Physiologic Modeling of the Intravenous Glucose Tolerance Test in Type 2 Diabetes: A New Approach to the Insulin Compartment

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The minimal model of Bergman et al has been used to yield estimates of insulin sensitivity (Si) and glucose effectiveness (Sg) in type 2 diabetes by incorporating exogenous insulin protocols into the regular intravenous glucose tolerance test (IVGTT). These estimates, however, are influenced by the degree to which the dose of exogenous insulin is greater than the physiologic response to a glucose load. Moreover, most studies have related to type 2 diabetes subjects whose diabetes was relatively mild in terms of therapeutic requirements. To develop a "minimal disturbance" approach in estimating Si and Sg in type 2 diabetes, we have used a reduced glucose load (200 mg/kg) and a "physiologic" insulin infusion throughout the IVGTT in a series of 8 patients, 5 of whom were insulin-requiring. Data from this approach were analyzed using the modelling program CONSAM to apply the Bergman model, either unmodified (BMM), or incorporating an additional delay element between the plasma and "remote" insulin compartments (MMD). Application of the MMD and extension of the IVGTT from 3 to 5 hours improved successful resolution of Si and Sg from 37.5% (BMM, 3-hour IVGTT) to 100% (MMD, 5-hour IVGTT). Si was reduced in these type 2 diabetes patients compared with normal subjects (1.86 ± 0.60 *v* 8.65 ± 2.27 $min^{-1} \cdot \mu U^{-1} \cdot mL \times 10^4$ $P < .01$). The results were validated in the type 2 diabetes group using a 2-stage euglycemic clamp ($^{Si}CLAMP = 2.02 \pm 0.42$ $min^{-1} \cdot \mu U^{-1} \cdot mL \times 10^4$ $P > .4$). Sg was not significantly reduced (2.00 ± 0.25 type 2 diabetes *v* 1.55 ± 0.26 normal $min^{-1} \times 10^2$). Data from a group of normal nondiabetic subjects was then analyzed using the MMD, but this approach did not enhance the fit of the model compared with the BMM. This result indicates that the delay in insulin action in type 2 diabetes represents an abnormality whereby the onset of insulin action cannot be described as a single phase in the transfer of insulin from plasma to the remote compartment. It is postulated that the physiologic basis for this delayed action may relate to transcapillary endothelial transfer of insulin, this process limiting the rate of onset of insulin action.

INSULIN RESISTANCE IS an important component of type 2 diabetes and is thought to be a risk factor for coronary artery disease.[1,2] Identification and modification of factors, which in turn lead to the development of insulin resistance, should improve management of type 2 diabetes and potentially result in reduction of macrovascular disease in these patients. The parameters associated with "insulin resistance" as measured with the euglycemic clamp include reduced non–insulin-mediated glucose disposal, as well as reduced insulin sensitivity. An effective method of measurement of both Sg, (glucose effectiveness index or glucose-mediated glucose disposal), and Si (insulin sensitivity) should therefore be used in quantitative assessment of insulin resistance in type 2 diabetes.

The minimal model method of computer analysis of the intravenous glucose tolerance test (IVGTT) has provided a method of estimation of Si and Sg in a single test in normal subjects.[3-5] The model represents the effects of the endogenous insulin and glucose itself on glucose kinetics (Si and Sg, respectively) and the feedback effect of glucose on insulin secretion (Fig 1). It has been shown that this model can be applied to insulin-dependent diabetes (type 1 diabetes) if exogenous insulin is given during the IVGTT.[6,7] This modified method for IVGTT analysis is able to fit data in type 1 diabetes with standard glycemic control[7] and in perturbed states.[8,9]

A study reported by Ng[10] used a modification of the minimal model to analyze data from the IVGTT in mild type 2 diabetes, in which subjects had sufficient endogenous insulin secretion to enable analysis. A further study in type 2 diabetes patients without adequate endogenous insulin was described by Welch et al,[11] in which an IVGTT incorporating an exogenous insulin protocol was analyzed using the minimal model. In this method, a fixed bolus of 0.05 U/kg was delivered 20 minutes after the glucose bolus, producing supraphysiologic plasma insulin levels (mean, 1,150 mU/L). In the 11 studies reported, baseline glucose levels were high, and no hypoglycemia occurred. Using a similar protocol in normal subjects, however, the insulin bolus being 0.02 U/kg, hypoglycemia or "undershoot" of glucose below basal level was a significant problem,[11] leading to possible counterregulatory changes in Sg and Si. In other studies, the minimal model has been used in type 2 diabetes patients with cirrhosis to analyze the IVGTT using tolbutamide to enhance the insulin response[12] and also in a group of type 2 diabetes patients with mild fasting hyperglycemia managed by dietary restriction alone, in this case giving a 5-minute exogenous insulin infusion 20 minutes after the IVGTT glucose bolus.[13] Type 2 diabetes patients with more marked fasting hyperglycemia requiring insulin therapy were not, however, included in these analyses.

Our plan in designing the present study protocol was to develop a modification of our type 1 diabetes protocol, which would overcome problems such as hypoglycemia and also to include type 2 diabetes subjects with a wider range of Si and fasting hyperglycemia than was seen in the other studies, in-

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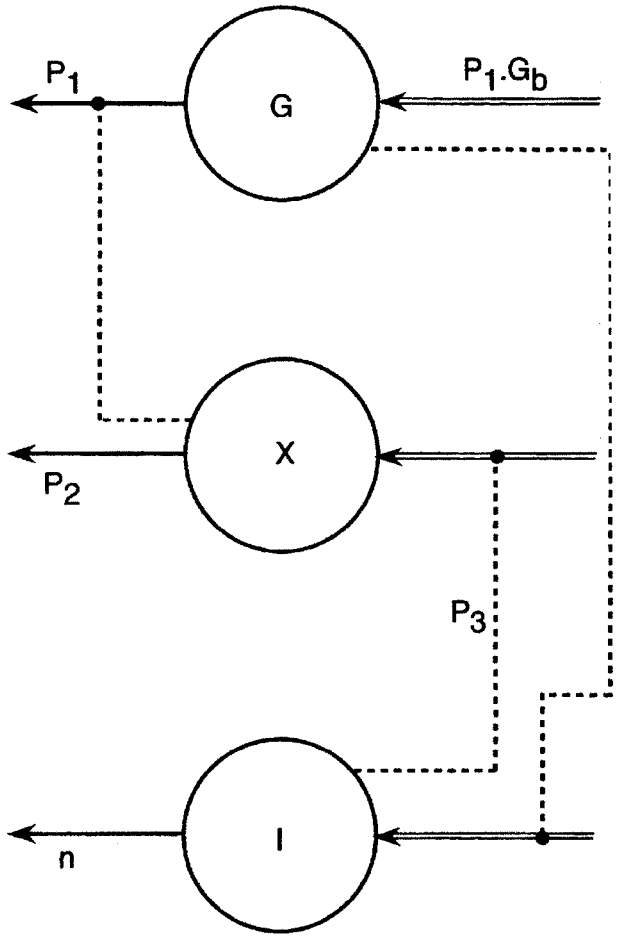

Fig 1. Minimal model of Bergman et al (BMM). This represents both glucose and insulin models as implemented in nondiabetic subjects using the SAAM compartmental modeling process.[29] Coupled Compartments: "G" represents plasma glucose; "I" represents plasma insulin; "X" represents insulin remote from the plasma. Dashed lines represent interaction between compartments. Double arrows represent input flow. Single arrows represent output flow. Gb, basal glucose; P1, insulin-independent fractional glucose clearance; P2, removal of insulin from the remote compartment; P3, "insulin action"; n, fractional insulin clearance (equations 1a to 3a).

cluding insulin-requiring type 2 diabetes.[10,12,13] It was decided to use a variable insulin infusion, adjusted to prevent hypoglycemia, and at physiologic levels as in previous type 1 diabetes studies rather than using a fixed supraphysiologic insulin bolus as described by Welch et al.[11] It has been reported that the dose of exogenous insulin has influenced the results of the estimation of Si. In view of this, it appears desirable to approximate as closely as possible the physiologic profiles of insulin and glucose that are obtained during IVGTT testing in nondiabetic subjects. We therefore compared the results with those obtained by our "phasic" protocol with those from previous IVGTT testing in some of the type 2 diabetes subjects in our study group, using 2 less physiologic exogenous insulin protocols as described by Welch et al[11] and Taniguchi et al.[13]

The aim of our approach was to enable us to quantify abnormalities of the kinetics of onset of insulin action in type 2 diabetes by using an additional ('time delay') modification to the Bergman minimal model.

MATERIALS AND METHODS

Patients

Eight type 2 diabetic patients were studied on 2 occasions (Table 1). There were 7 males and 1 female, mean age, 53 ± 4.8 years. The duration of diabetes was 10.3 ± 1.8 years. Body mass index (BMI) was 29.3 ± 1.7 kg/m^2, and mean glycosylated hemoglobin (HbA_{1c}) was 7.1% (range, 6.7% to 7.6%). Three patients were being treated with diet and oral hypoglycemic agents (gliclazide, 160 mg daily in 2 cases and glibenclamide, 5 mg plus metformin 1 G daily in 1 case), while 5 patients were insulin-requiring (47 ± 13 U daily). One patient had proliferative retinopathy, which had been treated with laser therapy; 2 patients had mild hypertension (blood pressure [BP] 160/90 and 140/90), managed with captopril and enalapril, respectively; and 1 patient with a history of hypercholesterolemia was taking gemfibrozil. No treatment regimen was altered during the period of the studies. All patients gave informed written consent, and the studies were approved by the St. Vincent's Hospital Human Research Ethics Committee.

Protocol

Two studies were performed, each on separate days and in random order. Patients were admitted to the Metabolic Ward on both occasions, after an overnight fast and without having taken any morning medication. On 1 occasion, a 5-hour frequently sampled IVGTT was performed: the aim of increasing the length of the test from its usual 3 hours was to ensure that glucose levels returned to basal level, which can be unpredictable in type 2 diabetes in the shorter IVGTT. Our aim was to facilitate computer analysis of the "offset" period in the IVGTT and to evaluate the importance of the 3- to 5-hour data in fitting the

Table 1. Clinical Data on the Type 2 Diabetic Patients

Type 2 Diabetic Patients	Age (yr)	Weight (kg)	Height (m)	BMI (kg · m^{-2})	Fasting Plasma Glucose (mmol/L)
1	58	77	1.72	27.0	8.3
2	68	75	1.65	27.5	6.1
3	42	81	1.73	27.1	9.3
4	63	93	1.78	29.4	5.4
5	30	126	1.90	34.9	7.9
6	65	94	1.60	36.7	8.4
7	41	59	1.67	21.2	8.5
8	55	82	1.64	30.5	9.1
Average (SEM)	52.8 (4.8)	85.9 (6.9)	1.71 (0.03)	29.3 (1.7)	7.9 (0.5)

minimal model (see Computer Analysis below). On the other occasion, a 2-stage euglycemic clamp (EUCL) was performed, clamping blood glucose at fasting levels, with insulin infusion rates of 40 mU/kg/h (first stage) and 100 mU/kg/h (second stage).

Study 1: IVGTT

A glucose bolus of 200 mg/kg (to a maximum of 27 mL of 50% dextrose) was given over 60 seconds at time 0, and blood samples were collected for glucose and free-insulin measurements at −20, −10, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, 180, 200, 220, 240, 260, 280, and 300 minutes. In addition, samples were taken for C-peptide, glucagon, and nonesterified fatty acids (NEFA) at −1, 5, 30, 60, 180, and 300 minutes. Incremental insulin was infused in these type 2 diabetes patients to simulate dynamic plasma free-insulin according to a protocol, which was a modification of one previously described for use in type 1 diabetes.[7] Modification of the IVGTT protocol was necessary to compensate for endogenous insulin secretion in the type 2 diabetes patients and was implemented by reduction of the insulin infusion rate to 50% of that used in the type 1 diabetes studies,[9] ie, after administration of the glucose bolus, insulin infusion rates were: phase 1 (2 to 4 minutes) 3.5 mU/kg/min; phase 2 (7 to 17 minutes) 0.5 mU/kg/min; phase 3 (17 to 50 minutes) 0.25 mU/kg/min; and "basal" insulin infusion rate (50 to 300 minutes) 0.1 mU/kg/min (Fig 2). Blood glucose levels were monitored at the bedside at 10-minute intervals throughout the IVGTT, and basal insulin infusion rate was adjusted if blood glucose decreased below 6 mmol/L.

Study 2: Euglycemic Clamp

A 2-stage euglycemic clamp was performed in the same type 2 diabetes patients using the technique previously described.[14,15] The blood glucose was clamped at the patient's actual fasting level (or at 7.8 mmol/L if the fasting blood sugar was elevated above this level on the day of the study). Insulin infusion rate was 40 mU/kg/h in the first stage and 100 mU/kg/h in the second stage, with simultaneous infusion of 20% dextrose at the rate calculated by the Ox Clamp computer program.[16] Blood glucose was estimated at 5-minute intervals at the bedside throughout the clamp study, using the Yellow Springs Instruments glucose analyzer (Yellow Springs, OH), and the rate of the dextrose infusion was adjusted to maintain euglycemia. At the same time, samples were taken for assay of plasma concentrations of glucose and insulin during the steady state stage of the insulin infusion.

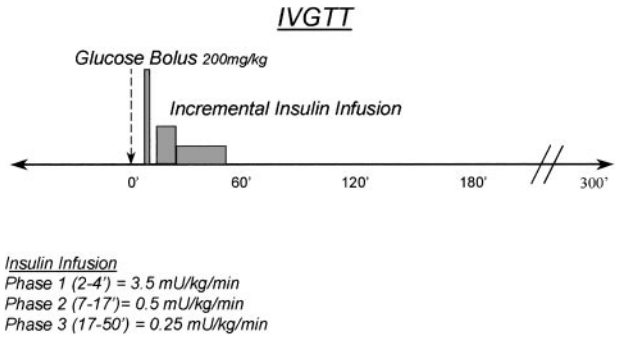

Fig 2. IVGTT exogenous insulin protocol. After administration of the glucose bolus, insulin infusion rates were: phase 1 (2 to 4 minutes) 3.5 mU/kg/min; phase 2 (7 to 17 minutes) 0.5 mU/kg/min; phase 3 (17 to 50 minutes) 0.25 mU/kg/min; and "basal" insulin infusion rate (50 to 300 minutes) 0.1 mU/kg/min.

Assays

Plasma glucose concentrations were measured with a Yellow Springs Instruments glucose analyzer using the glucose oxidase method. Plasma insulin and glucagon were measured by radioimmunoassay using charcoal separation of bound from free fractions.[17,18] With this assay, the normal fasting insulin range is less than 10 mU/L, and the interassay coefficient of variation is 6.3% at 10.5 mU/L and 8.4% at 22.0 mU/L. In patients who had previous insulin therapy, plasma-free insulin was measured after precipitation of bound insulin[19] by polyethylene glycol precipitation on the day of the study to avoid disturbing the equilibrium between free and antibody-bound insulin.[20] Free C-peptide was assayed using the Novo C-peptide radioimmunoassay kit (Novo Research Institute, Copenhagen, Denmark), using synthetic human C-peptide and guinea pig antihuman C-peptide antiserum,[21] the normal fasting range being 0.18 to 0.63 nmol/L. NEFA were estimated by an enzymatic colorimetric method using a kit (Wako Pure Chemical Industries, Osaka, Japan).

Computer Analysis

A computer analysis of plasma insulin and glucose concentrations during the IVGTT was made based on the minimal models of glucose[3] and insulin[4] kinetics of Bergman et al and the simulation and modelling program CONSAM.[22] As previously reported,[23] 3-hour IVGTTs had been performed in a group of type 2 diabetes subjects (controlled by diet alone or oral hypoglycemic agents), and the minimal model method of analysis as modified for type 1 diabetes[7] had been applied. However, this modification was found to be inadequate for those type 2 diabetic subjects who had higher therapeutic needs, because (1) the early stages of glucose disappearance as predicted by the model differed markedly from the actual values, and (2) poor estimates of the model parameters were obtained. We therefore investigated the following adaptations to enhance fitting of the model to the data in the current group of type 2 diabetes patients that include a wider range of therapeutic requirements: (1) extension of the IVGTT from 3 to 5 hours to ensure sufficient time for equilibration of plasma glucose to a basal state, (2) introduction of a "delay element" in the part of the model relating to the onset of insulin action.

The degree of similarity of the MMD to the BMM can also be seen from the sets of state equations characterizing responses of each system, viz:

BMM

$$\dot{G} = P1.\ Gb - G.\ (P1 + X) \quad (1a)$$

$$\dot{X} = P3.\ I - P2.\ X \quad (2a)$$

$$\text{and } \dot{I} = g.t.(G - h) - n.I \quad (3a)$$

MMD

$$\dot{G} = P1.\ Gb - G.\ (P1 + X) \quad (1b)$$

$$\dot{X} = P3.\ I - P2.\ X \quad (2b)$$

$$\text{and } I = L(0_i) \quad (3b)$$

Here, as in the models of Bergman et al,[3,4] the state variables are: G = plasma glucose, X = remote insulin, I = plasma insulin, P1 = Sg, Gb = basal glucose and Si = P3/P2 (other parameters are as described by Bergman et al[3,4]).

It can be seen that 2 principal variations have been applied to our previous type 1 diabetes model to derive the MMD. First, the compartmental modelling of the insulin infusion was removed, replacing the

insulin response with a quasi-response function.

$$I = L(0_i) \quad (3b)$$

This permits, indirectly, the remote insulin to be driven by the observed plasma insulin observations (O_i). Where a solution for the stated equations is called for at a point for which no observation exists, a linearly interpolated value is calculated and used. Thus, it is similar to other implementations of the BMM that use linear interpolation between the insulin data points. Second, a major problem associated with using the BMM to fit type 2 diabetes IVGTT data was its inability to resolve insulin-mediated glucose elimination from Sg. In practice, Si, identified in conjunction with P3 and P2, could not reliably be determined. This suggested to us that in keeping with the work of DeFronzo et al,[24] the effect of insulin action may be substantially more delayed in type 2 diabetes responses than in either type 1 diabetes or normal subjects. To reflect this, we experimented (numerically) with the introduction of a delay element in the insulin action path of the model (Fig 3), removing the feedback effect of glucose on insulin secretion. As the new model was set up to use the actual plasma insulin levels as input values, the source of the insulin does not affect the modelling, therefore the feedback effect of glucose on insulin secretion was not relevant in this model. The best resolution of the value for this delay (DT18) and other model parameters (estimated from the type 2 diabetes IVGTT responses) was achieved when the delay was inserted between the plasma and the remote insulin compartments. We used this model structure also because it seemed consistent with physiologic considerations,[25] as well as preserving a high degree of basic conformity with the minimal model of Bergman et al.[3,4]

In applying the MMD to the type 2 diabetes data, it was not appropriate to use extrapolation of the early insulin data points to 0. Rather, we used interpolation between actual data points. By applying the same process to the data from normal subjects, it was shown that this process led to better precision of estimation of Si and Sg compared with our previous implementation of the Bergman model, in which extrapolation of the early insulin data to 0 was used. In this new model for type 2 diabetes, (MMD), the baseline insulin was subtracted from the insulin values as in the standard minimal model. In these analyses, therefore, the early insulin data were represented by linear interpolation between the actual data points rather than by extrapolation to 0 to obtain initial conditions for the insulin compartment.

Comparison of MMD results with other methods of estimating Sg and Si. Data from 3 other previous studies were also analyzed using the BMM and the MMD methods; the results for Sg and Si being compared with the current type 2 diabetes studies. One was a 3-hour IVGTT study in 6 normal nondiabetic subjects; the second study was a 3-hour IVGTT in 5 of the type 2 diabetes patients using the incremental insulin protocol described above; and the third was a 3-hour IVGTT in 6 mild type 2 diabetes patients (controlled by diet or low-dose hypoglycemic agents alone), using the protocol incorporating a 5-minute insulin infusion at 20 mU/kg 20 minutes after the 300 mU/kg glucose bolus, as described by Tanaguchi et al.[13]

RESULTS

Study One

IVGTT. Basal fasting plasma glucose levels were 9.1 ± 0.8 SEM mmol/L, fasting C-peptide was 0.59 ± 0.11 nmol/L, fasting glucagon was 38 ± 5 ng/L, and fasting NEFA was 0.57 ± 0.1 mmol/L. The plasma insulin and glucose profiles showed a similar pattern within the group studied (Fig 4), and mean glucose disappearance rate (Kg) was 0.77 ± 0.2 $min^{-1} \times 10^2$. Glucose had not achieved steady state by 180 minutes, and there was a further decrease in glucose of 10% by 300 minutes.

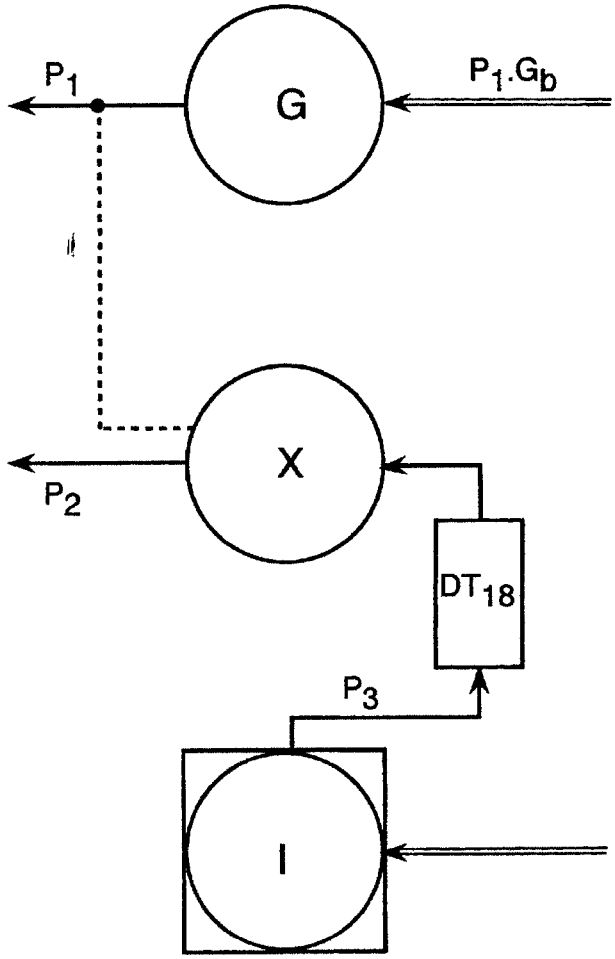

Fig 3. Modification of minimal model with delay (MMD). Feedback effect of glucose on insulin secretion has been removed, which is therefore the same model as the "glucose model" described by Bergman et al.[3,4] However in addition DT18 representing a time delay in transfer of plasma insulin to remote insulin compartment has been added, corresponding to a delay in onset of insulin action. The square around compartment "I" indicates that observed insulin values drive the system, so fractional insulin clearance (n) need no longer be considered. Other symbols are as in Fig 2.

C-peptide levels had increased by 10% at 30 minutes, returned to basal level by 180 minutes, and were significantly reduced at 300 minutes ($P = .04$) (Table 2). There was no significant change in glucagon levels, but NEFA decreased during the IVGTT, returning to baseline levels by 180 minutes (Table 2), before increasing above baseline levels by 300 minutes, reflecting a reduction in the rate of basal insulin infusion required to maintain euglycemia during the latter part of the IVGTT study.

Computer analysis. Using the BMM, evaluation of Si and Sg by computer analysis of IVGTT results gave estimates of poor quality, and few cases could be satisfactorily resolved. Inclusion of the delay element (64 ± 17 minutes) in the MMD dramatically affected its capacity to estimate Si and Sg. The computer analysis results are summarized in Table 3. Satisfactory identification of Si and Sg, without using a delay element, was only achieved in 3 of 8 of cases in the 3-hour study and in

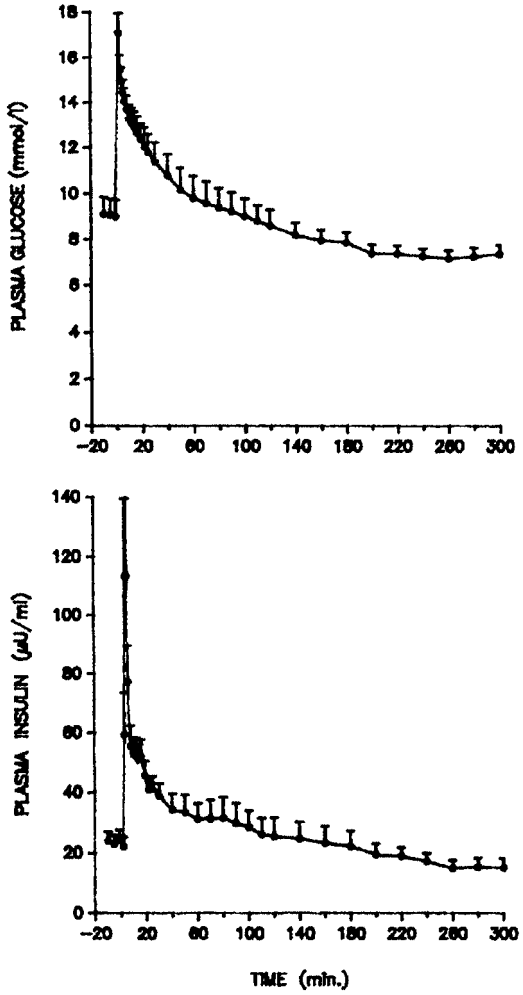

Fig 4. IVGTT in type 2 diabetes subjects (N = 8). Mean (± SEM) plasma glucose and insulin profiles over the 5-hour period after an intravenous glucose load. Exogenous insulin was infused as described in Materials and Methods to simulate a physiologic profile of insulin release.

5 of 8 in the 5-hour study (the satisfactory identification level was selected at $P < .05$, Student's $t > 2$, and fractional standard deviation [FSD] < 0.05). When the delay element was included, identification improved to 7 of 8 (3-hour study) and 8 of 8 (5-hour study) (mean Si being 1.86 ± 0.6 min$^{-1} \cdot \mu$U$^{-1} \cdot$ mL $\times 10^4$ and Sg being 2.00 ± 0.25 min$^{-1} \times 10^2$). Insertion of the delay in the MMD increased the FSD of Si from 8.4% to 22.9% in the 3-hour study, whereas the FSD decreased from 17.7% to 13.7% in the 5-hour study, which would underscore the value of extending the measurements to 5 hours when using the MMD.

Study Two

Euglycemic clamp. On admission, basal fasting plasma glucose was 9.7 ± 0.35 mmol/L, C-peptide was 0.67 ± 0.1 nmol/L, fasting glucagon was 54 ± 10 ng/L, and fasting NEFA was 0.60 ± 0.06 mmol/L. As basal fasting glucose levels were above 8 mmol/L in all cases, the glucose clamp level was programmed at a uniform level of 7.8 mmol/L. During the EUCL, C-peptide decreased during the first stage infusion and was significantly reduced when plateau was reached in the second stage of the clamp. Glucagon and NEFA also decreased during the euglycemic clamp (Table 2).

Correlation with IVGTT. When Si was calculated from the type 2 diabetes EUCL data using the method of Bergman et al,[14] the results were similar to those obtained from the 5-hour IVGTT with the delay element included (Sip [EUCL] *v* Si [IVGTT] $= 2.02 \pm 0.42$ *v* 1.86 ± 0.6 min$^{-1} \cdot \mu$U$^{-1} \cdot$ mL $\times 10^4$) (Fig 5). These means were not significantly different using Wilcoxon's matched-pairs signed-rank test (Si [IVGTT] *v* Si_p, $P = .94$). Si calculated by IVGTT method and Si_p were significantly correlated (Spearman rank order correlation, $rs = 0.76$, $P < .05$).

Analysis of Data From Other Studies Using MMD

Nondiabetic IVGTT. Computer analysis of data obtained from the 6 normal nondiabetic patients showed that when identical insulin values were input into the BMM and the MMD, then similar Si and Sg values were obtained when the delay element was omitted from the MMD (Table 4). When the delay element was added into the MMD, no improvement with model fit was seen, with no significant improvement in the residual sum of squares and no change to Si. However, we observed a significant elevation of Sg when including the delay element versus Sg without the delay (2.77 ± 0.47 *v* 1.64 ± 0.25 min$^{-1} \times 10^2$), which may be related to the small size of the delay value (7.5 ± 1.7 min).

Other type 2 diabetes protocols. The Si results obtained with the protocol of Taniguchi et al[13] were not significantly different from those from the phasic protocol and correlated well with them ($r = .90$, $P < .05$, Table 5). However, the Si values obtained with the protocol of Welch et al[11] were significantly below the phasic results and showed a poorer correlation with them ($r = .67$, $P = .22$, Table 5). The Sg values from

Table 2. Plasma C-Peptide, Glucagon, and NEFA Concentrations in Type 2 Diabetes Studies

	Plasma Concentrations (mean + SEM)		
Time in Minutes	C-Peptide (nmol/L)	Glucagon (ng/L)	NEFA (mmol/L)
Study 1: IVGTT			
0	0.59 + 0.1	38 + 5	0.56 + 0.1
5	0.55 + 0.1	41 + 5	0.57 + 0.1
10	0.59 + 0.1		0.43 + 0.16
30	0.64 + 0.12*	44 + 7	0.39 + 0.08
60	0.62 + 0.15	42 + 3	0.31 + 0.07*
180	0.49 + 0.14	34 + 6	0.51 + 0.06
300	0.45 + 0.09*	39 + 6	0.84 + 0.06
Study 2: EUCL			
0	0.67 + 0.1	54 + 10	0.60 + 0.06
First stage plateau†	0.50 + 0.1	37 + 7	0.16 + 0.02*
Second stage plateau‡	0.39 + 0.07*	19 + 5*	0.10 + 0.01*

* $P < .05$ compared with baseline (0 min) value.
† Insulin infusion at 40 mU/kg/h.
‡ Insulin infusion at 100 mU/kg/h.

Table 3. Analysis of Data From the Eight Type 2 Diabetic Patients Using MMD

Model Variation*	Data Extent (h)	Percent Identified	Mean SG $(min^{-1}) \times 10^2$	FSD of Mean SG	Mean SI $(min^{-1} \cdot \mu U^{-1} \cdot mL) \times 10^4$	FSD of Mean SI
No delay	3	37.5	2.3	19.6	2.8	8.4
No delay	5	62.5	1.8	17.9	2.6	17.7
Delay	3	87.5	2.0	5.3	1.7	22.9
Delay	5	100.0	2.0	7.5	1.9	13.7

Abbreviation: FSD, fractional standard deviation, expressed as percentage of the mean.

* Improvement in parameter estimability was achieved by extending the IVGTT to 5 hours and by including the delay element in MMD analysis of the type 2 diabetes data sets.

both methods were not significantly different from those from the Phasic protocol (Table 5).

DISCUSSION

Type 2 diabetes covers a wide spectrum of abnormality of insulin and glucose kinetics, analysis of which is not readily accommodated by standard techniques in every case. One of our major aims in the present study was to gain further insight into the kinetics of insulin action after a glucose load under near physiologic conditions in type 2 diabetes. We therefore combined a reduced glucose load with a phasic insulin infusion simulating a physiologic plasma insulin response in the IVGTT, facilitating the analysis of the full spectrum of type 2 diabetes including insulin-requiring subjects.

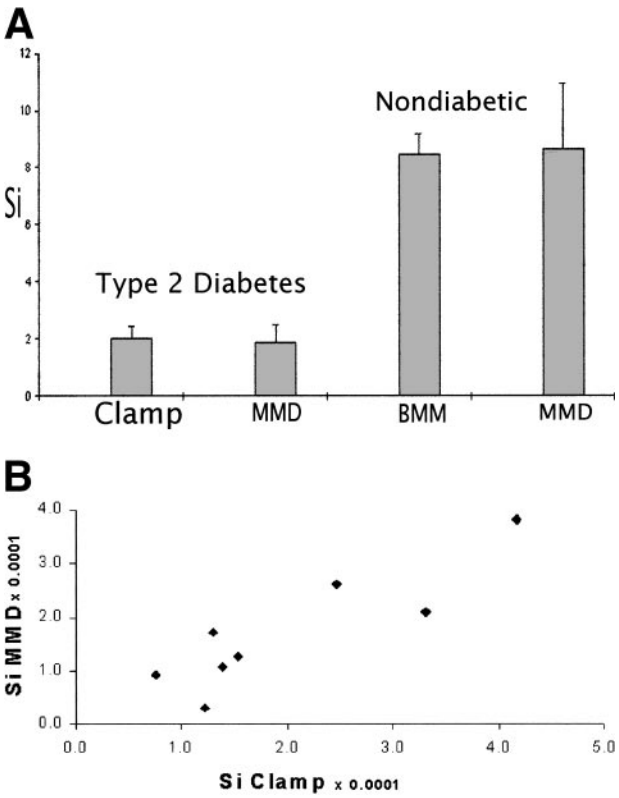

Fig 5. (A) Si from IVGTT and glucose CLAMP in type 2 diabetes and from IVGTT in normal subjects. The histograms of the type 2 diabetes represent mean (± SE) by 2 measures of insulin sensitivity: (1) from MMD analysis (with the delay element) of the IVGTT, (2) from clamp plateau values (Sip). The results show that the same estimates for Si were calculated by the 2 different techniques. The histograms of the normal subject data illustrate the effect of the addition of the delay element in MMD applied to this group of subjects. No change in Si is obtained in contrast to the effect in type 2 diabetes. (B) Correlation of 2 different methods of estimating Si in the type 2 diabetic subjects. Scatterplot is shown of Si estimated by minimal model analysis with delay (MMD) of IVGTT *v* the Si estimated by the 2-step hyperinsulinemic glucose clamp technique.

Our results indicate that the minimal model of Bergman et al[3,4] can be modified to fit data from IVGTT in type 2 diabetics over a wide range of Si and fasting hyperglycemia, if exogenous insulin is infused to simulate the endogenous insulin secretion. Our earlier model modification for analysis of type 1 diabetes data[7] could not accommodate the type 2 diabetes data in all cases. Poor fitting of the insulin data was ascribed to the unpredictable contribution of the endogenous insulin secretion in type 2 diabetes, which could not be accommodated by simple modeling of the insulin infusion as in type 1 diabetes studies. In our initial modifications to the Bergman model for use in type 1 diabetes, the insulin compartment was described by compartmental modeling[22] of the exogenous insulin infusion into the plasma insulin compartment. In the present study, further modifications to analyze the type 2 diabetes data included (1) linear interpolations between the actual plasma insulin values in constructing the plasma insulin curve; and, in the MMD (2) introduction of a delay element between the plasma and remote insulin compartments.

Extension of the IVGTT from 3 to 5 hours only improved the number of cases, which were satisfactorily "fitted" by the BMM from 3 to 5 of 8 subjects without using the delay. The original minimal model does contain an implicit delay in the onset of insulin action represented by the transfer of insulin from the plasma to the "remote" insulin action compartment (X). In the original model, this process is represented by a single phase. In our new model, however, the transfer from the plasma to remote compartment X is satisfactorily represented by more than 1 phase, with an associated additional delay.

The cause of the additional delay in onset of insulin action in the IVGTT in type 2 diabetes is not immediately evident. It is possible that the the delay could be related to abnormal transfer across the endothelium, and Ader et al[25] have already shown that the "normal" delay in onset of insulin action is almost entirely due to the transfer across the endothelium. However, in that case, the onset of insulin action would be represented by 1 compartment, which would not be consistent with our findings. The existence of an abnormality in the cellular interaction of

Table 4. Analysis of IVGTT in Normal Subjects (n = 6)

Model Variation	Data Extent (h)	Percent Identified	Mean SG (min^{-1}) $\times 10^2$	FSD of Mean SG	Mean SI ($min^{-1} \cdot \mu U^{-1} \cdot mL$) $\times 10^4$	FSD of Mean SI	Residual SS (6)§
BMM*	3 h	100	1.55	33%	8.65	11%	
MMD, delay = 0†	3 h	100	1.64	15%	8.43	9%	133 + 26
MMD & DELAY‡	3 h	100	2.7	6%	7.86	16%	132 + 26

Abbreviations: FSD, fractional standard deviation, expressed as % of mean value; SG, glucose effectiveness index; SI, insulin sensitivity index.
* Bergman minimal model fitting both glucose and insulin models simultaneously using SAAM.[28]
† Modified minimal model using SAAM to fit only glucose model with no delay element.[3]
‡ Modified minimal model with addition of delay element, which is estimated in fitting process.
§ Residual sum of squares of compartment 6 (glucose) ± SEM.

insulin is another possible reason for the additional delay in the study of type 2 diabetes patients, although previous evidence suggests that cellular interaction is rapid. It is more likely, however, that the abnormality might relate to delayed transit of insulin through the interstitial fluid, which would be consistent with the observed thickening of the basement membrane in type 2 diabetes patients.

The results of our studies showed that while Si was impaired in type 2 diabetes compared with nondiabetics, Sg was not significantly reduced. Our findings were similar to the results of Baron et al,[26] although Kruszynska et al[12] found significant reduction of Sg in type 2 diabetes subjects with cirrhosis.

In our present studies, the estimates of Si obtained by the new MMD with the delay element were verified against another technique for measuring Si, namely the 2-stage euglycemic clamp.[14,15] Our studies indicate that similar results for Si are obtained by both methods.

In type 2 diabetes subjects, the IVGTT response to the glucose and insulin (Fig 4), showed that the glucose levels do not reach a steady state by 3 hours, but have reached a plateau by 5 hours. We fitted the new model to glucose and insulin data obtained from IVGTT in normal subjects to explore the consistency with the original Bergman model. This showed that the results obtained by both methods (BMM and MMD) were similar for Si and Sg, if identical insulin values were used as input to the MMD and BMM. This also indicated that the process of linear interpolation between the actual data points did not result in any change in the estimates of Sg and Si or in the precision of these estimates.

Table 5. Estimates of Si and Sg From Fitting of Minimal Model to IVGTT Data From Phasic, Tanaguchi, and Welch Protocols

Parameter/Protocol	No.	Mean	SEM
Si/clamp	8	2.0	0.4
Si/Taniguchi	5	3.1	1.2
Si/Welch	5	0.7*†	0.2
Si/5 h phasic	8	1.8	0.6
Si/3 h phasic	9	1.5	0.4
Sg/Taniguchi	5	1.05	0.23
Sg/Welch	5	0.86	0.16
Sg/5 h phasic	8	1.93	0.48
Sg/3 h phasic	9	1.82	0.43

NOTE. Si units: $min^{-1} \cdot \mu U^{-1} \cdot mL \times 10^4$. Sg units: $min^{-1} \times 10^2$.
* $P < .05$ *v* clamp estimate.
† $P < .05$ *v* 3 h phasic estimate.

Our results indicate that the need for the delay element does not occur in fitting data from normal subjects compared with the type 2 diabetes subjects, because when the MMD with the delay element was applied to the normal subject IVGTTs, there was no improvement in the "goodness of fit" to the glucose data, and in addition, the delays, which were resolved, were small compared with those in the type 2 diabetes study. This indicates that the prolonged delay in onset of insulin action may be associated with a specific pathophysiologic abnormality in type 2 diabetes, although whether this also occurs in other insulin-resistant states will need further investigation.

Our new technique provides a method of accurate and precise measurement of Sg and Si in type 2 diabetes. It has been shown that IVGTT data can be analyzed in cirrhotic patients with diabetes, using the Bergman minimal model with little adaptation[12]: tolbutamide was used in this study to enhance the endogenous insulin secretory response. The BMM has also been shown by Taniguchi et al[13] to be suitable for analysis of IVGTT data from recently diagnosed nonobese type 2 diabetes patients, who are well-controlled on diet alone and are not significantly insulin resistant: in these cases, a short bolus of insulin was infused 20 minutes after the glucose bolus. Our study design was intended to be appropriate for a more representative range of type 2 diabetes subjects, particularly those with demonstrable insulin resistance. We used a slower infusion of insulin, which more clearly replicates the normal physiologic pattern of plasma insulin after IVGTT, and hypoglycemia was avoided. It is possible that with the use of a higher insulin infusion rate, resolution of the delay element in computer analysis might be obscured.

The modification to the model including a delay element allows fitting of data from type 2 diabetes subjects over a wide range of fasting hyperglycemia and Si values (0.4 to 5.7 min^{-1} per mU/L $\times 10^4$). Furthermore, the kinetics of insulin action are examined over a physiologic range of plasma insulin values. Over this physiologic range, the delay in onset of insulin action can be identified, although the precise correlation of its numerical value with physiologic processes needs further clarification. Nevertheless, we can conclude that the onset of action of physiologic levels of insulin in type 2 diabetes cannot be described as a single phase in the transfer of insulin from plasma to the remote compartment.

The need for more than 1 phase in analyzing the onset of insulin action over the physiologic range is also consistent with the results of Ader et al,[25] who have shown that a rate-limiting

step in the onset of insulin action is transportation across the capillary wall into the interstitial fluid, the second step being binding to the cell surface and cellular insulin action. These 2 phases do not appear to be resolveable in analyzing the IVGTT in normal subjects, but our data suggests that the 2 phases may be separately identifiable in type 2 diabetes insulin-resistant subjects at physiologic insulin levels. Although little detail is known about disorders of the onset of insulin action in pathophysiologic states, abnormal delays in onset have been found in obesity[27] and in leg glucose uptake in type 2 diabetes.[24] Moreover, in diabetic rats, reduced binding of insulin to endothelial cells has been reported, and this might regulate transepithelial transport.[25] Further studies would be valuable to explore the existence of a delay factor in insulin action in situations, such as obesity and other insulin-resistant states, to determine whether this abnormal delay is specific to type 2 diabetes.

The incorporation of a delay factor in our model of the 5-hour IVGTT data in type 2 diabetes has enabled successful resolution of Si and Sg to occur in all cases, thereby extending the range of patients whose data can be analyzed. In our experience, results obtained with other short "square wave" exogenous-insulin protocols produced similar estimates of Si and Sg when the low-dose of insulin described by Taniguchi et al[13] was used, but significant underestimation of Si occurred when the high-dose protocol as described by Welch et al[11] was used, confirming that the dose of exogenous insulin influences the estimates of Si. This suggests that doses of exogenous insulin closest to physiologic levels would be more likely to give estimates of Si and Sg comparable to those in nondiabetic subjects. It also implies that the simpler protocol of Taniguchi et al[13] would be a reasonable choice if the aim is only to estimate Si and Sg, but that our phasic technique should be used if additional characterization of the compartmental nature of the onset of insulin action is required.

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REFERENCES

1. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1606, 1988

2. Stout RW: Diabetes and atherosclerosis—The role of insulin. Diabetologia 16:141-150, 1979

3. Bergman RN, Ziya-Ider Y, Bowden CR, et al: Quantitative estimation of insulin sensitivity. Am J Physiol 236:E667-R677, 1979

4. Bergman R, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man. J Clin Invest 48:1456-1457, 1981

5. Ward GM, Walters JM, Aitken PA, et al: Effects of prolonged pulsatile hyperinsulinemia in humans. Enhancement of insulin sensitivity. Diabetes 39:501-507, 1990

6. Finegood DT, Hramiak IM, Dupre J: A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. J Clin Endocrinol Metab 70:1538-1549, 1990

7. Ward GM, Weber KM, Walters JM, et al: A modified minimal model analysis of insulin sensitivity and glucose-mediated glucose disposal in insulin-dependent diabetes. Metabolism 40:4-9, 1991

8. Walters JM, Ward GM, Kalfas A, et al: The effect of epinephrine on glucose-mediated and insulin-mediated glucose disposal in insulin-dependent diabetes. Metabolism 41:671-677, 1992

9. Ward GM, Walters JM, Kalfas A, et al: Minimal model analysis of the effect of hyperglycemia in insulin dependent diabetes. Diabetologia 35:100, 1992 (suppl 1, abstr)

10. Ng LL: Application of modelling techniques to the assessment of insulin sensitivity in man. Diabet Med 5:217-222, 1988

11. Welch S, Gebhart SSP, Bergman RN, et al: Minimal model analysis of intravenous glucose tolerance test–derived insulin sensitivity in diabetic subjects. J Clin Endocrinol Metab 71:1508-1518, 1990

12. Kruszynska YT, Harry DS, Bergman RN, et al: Insulin sensitivity, insulin secretion and glucose effectiveness in diabetic and non-diabetic cirrhotic patients. Diabetologia 36:121-128, 1993

13. Taniguchi A, Nakai Y, Fukushima M, et al: Pathogenic factors responsible for glucose intolerance in patients with NIDDM. Diabetes 41:1540-1546, 1992

14. Bergman RN, Prager R, Volund A, et al: Equivalence of the insulin sensitivity index in man derived by the minimal model method and euglycaemic clamp. J Clin Invest 79:790-800, 1987

15. Proietto J, Harewood M, Aitken P, et al: Validation of a practical in vivo insulin dose-response curve in man. Metabolism 31:345-360, 1982

16. Matthews DR, Edge JA, Dunger DB: An unbiased glucose clamp method using a variable insulin infusion: Its application in diabetic adolescents. Diabet Med 7:246-251, 1990

17. Albano JDM, Ekins RP, Maritz G, et al: A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol 70:487-509, 1972

18. Alford FP, Bloom SR, Nabarro J: Glucagon levels in normal and diabetic subjects: Use of a specific immunoabsorbent for glucagon radioimmunoassay. Diabetologia 13:1-6, 1977

19. Nakagawa S, Nakayama H, Sasaki T, et al: A simple method for the determination of serum free insulin levels in insulin-treated patients. Diabetes 22:590-600, 1973

20. Hanning I, Home PD, Alberti KGMM: Measurement of free insulin concentrations: The influence of the timing of extraction of insulin antibodies. Diabetologia 28:831-835, 1985

21. Heding L: Radioimmunological determination of human C-peptide in serum. Diabetologia 11:541-548, 1975

22. Boston RC, Greif PC, Berman M: Conversational SAAM—An interactive program for kinetic analysis of biological systems. Comp Prog Biomed 13:111-119, 1981

23. Walters JM, Ward GM, Barton J, et al: The effect of norepinephrine upon insulin secretion and glucose effectiveness in NIDDM. Metabolism 46:1448-1453, 1997

24. Defronzo RP, Gunnarsson R, Bjorkman O, et al: Effects of insulin on peripheral and splanchnic glucose metabolism in non insulin-dependent (type II) diabetes mellitus. J Clin Invest 76:149-155, 1985

25. Ader M, Poulin RA, Yang YJ, et al: Dose-response relationship between lymph insulin and glucose uptake reveals enhanced insulin sensitivity of peripheral tissues. Diabetes 41:241-253, 1992

26. Baron AD, Kolterman OG, Bell J, et al: Rates of noninsulin-mediated glucose uptake are elevated in type II diabetic subjects. J Clin Invest 76:1782-1788, 1985

27. Prager R, Wallace P, Olefsky JM. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. J Clin Invest 78:472-481, 1986

28. Weber K, Martin IK, Best JD, et al: Alternative method for minimal model analysis of intravenous glucose tolerance data. Am J Physiol 256:E524-E535, 1989