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Cobelli, Claudio, Andrea Caumo, and Matteo Omen-etto. Minimal model S_G overestimation and S_I underestimation: improved accuracy by a Bayesian two-compartment model. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E481–E488, 1999.—The intravenous glucose tolerance test (IVGTT) single-compartment minimal model (1CMM) method has recently been shown to overestimate glucose effectiveness and underestimate insulin sensitivity. Undermodeling, i.e., use of single- instead of two-compartment description of glucose kinetics, has been advocated to explain these limitations. We describe a new two-compartment minimal model (2CMM) into which we incorporate certain available knowledge on glucose kinetics. 2CMM is numerically identified using a Bayesian approach. Twenty-two standard IVGTT (0.30 g/kg) in normal humans were analyzed. In six subjects, the clamp-based index of insulin sensitivity (S_I^c) was also measured. 2CMM glucose effectiveness (S_G^2) and insulin sensitivity (S_I^2) were, respectively, 60% lower ($P < 0.0001$) and 35% higher ($P < 0.0001$) than the corresponding 1CMM S_G^1 and S_I^1 indexes: 2.81 ± 0.29 (SE) vs. $S_G^1 = 4.27 \pm 0.33$ ml·min^{-1}·kg^{-1} and $S_I^2 = 11.67 \pm 1.71$ vs. $S_I^1 = 8.68 \pm 1.62$ 10^2 ml·min^{-1}·kg^{-1} per μU/ml. S_I^2 was not different from $S_I^c = 12.61 \pm 2.13$ 10^2 ml·min^{-1}·kg^{-1} per μU/ml (nonsignificant), whereas S_I^1 was 60% lower ($P < 0.02$). In conclusion, a new 2CMM has been presented that improves the accuracy of glucose effectiveness and insulin sensitivity estimates of the classic 1CMM from a standard IVGTT in normal humans.

glucose effectiveness; insulin sensitivity; glucose kinetics; glucose clamp technique

THE SINGLE-COMPARTMENT minimal model (1CMM) method (4) is widely used in clinical and epidemiological studies to estimate indexes of glucose effectiveness (S_G) and insulin sensitivity (S_I) from an intravenous glucose tolerance test (IVGTT). However, recent reports indicate that S_G is overestimated (11, 18, 20) and S_I underestimated (22). Undermodeling of glucose kinetics by 1CMM during the highly dynamic IVGTT perturbation, i.e., the use of a single- instead of a two-compartment description, has been advocated to explain S_G overestimation and S_I underestimation (8–10). Unfortunately, a two-compartment model is only resolvable if a tracer is added to the IVGTT bolus (7, 14, 23). However, the labeled IVGTT is not going to reach the widespread application of the standard IVGTT because of the additional technicalities and costs involved. It is therefore of interest to determine whether use of cer-

tain available a priori knowledge on glucose kinetics allows us to resolve a two-compartment model.

This was exactly the aim of this paper. We formulated a two-compartment minimal model (2CMM) by appending a second, nonaccessible compartment to the classic 1CMM. Theory shows that resolution of this 2CMM from an IVGTT requires a priori knowledge on glucose exchange kinetics. We incorporated such knowledge (12, 13, 17, 19) into the 2CMM in a probabilistic context by using the Bayesian approach (24). Indexes of glucose effectiveness and insulin sensitivity were then derived from the 2CMM and compared with those provided by the 1CMM. In addition, in a subset of six subjects (6 of 22), the indexes of insulin sensitivity provided by the two minimal models were also compared with the insulin sensitivity index provided by the glucose clamp technique. Our results in normal humans show that this approach is able to improve the accuracy of S_G and S_I estimation of the 1CMM from a standard IVGTT.

MATERIALS AND METHODS

The IVGTT Data Base

Twenty-two standard IVGTT [dose 302 ± 7 (SE) mg/kg] performed in normal humans (age 28 ± 1 yr; body weight 72 ± 2 kg) were considered. Sixteen IVGTT have already been published (2, 3, 23), whereas six are new. In these last six subjects, insulin sensitivity was also measured by the euglycemic hyperinsulinemic clamp technique, with insulin infused at 1 mU·min^{-1}·kg^{-1} (16; and unpublished data of Dr. R. C. Bonadonna). The protocol was approved by the ethical committee of the University of Verona, School of Medicine, Verona, Italy).

The Single-Compartment Minimal Model

The classic 1CMM (Fig. 1*A*) (4, 13) can be conveniently written with its uniquely identifiable parameters as

$$\dot{Q}(t) = -[p_1 + X(t)]Q(t) + p_1 Q_b \qquad Q(0) = Q_b + D \qquad (1)$$

$$\dot{X}(t) = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \qquad (2)$$

$$G(t) = Q(t)/V \qquad (3)$$

where Q is glucose mass (mg/kg), with Q_b denoting its basal (end-test) steady-state value; D is the glucose dose (mg/kg); X is a variable related to insulin concentration (deviation from basal) in a compartment remote from plasma, $X(t) = (k_4 + k_6)I'(t)$, where k_4 and k_6 are rate parameters (min^{-1}); $I(t)$ is plasma insulin concentration (μU/ml), with I_b denoting its basal value; G is plasma glucose concentration, with G_b denoting its basal value; V is the distribution volume per unit body weight (ml/kg); and $p_1 = k_1 + k_5$, $p_2 = k_3$, and $p_3 = k_2(k_4 + k_6)$ are rate parameters expressed in min^{-1}, min^{-1}, and
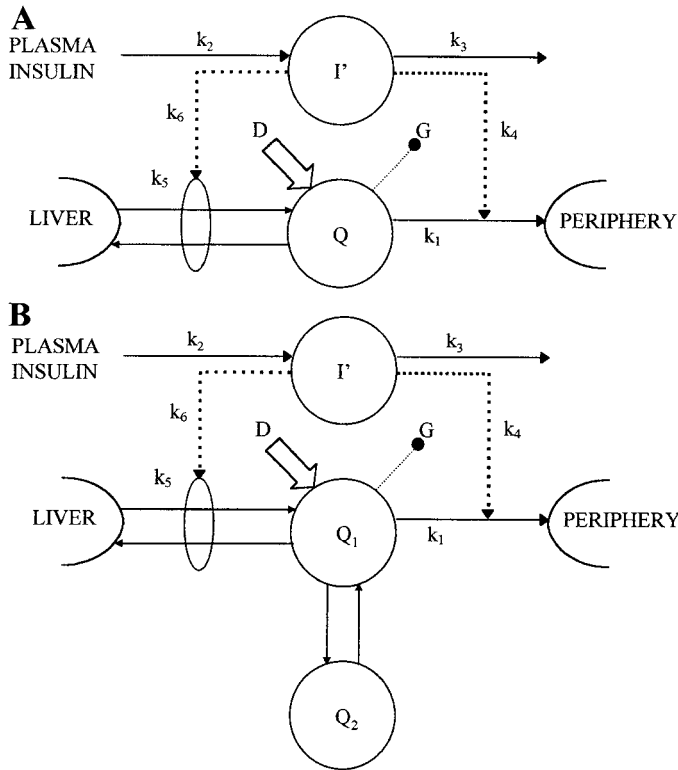
A

PLASMA
INSULIN

B

PLASMA
INSULIN

Fig. 1. The classic single-compartment minimal model (1CMM, *A*) and the two-compartment minimal model (2CMM, *B*). For meaning of abbreviated terms, see MATERIALS AND METHODS.

$min^{-2} \cdot \mu U^{-1} \cdot ml$, respectively. Clearly one has $Q_b = G_b V$. From the 1CMM one can derive indexes of glucose effectiveness (S_G^1) and insulin sensitivity (S_I^1)

$$S_G^1 = -\frac{\partial \dot{Q}(t)}{\partial G(t)} = p_1 V = S_G V \; (ml \cdot min^{-1} \cdot kg^{-1}) \qquad (4)$$

$$S_I^1 =$$
$$-\frac{\partial^2 \dot{Q}(t)}{\partial I(t) \partial G(t)} = \frac{p_3}{p_2} V = S_I V \; (ml \cdot min^{-1} \cdot kg^{-1} \; per \; \mu U/ml) \qquad (5)$$

where the subscript ss denotes steady state.

It is important to note that S_G^1 and S_I^1, at variance with the fractional (i.e., per unit volume) indexes S_G and S_I commonly expressed elsewhere, have the same units of the analogous glucose clamp indexes, thus allowing a direct comparison (10). Also, because the 1CMM and 2CMM have different accessible pool volumes (see *Model structure*), the choice of these indexes is the most appropriate for comparing the two models.

The 1CMM parameters were estimated (4, 6, 14) by weighted nonlinear least squares [using the ADAPT software (15), see *Bayesian identification*] with weights optimally chosen, i.e., equal to the inverse of the variance of the glucose measurement error assumed to be additive, independent, Gaussian zero mean, and with a constant coefficient of variation (CV) of 2%. Precision of parameter estimates was obtained from the inverse of the Fisher information matrix (6). As normally done with the 1CMM, the first 8- to 10-min glucose samples were ignored in model identification. The glucose dose administration was described as a 1-min rectangular infusion.

The Two-Compartment Minimal Model

Model structure. The 2CMM is the natural evolution of the classic 1CMM: a second, nonaccessible compartment is appended to it (Fig. 1, *B*), and the only difference is the exchange between the accessible and nonaccessible pools. It is described by

$$\dot{Q}_1(t) = -[p_1 + k_{21} + X(t)]Q_1(t) + k_{12}Q_2(t) + p_1 Q_{1_b}$$
$$Q_1(0) = Q_{1_b} + D \qquad (6)$$

$$\dot{Q}_2(t) = k_{21}Q_1(t) - k_{12}Q_2(t) \qquad Q_2(0) = Q_{2b} \qquad (7)$$

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

$$G(t) = Q_1(t)/V_1 \qquad (9)$$

where Q_1 and Q_2 (mg/kg) denote the glucose masses in the accessible and nonaccessible compartments, respectively, with subscript b denoting their basal (end-test) steady-state values; V_1 is the volume of the accessible compartment (ml/kg); k_{12} and k_{21} (min^{-1}) are rate parameters describing glucose exchange kinetics; D, G, I, X, p_1, p_2, and p_3 are variables and parameters already defined for the 1CMM. One has $Q_{1b} = G_b V_1$, and thus $Q_1(0)$ has a similar expression to $Q(0)$ of *Eq. 1*, with V_1 in place of V. $Q_2(0)$ is $k_{21}Q_{1b}/k_{12}$ from the steady-state constraint.

From the 2CMM one can calculate, as for the 1CMM, indexes of glucose effectiveness (at basal insulin) and insulin sensitivity. The 2CMM glucose effectiveness (S_G^2; superscript "2" denotes the second compartment) is

$$S_G^2 = -\frac{\partial \dot{Q}_1(t)}{\partial G(t)} = p_1 V_1 = S_G V_1 \; (ml \cdot min^{-1} \cdot kg^{-1}) \qquad (10)$$

and insulin sensitivity (S_I^2) is

$$S_I^2 = -\frac{\partial^2 \dot{Q}_1(t)}{\partial I(t) \partial G(t)} = \frac{p_3}{p_2} V_1$$
$$= S_I V_1 \; (ml \cdot min^{-1} \cdot kg^{-1} \; per \; \mu U/ml) \qquad (11)$$

The 2CMM differs from the 1CMM only in allowing an exchange of glucose between the accessible and the nonaccessible compartment, i.e., the terms $k_{21}Q_1$ and $k_{12}Q_2$ in *Eqs. 1* and *2* (cf. Fig. 1). Unfortunately, this small added complexity brings a priori identifiability problems. By employing the a priori identifiability analysis method for nonlinear models described in Ref. 6, it can be shown (see APPENDIX A) that only V_1, p_2, and p_3 are uniquely identifiable, whereas p_1, k_{21}, and k_{12} are not. In particular, one can only estimate their aggregates $p_1 + k_{21}$ and $k_{21}k_{12}$. This means that unique identifiability of the 2CMM can only be reached by resorting to additional independent knowledge of glucose exchange kinetic parameters k_{21} and k_{12}.

Glucose tracer kinetic studies do in principle contain this information. We have reanalyzed published tracer bolus injection data obtained in the basal state in normal humans (12, 13, 17, 19) with a two-compartment model corresponding to that of Fig. 1*B*, i.e., with no irreversible loss from the nonaccessible pool. The model has been numerically identified in fourteen subjects, and population values of k_{21} and k_{12} were obtained (in addition to values of V_1 and k_{01}). This kinetic knowledge has been used to resolve the 2CMM with the strategy described in *Bayesian identification*.

Bayesian identification. Bayesian estimation allows a flexible, theoretically sound incorporation of a priori knowledge

into model identification. In particular, the so-called Maximum a Posteriori (MAP) Bayesian estimator (24) was chosen. Briefly, the unknown model parameters are partitioned into two uncorrelated components. The first is formed by V_1, p_1, p_2, and p_3, of which we assume to have no a priori knowledge, i.e., their estimates will be data driven. The second component is formed by the glucose exchange kinetic parameters k_{21} and k_{12}, of which we assume to have some prior knowledge available, i.e., their estimates will be both data and a priori knowledge driven. In particular, from the above reanalysis of basal state tracer data, k_{21} and k_{12} are assumed to be normally distributed, with means and standard deviations of 0.050 ± 0.013 and 0.070 ± 0.018 min^{-1}, respectively, and with a correlation coefficient of 0.90.

The cost function for a MAP Bayesian estimator is similar to that of weighted nonlinear least squares, with an additional term pertaining to k_{21} and k_{12} a priori knowledge (APPENDIX B, *Eq. B3*). As for the 1CMM, weights were chosen optimally, and precision of parameter estimates was obtained from the inverse of the Fisher information matrix (6). All glucose data (usually starting from 2 min) were used in model identification. A 1-min rectangular infusion was used to describe the glucose administration format. Parameter estimation was performed with the ADAPT software (15), which contains a MAP Bayesian estimation feature.

Glucose Clamp Insulin Sensitivity

Insulin sensitivity measured with the euglycemic hyperinsulinemic glucose clamp technique (S_I^c) was calculated as in Ref. 3a

$$S_I^c = \frac{\Delta GIR}{G_b \cdot \Delta I} \qquad (\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{ per } \mu\text{U/ml}) \qquad (12)$$

where ΔGIR and ΔI are increments of the exogenous glucose

infusion rate and plasma insulin concentration, respectively, and G_b is basal plasma glucose concentration.

Statistical Analysis

Results are given as means $\pm$ SE. Student's *t*-test for paired data was used to evaluate differences between indexes estimated with 1CMM, 2CMM, and the glucose clamp. In addition, the value of S_I^c has been compared with the 2CMM and the 1CMM by the use of a statistical approach that assesses the agreement between two methods for measuring a clinical variable by displaying on the *y*-axis the difference between methods and on the *x*-axis the mean of the two methods (1).

RESULTS

The individual results of Bayesian identification of the 2CMM are shown in Tables 1 and 2; Fig. 2 shows the mean weighted residuals. The residuals (Fig. 2) have a satisfactory behavior, in terms of both pattern and amplitude: in particular, the 2CMM (*A*) is able to describe the initial portion of the IVGTT (8–10 min), which is not possible with the 1CMM (*B*). Parameters were generally estimated with an acceptable precision (Tables 1 and 2). In a few circumstances, the exchange kinetic parameters, and particularly so k_{12}, were difficult to resolve with precision. As expected, the 2CMM accessible pool volume was lower than the 1CMM volume (128.9 ± 7.4 vs. 152.9 ± 5.2 ml/kg).

The individual estimates of 2CMM (S_G^2, S_I^2) and 1CMM (S_G^1, S_I^1) and glucose effectiveness and insulin sensitivity shown in Table 2 are summarized in Fig. 3,

Table 1. *2CMM parameter estimation results*

Subject No.				Parameters		
	V_1, ml/kg	k_{21}, min^{-1}	k_{12}, min^{-1}	p_1, min^{-1}	p_2, min^{-1}	p_3, 10^3 ml·min^{-2}·kg^{-1} per μU/ml
1	129.5 (3)	0.0681 (14)	0.1110 (11)	0.021 (34)	0.0445 (29)	1.657 (43)
2	141.1 (2)	0.0044 (213)	0.0098 (140)	0.030 (34)	0.0140 (25)	0.738 (58)
3	132.0 (2)	0.0060 (171)	0.0041 (340)	0.023 (51)	0.0190 (136)	0.389 (66)
4	151.1 (2)	0.0057 (174)	0.0141 (111)	0.010 (105)	0.0489 (40)	1.256 (27)
5	97.7 (3)	0.1113 (9)	0.1842 (7)	0.039 (13)	0.0374 (14)	4.190 (14)
6	122.5 (3)	0.1226 (9)	0.1971 (7)	0.029 (26)	0.0515 (14)	4.406 (27)
7	101.7 (6)	0.1616 (7)	0.2553 (6)	0.058 (28)	0.0603 (32)	5.198 (44)
8	196.2 (4)	0.0111 (95)	0.0018 (740)	0.013 (94)	0.0216 (68)	4.524 (32)
9	178.3 (2)	0.0129 (54)	0.0037 (101)	0.006 (116)	0.0333 (36)	2.933 (26)
10	156.9 (3)	0.0077 (85)	0.0208 (62)	0.022 (16)	0.0540 (43)	1.230 (28)
11	145.1 (4)	0.0504 (19)	0.1087 (13)	0.022 (42)	0.0385 (58)	3.846 (66)
12	129.5 (3)	0.0240 (35)	0.0150 (47)	0.009 (145)	0.0582 (23)	5.657 (23)
13	72.8 (3)	0.1110 (8)	0.1610 (6)	0.037 (10)	0.0314 (10)	6.356 (16)
14	95.3 (4)	0.1042 (10)	0.1544 (9)	0.021 (26)	0.0476 (6)	8.153 (12)
15	164.5 (5)	0.0047 (215)	0.0152 (87)	0.016 (40)	0.0367 (61)	3.854 (32)
16	168.9 (1)	0.0089 (116)	0.0018 (755)	0.014 (77)	0.0142 (165)	1.235 (22)
17	173.1 (5)	0.0163 (51)	0.0035 (241)	0.004 (371)	0.0272 (83)	2.967 (62)
18	124.4 (2)	0.0120 (92)	0.0173 (89)	0.030 (48)	0.0147 (44)	0.906 (128)
19	109.0 (4)	0.0958 (11)	0.1552 (8)	0.017 (69)	0.0394 (34)	3.788 (61)
20	94.1 (7)	0.1099 (9)	0.1653 (7)	0.027 (69)	0.0283 (65)	3.092 (102)
21	84.6 (5)	0.1254 (8)	0.1920 (7)	0.067 (21)	0.0356 (65)	2.814 (101)
22	68.2 (5)	0.1019 (10)	0.1552 (9)	0.020 (41)	0.0167 (26)	0.771 (30)
Mean $\pm$ SE	128.9 ± 7.4	0.0580 ± 0.011	0.0885 ± 0.018	0.024 ± 0.003	0.0351 ± 0.003	3.180 ± 0.43
Mean Precision	(4)	(64)	(127)	(67)	(9)	(46)

Values in parentheses show precision of estimate expressed as percent coefficient of variation. 2CMM, two-compartment minimal model. For meaning of parameters, see MATERIALS AND METHODS.

Table 2. *Glucose effectiveness and insulin sensitivity estimated from the 2CMM and the 1CMM minimal models, and insulin sensitivity measured in 6 subjects by the glucose clamp technique*

Subject No.	Glucose Effectiveness, ml·min⁻¹·kg⁻¹		Insulin Sensitivity, 10² ml·min⁻¹·kg⁻¹ per μU/ml		
	S_G^2	S_G^1	S_I^2	S_I^1	S_I^c
1	2.72 (33)°	5.32 (10)	4.82 (17)	2.52 (15)	
2	4.26 (34)	5.07 (3)	7.47 (71)	5.74 (9)	
3	3.02 (50)	3.84 (39)	2.70 (171)	1.78 (108)	
4	1.45 (104)	1.84 (79)	3.88 (34)	3.56 (19)	
5	3.82 (11)	4.35 (13)	10.94 (3)	10.50 (4)	
6	3.55 (23)	6.79 (36)	10.48 (17)	2.75 (198)	
7	5.91 (23)	7.43 (71)	8.77 (18)	5.47 (113)	
8	2.63 (92)	5.13 (130)	41.15 (53)	39.07 (6)	
9	1.11 (115)	3.95 (24)	15.71 (28)	9.31 (14)	
10	3.49 (16)	2.88 (13)	3.58 (24)	3.88 (10)	
11	3.21 (40)	4.07 (36)	14.51 (11)	10.34 (23)	
12	1.12 (142)	6.77 (45)	12.58 (25)	3.88 (105)	
13	2.69 (9)	3.13 (8)	14.73 (9)	13.98 (9)	
14	2.04 (23)	3.24 (17)	16.33 (11)	12.32 (15)	
15	2.68 (41)	3.95 (21)	17.28 (36)	11.88 (23)	
16	2.29 (77)	3.55 (24)	14.73 (179)	12.54 (15)	
17	0.64 (366)	4.71 (58)	18.86 (55)	10.21 (41)	18.15
18	3.72 (48)	3.98 (11)	7.65 (101)	4.87 (28)	8.50
19	1.87 (66)	2.99 (152)	10.48 (31)	7.50 (147)	19.20
20	2.58 (62)	2.85 (168)	10.29 (43)	9.93 (121)	10.50
21	5.68 (16)	6.44 (62)	6.68 (42)	6.01 (177)	14.72
22	1.33 (37)	1.75 (66)	3.16 (8)	2.93 (13)	4.60
Mean ± SE	2.81 ± 0.29	4.27 ± 0.33	11.67 ± 1.71	8.68 ± 1.62	12.61 ± 2.13
Mean Precision	(65)	(49)	(45)	(55)	$S_I^2 = 9.52 ± 1.98$ $S_I^1 = 6.91 ± 1.07$

Values in parentheses show precision of estimate expressed as percent coefficient of variation. S_G^2 and S_G^1, glucose effectiveness in the 2CMM and single-compartment minimal model (1CMM), respectively. S_I^2 and S_I^1, insulin sensitivity in the 2CMM and 1CMM, respectively. S_I^c, S_I measured in *subjects 17–22* by glucose clamp technique.

together with the insulin sensitivity clamp index (S_I^c). S_G^2 was almost one-half of ($P < 0.0001$) S_G^1 [2.81 ± 0.29 (SE) vs. 4.27 ± 0.33 ml·min⁻¹ ·kg⁻¹] and S_I^2 was 35% higher ($P < 0.0001$) than S_I^1 (11.67 ± 1.71 vs. 8.68 ± 1.62 10² ml·min⁻¹·kg⁻¹ per μU/ml). Precision of the 2CMM indexes was comparable to that of the 1CMM indexes: standard deviations are virtually the same for S_G^2, S_G^1 and S_I^2, S_I^1, whereas the CV is greater for S_G^2 (less for S_I^2), because the parameter estimate is less (greater for S_I^2).
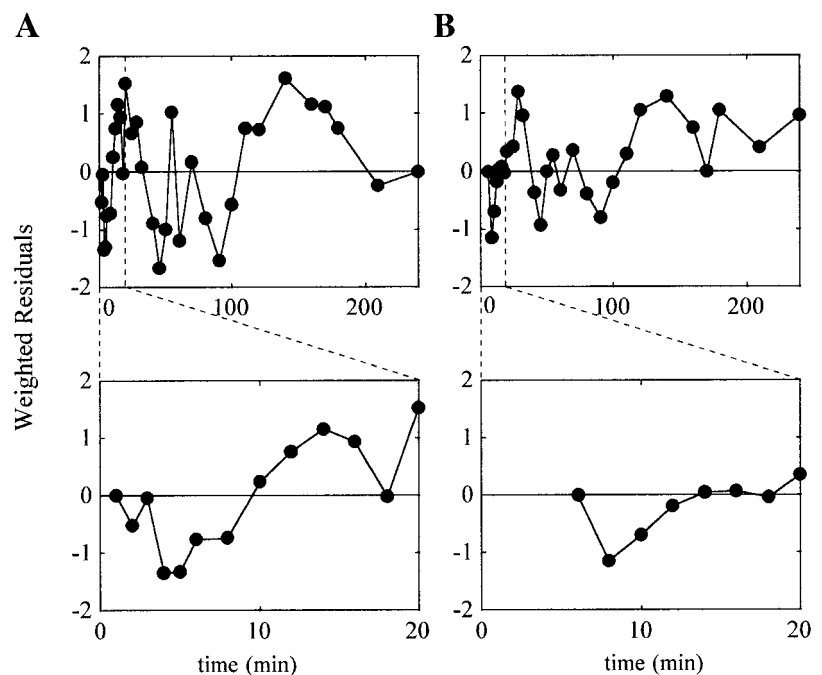

Fig. 2. Mean weighted residuals, i.e., difference between data and model predictions divided by the standard deviation averaged over all subjects, of the 2CMM (*A*) and 1CMM (*B*).
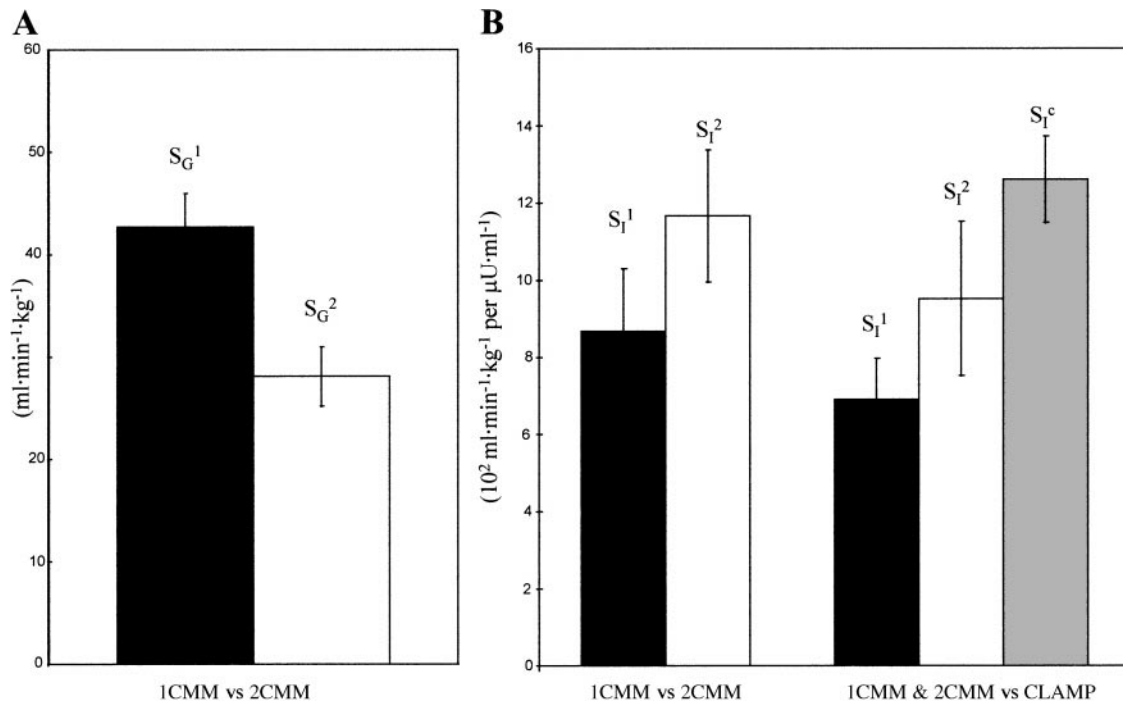
Fig. 3. Indexes of glucose effectiveness (S_G) and insulin sensitivity (S_I) for the 1CMM (S_G^1, S_I^1) and the 2CMM (S_G^2, S_I^2). Insulin sensitivity measured with the glucose clamp technique (S_I^c) is also shown and compared with S_I^1 and S_I^2 in the same subset of subjects. Data are expressed as means $\pm$ SE.

Of interest is the comparison of S_I^2 and S_I^1 with the glucose clamp measure S_I^c in the subsample of six subjects. S_I^2 was not statistically different from S_I^c [9.52 ± 1.98 (SE) vs. 12.61 ± 2.13 10^2 ml·min^{-1}·kg^{-1} per µU/ml], whereas S_I^1 (6.91 ± 1.07) was significantly lower ($P < 0.02$). To better assess the agreement of S_I^2 and S_I^1 with S_I^c, the "difference against average of methods" comparison plots were examined (Fig. 4). The small amount of data prevents any definite conclusion. However, one can safely say that 2CMM S_I^2 underestimates S_I^c (B) much less than 1CMM S_I^1 (A).

DISCUSSION

One of the assumptions of the 1CMM method is that glucose exhibits single-compartment kinetics. To favor the domain of validity of this assumption, the initial portion of the glucose concentration data (usually the first 8–10 min) is not used in model identification. In fact, albeit the necessity of a two-compartment description of glucose kinetics in a highly dynamic nonsteady state like the IVGTT is a well-established notion, it is virtually impossible to resolve from an IVGTT a two-compartment model without the addition of a glucose tracer. However, evidence has become available that undermodeling of glucose kinetics, i.e., the use of a one- instead of a two-compartment description, is the major factor responsible for 1CMM overestimation of S_G and underestimation of S_I (8–11). The goal of this paper was to improve on these 1CMM limitations by exploiting available knowledge on glucose kinetics and by using a Bayesian approach to identify a 2CMM.

The new 2CMM provides estimates of glucose effectiveness, S_G^2, and insulin sensitivity, S_I^2, which improve
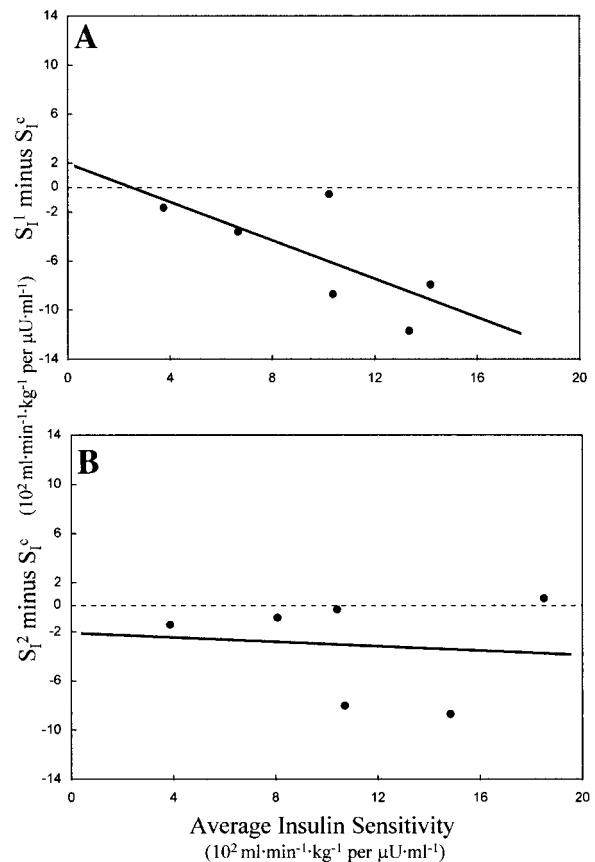

Fig. 4. Insulin sensitivity comparison of the glucose clamp method, with 1CMM (A) and the 2CMM (B) minimal model methods. Difference between methods (y-axis) is shown against the average of the methods (x-axis) with regression line.

the accuracy of the 1CMM S_G^1 and S_I^1 (Fig. 3): $S_G^2 = 2.81 \pm 0.29$ ml·min^{-1}·kg^{-1} is almost one-half of the value of S_G^1, and $S_I^2 = 11.67 \pm 1.71$ 10^2 ml·min^{-1}·kg^{-1} per μU/ml is 35% higher than S_I^1. These values are in agreement with recently published values measured with the glucose clamp technique in normal humans. Best et al. (5) report for glucose effectiveness measured with the glucose clamp, S_G^c, a value of 2.4 ml·min^{-1}· kg^{-1} and a value for the 1CMM S_G^1 (using the 1CMM volume of 150 ml/kg) 62% higher than S_G^c. Saad et al. (22) report for S_I^c a value of 10.1 10^2 ml·min^{-1}·kg^{-1} per μU/ml, and for the 1CMM S_I^1 (using the 1CMM volume of 150 ml/kg) a value 53% lower than S_I^c. This trend is also confirmed by the glucose clamp insulin sensitivity measurements we perfomed in a subset of subjects: S_I^2, but not S_I^1, was not different from S_I^c (Fig. 3), and association of S_I^2 with S_I^c is stronger than that with S_I^1 (Fig. 4).

Theory indicates that resolving a 2CMM from a standard (nonlabeled) IVGTT requires independent knowledge of glucose kinetics. A theoretically sound approach to incorporate such knowledge in probabilistic terms is the so-called MAP Bayesian approach (24), which, although frequently used in pharmacokinetic/ pharmacodynamic studies (see references in Ref. 15), has not yet been fully exploited in the endocrine-metabolic area. The glucose kinetic parameters k_{21} and k_{12} are described as Gaussian variables, with their mean, variance, and covariance determined from independent studies. The theory of this approach is well established (24), and software for Bayesian model identification is available (15). The results were very satisfactory both in terms of capability of the model to describe the data (Fig. 2) and in terms of parameter estimation (Tables 1 and 2).

The structure chosen for the 2CMM follows in some sense a minimum assumption strategy, i.e., the added complexity to the 1CMM (Fig. 1A) is simply a nonaccessible compartment attached to it (Fig. 1B). However, one should note that this description of glucose kinetics, i.e., with a time-varying irreversible loss in the accessible pool and no loss in the nonaccessible pool, is equivalent to that proposed by Radziuk et al. (21), which has become the most widely used model to analyze non-steady-state glucose kinetics. Although the description of glucose kinetics incorporated into the 2CMM is reasonable, the question arises of its physiological plausibility compared with other descriptions that have been proposed in the literature. Other commonly used structures also have a constrained irreversible loss in the nonaccessible pool (13, 17, 21). The use of an irreversible loss in the nonaccessible pool (even without the one in the accessible pool) means additional complexity: in fact, a new parameter is required in the 2CMM to separate the effect of insulin on glucose production from that on glucose utilization (this is not required with an irreversible loss in the accessible pool only). A priori knowledge of this additional parameter is scarce and would make even the Bayesian approach a difficult route to follow. Therefore, the proposed descrip-

tion of glucose kinetics is a reasonable necessity. An important plus of the chosen structure with a single irreversible loss in the accessible pool is that it is the one that makes the glucose exchange parameters k_{21} and k_{12} less dependent on insulin levels with respect to structures with an irreversible loss also, or exclusively, in the nonaccessible pool; (when the basal and elevated insulin data of Refs. 13 and 17 are reanalyzed with this model, k_{21} and k_{12} in the elevated insulin state are not statistically different from the basal ones). Thus with respect to other models, the chosen structure makes the assumption that k_{21} and k_{12} do not vary appreciably during the IVGTT more tenable.

The glucose clamp technique is considered in the literature the gold standard for measuring insulin sensitivity. The availability of this measure in a subset of subjects ($n = 6$) thus allows us to address the validity of the 1CMM and the 2CMM measurements. Usually this comparison is made in the literature by resorting to correlation plots and correlation coefficients (see, e.g., Ref. 22) as indicators of agreement. However, this strategy is not the most appropriate one (1). A plot of the difference against the mean of the methods is more informative (1) (Fig. 4). Albeit the amount of data is small, one can state that 2CMM S_I^2 is providing much closer values to S_I^c (B) than 1CMM S_I^1 (A). However, an underestimation is still present and, in addition, one can note an increase of the difference between the two methods with the increase of the insulin sensitivity value. Another issue of relevance here is more general: do we really have to expect a "one to one" concordance between the minimal model and the glucose clamp methods? In the literature there is almost a unanimous consensus on the glucose clamp technique being considered the gold standard. The answer is yes in theory, because both methods rely on the same insulin sensitivity definition. In practice, however, for S_I^2 (and S_I^1) to be equivalent to S_I^c, a number of conditions must be met (also described in Ref. 10), the most important of which are that 1) insulin dose independence of the glucose clamp technique across the insulin range experienced during an IVGTT, i.e., insulin effect of the aggregation of glucose production and utilization, increases linearly with insulin concentration, and that 2) the 2CMM description of glucose kinetics and their control by insulin is "correct." There is good evidence that neither of these requirements is fully met. For instance, the nonlinear effect of insulin on glucose production is a well accepted notion, and this renders the glucose clamp measurement of "local" validity, i.e., dose dependent. On the other hand, the way in which the 2CMM depicts glucose production, distribution, and utilization bears some approximation, e.g., glucose utilization may not be accurately described by the single accessible pool irreversible loss, and the description of glucose and insulin control on glucose production embodied in the 2CMM (and 1CMM) may be too rude. Given this scenario, one should interpret with caution the plots of Fig. 4: the reassuring "take home message" is the closer association with S_I^c of S_I^2 than of S_I^1.

In conclusion, a new 2CMM approach for the estimation of glucose effectiveness and insulin sensitivity from an IVGTT has been presented that improves on the 1CMM limitation. The present studies in normal humans are an obvious prerequisite, but further work is needed to assess the reliability of this approach. For instance, investigations are required to better define the role of the description of glucose production currently embodied in the 2CMM and to assess the reliability of the Bayesian approach in other situations, like the insulin-modified IVGTT, and pathophysiological states.

APPENDIX A

We analyze here the a priori identifiability of the 2CMM described by *Eqs. 6–9*. The model is nonlinear, and one has to resort to the Taylor series expansion of the measured variable, i.e., glucose concentration, around *time 0* (immediately after the bolus) to check a priori identifiability (6). The unknown parameters are p_1, p_2, p_3, k_{21}, k_{12}, and V_1. The exhaustive summary of the model is given by

$$G_b + \frac{D}{V_1} = G(0) \tag{A1}$$

$$-(p_1 + k_{21}) \cdot \frac{D}{V_1} = G^I(0) \tag{A2}$$

$$(p_1 + k_{21})^2 \cdot \frac{D}{V_1} + k_{12} \cdot k_{21} \cdot \frac{D}{V_1} = G^{II}(0) \tag{A3}$$

$$-(p_1 + k_{21})^3 \cdot \frac{D}{V_1} - 2 \cdot k_{12} \cdot k_{21} \cdot (p_1 + k_{21}) \cdot \frac{D}{V_1}$$
$$- k_{12}^2 \cdot k_{21} \cdot \frac{D}{V_1} + -p_3 \cdot \left(G_b + \frac{D}{V_1}\right) \cdot \dot{I}(0) = G^{III}(0) \tag{A4}$$

$$(p_1 + k_{21})^4 \cdot \frac{D}{V_1} + 3 \cdot k_{12} \cdot k_{21} \cdot (p_1 + k_{21})^2 \cdot \frac{D}{V_1} + 2 \cdot k_{12}^2 \cdot k_{21} \cdot (p_1 + k_{21}) \cdot \frac{D}{V_1} + k_{12}^2 \cdot k_{21} \cdot (k_{12} + k_{21}) \cdot \frac{D}{V_1} - p_3 \cdot \left(G_b + \frac{D}{V_1}\right) \cdot \ddot{I}(0) +$$
$$p_3 \cdot \left[3 \cdot (p_1 + k_{21}) \cdot \frac{D}{V_1} + p_2 \cdot \left(G_b + \frac{D}{V_1}\right) + (p_1 + k_{21}) \cdot \left(G_b + \frac{D}{V_1}\right)\right] \cdot \dot{I}(0) = G^{IV}(0) \tag{A5}$$

where $G(0)$, $G^I(0)$, $G^{II}(0)$, $G^{III}(0)$, and $G^{IV}(0)$ are the known glucose concentration and its first, second, third, and fourth derivatives at *time 0*; (the fifth derivative does not add independent knowledge).

By solving the system of *Eqs. A1–A5*, one sees immediately that whereas *Eqs. A1, A2,* and *A3* give V_1, $p_1 + k_{21}$, and $k_{12}k_{21}$, respectively, *Eqs. A4* and *A5* only provide a relationship between k_{12} and p_3 and among k_{12}, p_3, and p_2, respectively. Therefore, the model is a priori nonidentifiable, and unique identifiability can only be reached by using an independent additional relationship between k_{21} and k_{12}.

APPENDIX B

We briefly review here some fundamentals of Bayesian estimation.

Consider the problem of estimating a parameter vector $\mathbf{p} = (p_1, ..., p_P)^T$ from a set of N noisy measurements

$$z_i = y(t_i, \mathbf{p}) + v_i \qquad i = 1, \ldots, N \tag{B1}$$

where $y(t_i, \mathbf{p})$ is the model prediction at *time t_i*, and v_i denotes the (additive) error that affects the i-th measurement z_i. To solve the problem, a commonly used approach is nonlinear least squares (LS) (6). A more sophisticated but less used approach is maximum a posteriori (MAP) estimation. The major difference between these two approaches is that MAP estimation exploits not only the data but also certain a priori available statistical information on the unknown parameters. In other words, while LS is a Fisherian approach to parameter estimation, i.e., data are the only information supplied to the estimator, MAP is a Bayesian approach, i.e., a priori information, e.g., obtained from population studies, is used in addition to the data (termed a posteriori information) in the numerical estimation of the model parameters. Bayesian estimation can be of relevant interest because it can significantly improve the precision of parameter estimates with respect to Fisher estimation or allow (as in this paper) the adoption of more complex, and thus more physiologically plausible, models than those resolvable by a Fisherian approach. Clearly, one has to pay a price for this, i.e., the supply of a priori information.

Let's now turn to a more formal framework. As previously stated, Bayesian estimation is based on the concept of a priori information on the unknown parameter vector $\mathbf{p}$, mathematically specified by its a priori probability density function f_p. For instance, one can expect a priori, i.e., before having "seen" the data vector $\mathbf{z} = (z_1, ..., z_N)^T$, that the unknown vector $\mathbf{p}$ is sampled from a normal distribution with mean $\boldsymbol{\mu}$ and covariance matrix Ω. After having "observed" the data vector $\mathbf{z}$, the probability density function from which we expect that $\mathbf{p}$ is sampled obviously changes. This function, conditional on the data vector $\mathbf{z}$, goes under the name of an a posteriori probability density function and is denoted by $f_{p|z}(\mathbf{p}|\mathbf{z})$; ($\mathbf{p}|\mathbf{z}$ reads as "$\mathbf{p}$ given $\mathbf{z}$" and stays for "$\mathbf{p}$ given the data $\mathbf{z}$"). The MAP estimate of $\mathbf{p}$ is the vector $\hat{\mathbf{p}}$, which maximizes the a posteriori probability density function $f_{p|z}(\mathbf{p}|\mathbf{z})$

$$\hat{\mathbf{p}} = \arg \max_{\mathbf{p}} f_{p|z}(\mathbf{p}|\mathbf{z}) \tag{B2}$$

Equation B2 gives the general definition of the MAP estimator. In practical applications, the functional in the right side of *Eq. B2* depends on the specific form of both $f_p(\mathbf{p})$ and noise statistics in *Eq. B1*. For example, if vector $\mathbf{p}$ is Gaussian, with mean $\boldsymbol{\mu}$ and covariance matrix Ω, and the measurement errors v_i are also Gaussian, with zero mean and variance σ_i^2, by applying the Bayes theorem it is easily shown (see Ref. 24 for details) that *Eq. B2* turns into

$$\hat{p} = \arg \min_{\mathbf{p}} \left\{ \sum_{i=1}^{N} \left[\frac{z_i - y_i(t_i, \mathbf{p})}{\sigma_i^2} \right]^2 + [p - \mu]^T \Omega^{-1} [p - \mu] \right\} \tag{B3}$$

It is worth noting that in the cost function of *Eq. B3* there are two contributions. The first term, which coincides with the cost function of LS estimation (6), measures the goodness of fit, i.e., the adherence to the a posteriori information. The second term weights the adherence of the candidate estimate to the available a priori knowledge of the parameter vector. This is why Bayes estimators are said to establish a trade-off between a priori and a posteriori information, linked to expectations and data, respectively. It is also worth noting that if the a priori knowledge becomes weaker and weaker (i.e., the covariance matrix Ω tends to infinity), the last term of *Eq. B3* can be neglected, and the MAP estimator collapses into the LS estimator (only a posteriori information, i.e., the data, are exploited).

In the 2CMM, the vector $\mathbf{p}$ is made up of two components, i.e., $\mathbf{p} = [\mathbf{k}|\mathbf{q}]^T$, with $\mathbf{k} = [V_1, p_1, p_2, p_3]^T$, and $\mathbf{q} = [k_1, k_{12}]^T$; $\boldsymbol{\mu}$ is the vector of the a priori mean of $\mathbf{p}$, i.e., $\boldsymbol{\mu} = [\boldsymbol{\mu}_k, \boldsymbol{\mu}_q]^T$, and Ω is the a priori covariance matrix of $\mathbf{p}$. Ω is made up of two components, Ω_k and Ω_q, related to $\mathbf{k}$ and $\mathbf{q}$, respectively, which are uncorrelated, and this brings the zero value of the off-diagonal components

$$\Omega = \begin{bmatrix} \Omega_k & 0 \\ 0 & \Omega_q \end{bmatrix} \tag{B4}$$

Because no a priori knowledge is imposed on $\mathbf{k}$ (data-driven parameters), the 4×4 Ω_k matrix has its diagonal elements, i.e., the variances equal to infinity and its off-diagonal elements equal to zero. In contrast, we impose a priori knowledge on $\mathbf{q}$, i.e., Ω_q is a 2×2 matrix with its diagonal and off-diagonal elements given, respectively, by the population variances and covariances of k_{21} and k_{12}.

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