologically large. This overestimation generates undesired compensations in the parameters and variables of the model used with cold data. The large value of $p_1$ forces in turn $X(t)$ to stay at lower levels (see for instance Fig. 3), because from *Eq. 7*, $p_1 + X(t)$ can be viewed as the time-varying disappearance coefficient of $G(t)$. Forcing $X(t)$ to be low constrains also parameter $p_3$ to be low.

With respect to other parameters of the minimal model from cold data, let us focus on the insulin sensitivity index, $S_I$, which also takes into account parameters $p_2$ and $p_3$, being $S_I = p_3/p_2$. $S_I$ suffers from lower bias than the single $p_2$ and $p_3$. This can be explained by noting that $S_I$ is estimated, by definition (5), in a portion of the glucose disappearance curve where plasma insulin $I(t)$ changes slowly. In this situation the quasi steady-state pattern of $X(t)$ and $X^*(t)$ tends to be the same (see Fig. 3), as the insulin-inhibitory effect is relatively low. Thus, because from *Eq. 8* and *28* $X$ and $X^*$ are, respectively, proportional to $p_3/p_2$ and to $p_3^*/p_2^*$ via the quasi constant insulin, $S_I$ $(= p_3/p_2)$ and $S_I^*$ $(= p_3^*/p_2^*)$ are forced to take on a similar value. This simply explains why $S_I$ suffers lower bias than $p_2$ and $p_3$ as compared with the corresponding tracer-determined parameters. It should be emphasized, however, that estimated $S_I$ is significantly lower than $S_I^*$, a trend that is not expected on basic mathematical principles. Finally, because $S_I$ is defined as the ratio between $p_3$ and $p_2$, parameter $p_2$ is forced to assume values that are lower than $p_2^*$ and thus $X(t)$ exhibits a slower dynamics as compared with $X^*(t)$.

For all these reasons, we propose that the metabolic portrait provided from the labeled IVGTT data and the revised minimal model is more precise than the picture given by using only cold IVGTT data. In addition to test simplicity remaining, $S_I^*$ and $S_G^*$ are unequivocal parameters reflecting only glucose utilization processes, and, of particular significance, $S_G^*$ correctly measures the fractional glucose clearance at basal steady state.

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