The Effect of Insulin Dose on the Measurement of Insulin Sensitivity by the Minimal Model Technique

Evidence for Saturable Insulin Transport in Humans

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Abstract

Administration of exogenous insulin during an intravenous glucose tolerance test allows the use of the minimal model technique to determine the insulin sensitivity index in subjects with reduced endogenous insulin responses. To study the effect of different insulin administration protocols, we performed three intravenous glucose tolerance tests in each of seven obese subjects (age, 20–41 yr; body mass index, 30–43 kg/m^2). Three different insulin administration protocols were used: a low-dose (0.025 U/kg) infusion given over 10 min, a low-dose (0.025 U/kg) bolus injection, and a high-dose (0.050 U/kg) bolus injection, resulting in peak insulin concentrations of 1,167±156, 3,014±384, and 6,596±547 pM, respectively. The mean insulin sensitivity index was 4.80±0.95 × 10^{-5}, 3.56±0.53 × 10^{-5}, and 2.42±0.40 × 10^{-5} min^{-1}/pM respectively (x±SEM; $P = 0.01$). The association of higher peak insulin concentrations with lower measured insulin sensitivity values suggested the presence of a saturable process. Because results were not consistent with the known saturation characteristics of insulin action on tissue, a second saturable site involving the transport of insulin from plasma to interstitium was introduced, leading to a calculated K_m of 807±165 pM for this site, a value near the $1/K_d$ of the insulin receptor. Thus, the kinetics of insulin action in humans in these studies is consistent with two saturable sites, and supports the hypothesis for transport of insulin to the interstitial space. Saturation may have an impact on minimal model results when high doses of exogenous insulin are given as a bolus, but can be minimized by infusing insulin at a low dose. (*J. Clin. Invest.* 1996. 97:501–507.) Key words: biological transport • receptors, insulin • models, biological • kinetics • glucose tolerance test

Introduction

The minimal model of glucose kinetics is a commonly used method of measuring insulin sensitivity that uses mathematical modeling of the glucose and insulin results from an intravenous glucose tolerance test (IVGTT)[1] to quantify the insulin

sensitivity index (S_I). Recently, injection or infusion of exogenous insulin has been used during the IVGTT to allow use of the minimal model when insulin responses are reduced or absent (1–3). Since the level and time course of the induced exogenous insulin concentrations are under investigator control, it is necessary to select the most appropriate insulin dosing protocol.

Sufficient insulin must be used to identify accurately the minimal model parameters. However, evidence has been presented that insulin is transported from plasma to the interstitial space (4), where it then binds to cell surface receptors. Each of these sites may be subject to saturation. Thus, it may be necessary to limit the insulin dose to avoid saturation at these sites and to remain within the boundary where insulin sensitivity is independent of the insulin level. To evaluate whether a saturation effect is present, we performed IVGTTs on obese subjects with normal fasting glucose levels using three different protocols for administration of exogenous insulin.

Methods

Subjects. Seven obese subjects (five men, two women) with no history of major medical illness and taking no medications known to affect glucose metabolism participated in the study. Subjects were between 20 and 41 yr of age with all subjects having a body mass index (BMI) > 29 kg/m^2. Obese subjects were selected because they tend to be more insulin resistant (5, 6) and thus would be less likely to experience hypoglycemia with high doses of insulin. The study was approved by the Human Subjects Review Committee at the University of Washington, and all subjects gave written informed consent before participation in the study.

Procedures. Each subject underwent an IVGTT on three occasions to quantify the S_I using the minimal model of glucose kinetics (7). In addition to S_I, the IVGTT results were used to determine the glucose effectiveness at basal insulin (S_g), acute insulin response to glucose (AIRglucose), glucose disappearance rate constant (K_g), and the glucose effectiveness at zero insulin (GEZI), an index of insulin-independent glucose uptake. Three different insulin administration protocols were used. The order of the three tests was varied to minimize a sequence effect (see Table I). All tests were completed within an 8-d period except in one subject in which studies were completed in 18 d. The subjects were advised to maintain their normal patterns of exercise and diet. Women were studied during the follicular phase of the menstrual cycle.

The studies were performed on the metabolic ward of the Seattle Veterans Affairs Medical Center after an overnight fast. The subjects

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1. *Abbreviations used in this paper:* AIRglucose, acute insulin response to glucose; AUC, area under the curve; BMI, body mass index; COV, coefficient of variation; GEZI, glucose effectiveness at zero insulin; IVGTT, intravenous glucose tolerance test; K_g, glucose disappearance constant; S_g, glucose effectiveness at basal insulin; S_I, insulin sensitivity index.

were supine during the study, and blood samples were obtained through an 18-gauge plastic catheter placed in a forearm vein. This arm was maintained in a heating pad to arterialize the venous blood (8). Infusates were administered through a similar catheter inserted in the contralateral forearm. Both catheters were kept patent by a slow infusion of 0.9% saline.

The IVGTT procedure consisted of the collection of three baseline samples for insulin and glucose, followed by injection of 11.4 g/m² of glucose over 60 s commencing at time 0. Insulin was administered intravenously starting at $t = 20$ min using three different protocols: (*a*) low-dose (0.025 U/kg) infusion given over 10 min, (*b*) low-dose (0.025 U/kg) bolus injection, and (*c*) high-dose (0.050 U/kg) bolus injection. Blood samples were collected at 34 time points during the IVGTT at −15, −5, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 19, 21, 22, 23, 24, 25, 27, 30, 32, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. The presence of an apparent steady state was evaluated by comparing the glucose concentration of the 180-min sample to the 140- and 160-min values. In 13 studies, an apparent steady-state glucose concentration was not reached at 180 min, and additional samples were obtained at 200, 220, or 240 min. The samples were placed on ice until the plasma was separated and stored at −20°C until assayed for glucose and insulin.

Computations. Basal glucose and insulin levels were calculated as the average of the baseline samples. AIRglucose was determined as the mean of the incremental insulin levels at 2, 3, 4, 5, 6, 8, and 10 min after the administration of glucose. K_g was computed as the slope of the linear least square regression line to the natural logarithm of the glucose concentration versus time from 10 to 19 min after the administration of glucose. The S_I and glucose effectiveness at basal insulin concentration were obtained from the IVGTT results by identification of model parameters using a nonlinear least square technique (7, 9). Since the minimal model provides an estimate of glucose effectiveness at basal insulin levels, this value is influenced by both insulin sensitivity and basal insulin concentration. To eliminate these dependencies, GEZI was computed by subtracting glucose disposal mediated by the basal insulin concentration. Thus, $GEZI = S_g - I_b \cdot S_I$, where I_b is the basal insulin level, and S_g and S_I are parameters identified by the minimal model (10).

In addition to the minimal model equations described by Bergman (7), a modified set of minimal model equations that introduces two additional parameters to reflect potential sites of nonlinearity in insulin kinetics and action was used. The first additional parameter, K_{mI}, accounts for a potential nonlinear relationship in the transport of insulin from the plasma to the interstitial space. The second, K_{mA}, accounts for a potential nonlinearity between interstitial insulin levels and insulin action (Fig. 1). K_{mI} and K_{mA} are introduced as classical Michaelis-Menten parameters; thus, they represent the insulin level at which a half-maximal insulin effect occurs. Poor a posteriori identifiability prevented reliable determination of K_{mI} and K_{mA} simultaneously (11); thus, one parameter was set to a fixed value as described below, and the remaining parameters were obtained using a modification of the NL2SOL nonlinear least square program (12). Monte Carlo analysis was used to determine the reliability of parameter values, assuming a glucose assay coefficient of variation (COV) of 1.5% and an insulin assay COV of 8%. Additional details of the computational approach are provided in Appendix A.

Assays. Plasma glucose concentrations were measured in duplicate using a glucose oxidase method (Beckman Instruments, Palo Alto, CA). Plasma insulin was measured in duplicate using a modification of a double antibody radioimmunoassay (13), with all samples from the same individual measured in a single assay. Interassay COV for glucose and insulin was < 1.5 and < 8.0%, respectively, based on assay quality control statistics.

Statistics. Comparisons between the results from the three protocols were performed with ANOVA, and post-hoc comparisons used the Fisher's protected least significance difference (14). Data are expressed as mean±SE unless otherwise noted.
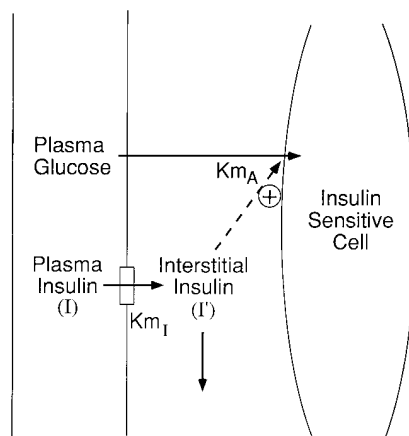

Figure 1. The hypothesized model of insulin transport and action. Saturation is hypothesized to occur at two sites, the first at the transport of insulin from plasma to the interstitial space with half-maximal rate constant K_{mI} and the second at the site of insulin action on cellular glucose uptake with half-maximal rate constant K_{mA}. The dashed arrow represents the control of glucose uptake by interstitial insulin.

Results

The mean age of the subjects was 33 yr, and the average BMI was 33.0 (range 29.7–42.8) kg/m². The characteristics of each subject along with fasting glucose and insulin concentrations are displayed in Table I. As shown in Fig. 2, a gradation in the peak insulin value was obtained between the low-dose infusion (1,167±156 pM), low-dose injection (3,014±384 pM), and high-dose injection (6,596±547 pM) protocols. With the infusion protocol, a gradual increase in insulin concentration was achieved while with the bolus protocols a peak in the insulin concentration was observed within 2 min after injection, followed by a rapid decay. The incremental area under the insulin curve (AUC) from 20 to 100 min was 16,205±3,181 pM·min for low-dose infusion, 18,079±2,556 pM•min for low-dose injection, and 36,298±3,334 pM·min for high-dose injection. The high-dose injection insulin AUC was significantly different

Table I. Subject Characteristics and Fasting Glucose and Immunoreactive Insulin Concentrations

| | | | Fasting glucose | | | Fasting insulin | | | |
			Low inf	Low inj	High inj	Low inf	Low inj	High inj	Study sequence
Subject	Age	BMI							
	yr	*kg/m²*		*mg/dl*			*pm*		
1	34	36.8	96.2	99.3	93.0	178	129	144	2-3-1
2	26	29.7	83.0	85.0	82.7	54	72	58	2-1-3
3	39	30.0	97.7	99.0	99.0	44	54	54	3-1-2
4	41	42.8	93.3	89.0	85.3	80	52	83	1-3-2
5	33	30.9	87.7	92.0	91.5	37	51	97	1-2-3
6	35	30.8	83.2	83.3	94.3	84	82	91	1-2-3
7	20	29.9	85.5	83.3	83.5	80	73	43	3-1-2
Mean	33	33.0	89.5	90.1	89.9	79	73	81	
±SEM	3	1.9	2.3	2.6	2.3	18	10	13	

1. *Low inf*, low-dose insulin infusion (0.025 U/Kg); 2. *Low inj*, low-dose insulin injection (0.025 U/Kg); 3. *High inj*, high-dose insulin injection (0.050 U/Kg).
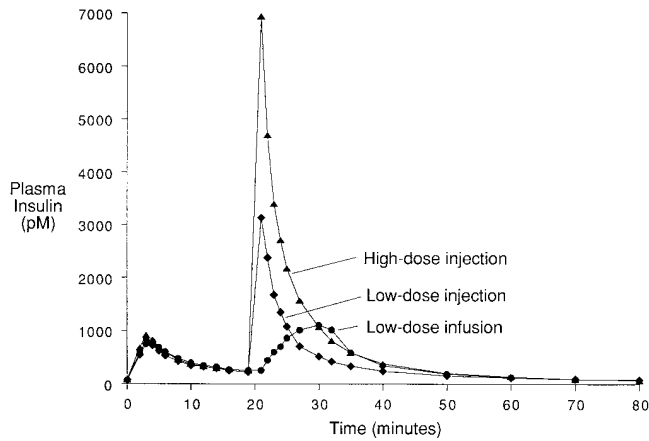
Figure 2. Plasma insulin levels versus time for three insulin adminis-
tration protocols: Low-dose infusion is 0.025 U/Kg over 10 min, low-
dose injection is 0.025 U/Kg given as a bolus, and high-dose injection
is 0.050 U/Kg given as a bolus.

from the other two protocols ($P < 0.0005$), and the insulin
AUC for the two low-dose protocols were similar ($P = 0.37$).

S_I differed significantly with the different protocols ($P = 0.01$, Fig. 3). The means were inversely related to the peak in-
sulin values, with the lowest computed S_I occurring with the
high-dose injection protocol. Since there is no hypothesis to
suggest that a lower S_I could occur with lower insulin concen-
trations, one-tailed tests were used for post-hoc analysis. Sig-
nificant differences were observed between the low-dose infu-
sion and high-dose injection ($P = 0.01$), low-dose injection and
high-dose injection ($P = 0.03$) and low-dose injection and low-
dose infusion ($P = 0.03$).

Since these data are consistent with a saturation of insulin
effect by dose, an estimate of the degree of saturation assum-
ing Michaelis-Menten kinetics was made by determining an
optimum value of the Michaelis-Menten saturation constants.
To do so, a model was used in which insulin is visualized as be-
ing transported from plasma into interstitial space by a satura-
ble mechanism, and/or insulin is assumed to bring about in-
creased glucose uptake into tissues by a saturable mechanism
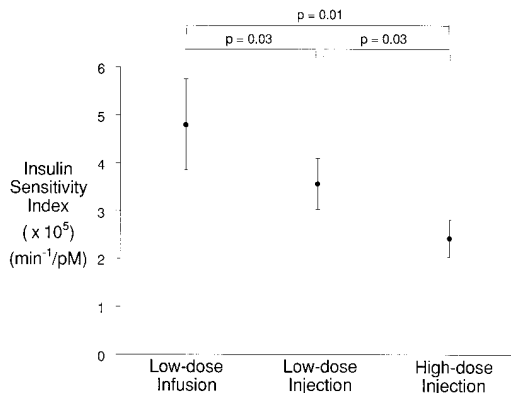(see Fig. 1). Reliable saturation parameters could not be deter-

mined for subject 4; thus, all subsequent results are based on
the results from the six remaining subjects.

For the first analysis, the saturation was assumed to occur
only at the site of insulin action on peripheral tissues; thus, K_{mI}
was fixed at infinity to represent a nonsaturable process. Using
this approach, K_{mA} was determined as 104±21 pM. Bergman
has reviewed data from studies using clamp methodology and
has determined that the half-maximal glucose uptake occurs at
a plasma insulin of 780 pM (15). However, this value must be
corrected for the decreased concentration of insulin in the in-
terstitium. Using the measured steady-state ratio of 0.6 be-
tween interstitial and plasma insulin concentrations (16–18),
one would calculate an apparent K_{mA} of 468 pM for the effect
of insulin at the peripheral tissue site. This measured value is
more than four times the above calculated value of 104 pM us-
ing a single saturable site ($P < 0.0001$) suggesting that this
model does not explain the findings. Based on the data sug-
gesting transport of insulin from plasma to the interstitial com-
partment (4), we next assumed a K_{mA} of 468 pM and computed
the apparent K_{mI} as 807±165 pM.

Parameter coefficient of variation as determined by Monte
Carlo simulation averaged 49% for K_{mI} and 39% for K_{mA}. To
test further the reliability of our results, we performed a sensi-
tivity analysis to evaluate the dependence of K_{mI} on the values
of K_{mA} and the steady-state ratio of plasma to interstitial insu-
lin. With this procedure, the value of K_{mA} is varied between
the limits of values measured by different investigators (15),
and a similar series of substitutions is performed for the
steady-state plasma to interstitium insulin ratio (16–18). Spe-
cifically, K_{mA} was varied between 284 and 515 pM, and the ra-
tio was varied between 0.5 and 1.0. With these alterations, the
mean K_{mI} varied between 761 and 1,046 pM.

The individual results for AIRglucose, K_g, and S_g and
GEZI are shown in Table II. No significant differences were
seen for these parameters. Additionally, since AIRglucose and
K_g would not be expected to be affected by the insulin admin-
istration protocol, and S_g and GEZI would have only small
changes with different insulin administration protocols, the re-
producibility of these values was determined as the COV as
shown in Table III. These data demonstrate that the day-to-
day reproducibility of these measures on three separate occa-
sions ranges from 3.7% for fasting glucose to 28.6% for GEZI.

Discussion

We observed a clear and consistent change in the measured
values of insulin sensitivity obtained from the minimal model
with different insulin administration protocols. One likely ex-
planation for this observation is that there is a saturable pro-
cess related to the insulin effect, since the minimal model will
interpret a saturation-induced decrease in insulin action as a
decrease in insulin sensitivity. The degree of saturation is not
solely related to the amount or time course of insulin adminis-
tration, but instead is dependent on both factors. Thus, the
same dose of insulin, when given quickly, will result in more
saturation than the same dose given over a longer time period
because of the higher insulin levels achieved. Additionally,
when the time course of insulin administration is similar, a
high insulin dose will result in a greater degree of saturation
than a low dose. Thus, if a saturable process is present, the
highest measured S_I would be expected from the low-dose in-
fusion, a lower measured S_I from the low-dose injection proto-

Figure 3. Mean S_I values from three insulin administration protocols.
The mean S_I values obtained from minimal model modeling are
shown for low-dose infusion (0.025 U/Kg infused over 10 min), low-
dose injection (0.025 U/Kg given as a bolus), and high-dose injection
(0.050 U/Kg given as a bolus) protocols.

Table II. IVGTT Results Using Unmodified Minimal Model Equations

Subject	AIRglucose			S_I			S_g			GEZI			K_g		
	Low inf	Low inj	High inj	Low inf	Low inj	High inj	Low inf	Low inj	High inj	Low inf	Low inj	High inj	Low inf	Low inj	High inj
	pM												%/min		
1	1,255	826	992	1.415	2.296	1.166	0.0214	0.0125	0.0220	0.0189	0.0096	0.0203	1.90	1.84	1.83
2	490	457	533	7.726	5.192	3.592	0.0205	0.0266	0.0259	0.0163	0.0228	0.0238	2.35	2.74	1.98
3	233	221	302	3.922	4.329	2.048	0.0164	0.0193	0.0165	0.0147	0.0169	0.0154	1.19	1.63	1.23
4	131	218	239	4.510	3.649	3.745	0.0146	0.0132	0.0206	0.0110	0.0113	0.0175	1.27	1.16	1.27
5	400	455	830	8.484	5.147	1.806	0.0215	0.0094	0.0184	0.0184	0.0068	0.0166	2.37	1.93	1.77
6	369	473	386	2.987	1.541	1.494	0.0137	0.0182	0.0248	0.0113	0.0170	0.0234	1.46	1.53	2.09
7	865	844	716	4.565	2.797	3.113	0.0205	0.0182	0.0172	0.0168	0.0162	0.0159	2.81	2.05	1.97
Mean	535	499	571	4.801	3.564	2.423	0.0184	0.0168	0.0208	0.0153	0.0144	0.0190	1.91	1.84	1.73
±SEM	149	96	107	0.949	0.535	0.395	0.0013	0.0021	0.0014	0.0012	0.0021	0.0013	0.24	0.19	0.13

Low inf, low-dose insulin infusion (0.025 U/Kg); *Low inj*, low-dose insulin injection (0.025 U/Kg); *High inj*, high-dose insulin injection (0.050 U/Kg).

col, and the lowest measured S_I from the high-dose injection protocol.

This expected relationship can be tested three times for each subject by comparison of the measured S_I from the high-dose injection to the low-dose injection and low-dose infusion, and the measured S_I from the low-dose injection to the low-dose infusion. The expected relationship was observed in 17 of the possible 21 comparisons and was found in all subjects when comparing the low-dose infusion to the high-dose injection (Table II). In three comparisons in which the expected relationship was not found, the difference in S_I was < 12%, which is within the day-to-day variation for S_I measurements (17–20%) (19–21). The fourth nonconsistent comparison occurred with subject 1 between the low-dose infusion and low-dose injection study, perhaps because of an unexplained alteration in metabolic status.

There are other possible explanations for our observation. First, rather than saturation of the insulin effect, a nonlinearity in the insulin assay may be present. We do not believe this to be the case, since samples were diluted when necessary to insure that they were measured on a reliable portion of the standard curve. Second, a difference in the measured S_I may be related to a difference between the injection and infusion protocol other than the insulin concentrations achieved. Since we observed a difference of measured S_I using the same injection protocol at high and low dose, it is unlikely that protocol differences per se are the explanation for the differences we observed. Third, a difference in the measured S_I could be re-

Table III. Reproducibility of Parameters Obtained from Three Intravenous Glucose Tolerance Tests in Seven Subjects

	COV
	%
Fasting glucose	3.7
Fasting immunoreactive insulin	26.3
Intravenous glucose tolerance (K_g)	15.5
Acute insulin response to glucose (AIRglucose)	23.0
Glucose effectiveness at basal insulin (S_g)	20.0
Glucose effectiveness at zero insulin (GEZI)	28.6

lated to a change in the rate of decrease of plasma glucose. Again, this seems unlikely because the low-dose infusion and low-dose injection protocols used the same total insulin dose, achieved similar incremental insulin area values, and achieved approximately the same rate of glucose disappearance, but showed a significant decrease in measured S_I during the injection protocol with its higher peak insulin levels. Thus, we believe that the present data are compatible with saturation of insulin effect.

This result has several implications for the use of the minimal model. To minimize the effect of saturation, the lowest insulin dose that allows accurate determination of the parameters should be used. Additionally, the insulin should be given as an infusion over a 10-min period rather than an injection, to avoid the high peak values that are likely to lead to saturation of insulin action. Finally, although we did not perform the tolbutamide protocol IVGTT in the present study, it is of interest to compare the insulin levels obtained from the insulin administration protocols with typical levels observed using the tolbutamide protocol. To estimate these values, we computed the average insulin levels from 169 tolbutamide protocol IVGTTs from our previous study (22). The mean peak insulin concentration from the tolbutamide-modified studies (1,070 pM) was similar to the mean peak insulin concentration from the low-dose insulin infusion protocol (1,167 pM). This suggests that the degree of saturation for the tolbutamide protocol is likely be small; however, this conclusion will require experimental confirmation.

In the modified minimal model we used, two sites were hypothesized to contribute to the saturation effect we observed: The first is the transport of insulin from the plasma into the interstitial space by vascular endothelial insulin transporters (4) by a saturable mechanism, and the second is the effect of interstitial insulin to increase peripheral glucose disposal by a saturable process. The results from the present study show that a single saturable site does not fit the data, but that saturation appears to be present at two sites. The apparent K_{mI} that we derived from the model we suggest is similar to $1/K_d$ of the insulin receptor and supports suggestions that have implicated the insulin receptor in this process (4, 23, 24). Although the K_{mA} is numerically lower than the K_{mI}, suggesting that saturation is more pronounced at the insulin action site, the contribu-

tion of each site to the overall saturation effect during an intravenous glucose tolerance test must consider that the insulin levels obtained in the interstitium are lower than those achieved in the plasma. In fact, further analysis indicates that 65% of the saturation is due to saturation at the plasma to interstitial space site with the low-dose infusion protocol, and it increases to 72% with the high-dose injection protocol (see Appendix B). Independent validation of this new model will require measurement of interstitial insulin concentrations. One approach is the use of microdialysis; preliminary results using this method have been reported by other investigators (25).

Since the various insulin administration protocols that were used would not be expected to affect K_g or AIRglucose and would have small effects on the values of GEZI and S_g, the current study provides an opportunity to evaluate the reproducibility of these measures. The reproducibility reflects the variability from parameter measurement as well as the day-to-day biological variations. The reproducibility in K_g, AIRglucose, and S_g from the present study are equal to or slightly less than values obtained from earlier studies, whereas the COV for GEZI is somewhat higher (19, 21).

In conclusion, use of different insulin doses and protocols during the insulin modified IVGTT leads to the calculation of different values for S_I. This finding is compatible with saturation of insulin transport across the endothelium in addition to saturation of insulin action at the cellular level. The effects of saturation at these sites can be minimized by use of an infusion protocol for insulin administration and by use of the lowest dose of insulin that will permit accurate identification of parameter values.

Appendix A

To allow the incorporation of saturation effects into the minimal model, the equations must be in a form with explicit expression of the interstitial insulin concentration (I'). Additionally, since the degree of saturation depends on the absolute insulin concentration rather than the difference from baseline values, the term ($I-I_b$) that appears in the original minimal model equations must be factored into its component parts. To develop a suitable equation, we define insulin sensitivity relative to interstitial insulin concentration:

$$S_{I'} = \frac{\partial\left(\frac{\partial\dot{G}}{\partial G}\right)}{\partial I'} \tag{A1}$$

Where I' is the interstitial insulin concentration, and $\dot{G} = \frac{dG}{dt}$

Integrating:

$$\int\frac{\partial\left(\frac{\partial\dot{G}}{\partial G}\right)}{\partial I'}\partial I' = \int S_{I'}\,\partial I' \tag{A2}$$

$$\frac{\partial\dot{G}}{\partial G} = S_{I'}I' + C_1 \tag{A3}$$

By definition, glucose effectiveness at zero insulin =

$$\overline{GEZI} = \frac{\partial\dot{G}}{\partial G} = C_1$$

$$\int\frac{\partial\dot{G}}{\partial G}\partial G = \int(S_{I'}I' + \overline{GEZI})\,\partial G \tag{A4}$$

$$\dot{G} = S_{I'}I'\ G + \overline{GEZI}\ G + C_2 \tag{A5}$$

Under baseline conditions $\dot{G} = 0$. Substituting baseline values for I' and G as I'_b and G_b:

$$C_2 = -[S_{I'}I'_bG_b + \overline{GEZI}\ G_b] \tag{A6}$$

Thus:

$$\dot{G} = S_{I'}I'G + \overline{GEZI}\ G - [S_{I'}I'_bG_b + \overline{GEZI}\ G_b] \tag{A7}$$

$$= \overline{GEZI}\,(G - G_b) + S_{I'}\,(I'G - I'_bG_b) \tag{A8}$$

Next, interstitial insulin (I') is expressed as a function of plasma insulin (I) using the one-compartment model described by the original minimal model (7):

$$\frac{\partial I'}{\partial t} = k_2I - p_2I' \tag{A9}$$

Since it is known from in vivo studies that $\frac{I'}{I} = 0.6$, and

$$\frac{\partial I'}{\partial t} = 0 \text{ under steady-state conditions:}$$

$$\frac{I'}{I} = \frac{k_2}{p_2} = 0.6;\ k_2 = 0.6\ p_2 \tag{A10}$$

$$\frac{\partial I'}{\partial t} = 0.6\ p_2I - p_2I' \tag{A11}$$

Equations A8 and A11 define the model and allow identification of $S_{I'}$, an index of interstitial insulin sensitivity. An index of plasma insulin sensitivity is defined in a similar manner:

$$S_I = \frac{\partial\left(\frac{\partial\dot{G}}{\partial G}\right)}{\partial I} \tag{A12}$$

Applying the chain rule:

$$S_I = \frac{\partial\left(\frac{\partial\dot{G}}{\partial G}\right)}{\partial I'}\ \frac{\partial I'}{\partial I} = S_{I'}\frac{\partial I'}{\partial I} \tag{A13}$$

Since $\frac{\partial I'}{\partial I}$ is a time-dependent term, S_I is physiologically relevant only under steady-state conditions. Using the experimental in vivo steady-state relationship $I' = 0.6\ I$:

$$\frac{\partial I'}{\partial I} = 0.6 \tag{A14}$$

From equation A13:

$$S_{I'} = \frac{S_I}{0.6} \tag{A15}$$

Substituting into equation A8:

$$\dot{G} = \overline{GEZI}\,(G - G_b) + \frac{S_I}{0.6}\,(I'G - I'_bG_b) \tag{A16}$$

The plasma S_I incorporates the $S_{I'}$ determined under the dynamic conditions during the IVGTT, and the reduction of insulin concentration between the plasma and interstitium under

steady-state conditions. Although these equations appear to be quite different from the original minimal model equations, they are equivalent. The equivalence of these equations was verified by using both the standard and modified set of minimal model equations to solve the 237 studies that formed the data base from our earlier study (22). The residual sum-of-squares, $\overline{GEZI}$, and S_I were identical in all cases.

Next, it is necessary to incorporate the effect of saturable processes into the modified minimal model equations A11 and A16. In general, the reaction for a receptor-mediated process is given by

$$Ligand + Receptor \rightleftharpoons Complex \tag{A17}$$
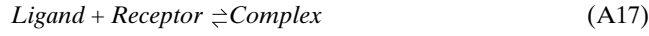
Substituting I as the ligand, R as the insulin receptor, IR as the complex, and K_d as the equilibrium constant:

$$\frac{[\overline{IR}]}{[I][R]} = K_d \tag{A18}$$

The velocity of insulin receptor mediated processes is proportional to $[\overline{IR}]$. If a process is nonsaturable, the unbound receptor concentration $[R]$ would remain constant; that is, there is an unlimited availability of receptors. Under these conditions, the reaction velocity is given by

$$[\overline{IR}] = [I][R]K_d \tag{A19}$$

With a saturable process, the receptor number is decreased for each complex that is formed. Let $[R_0]$ represent the initial receptor number:

$$[\overline{IR}] = [I]([R_0] - [\overline{IR}])K_d \tag{A20}$$

$$= \frac{[R_0][I]}{K_m + [I]} \tag{A21}$$

where $K_m = \dfrac{1}{K_d}$. This is the familiar Michaelis-Menten equation.

The effect of saturation can be expressed as a factor (SF), computed as the ratio between $[IR]$ with saturation (equation A21) and $[IR]$ without the presence of saturation (equation A19):

$$SF = \frac{\dfrac{[R_0][I]}{K_m + [I]}}{\dfrac{[R_0][I]}{K_m}} = \frac{K_m}{K_m + I} \tag{A22}$$

Thus, an insulin concentration multiplied by SF equals the insulin concentration that would have the same effect if saturation did not occur. This allows substitution of:

$$SF \cdot I = \frac{K_m}{K_m + I} \cdot I \tag{A23}$$

into equations A11 and A16 to adjust for the effect of saturation. In equation A16, K_{m_A} is used to represent the Michaelis-Menten constant for the action of insulin on peripheral tissues:

$$\dot{G} = \overline{GEZI}(G - G_b) + \frac{S_I}{0.6}\left(\frac{K_{m_A}}{K_{m_A} + I'}I'G - \frac{K_{m_A}}{K_{m_A} + I'_b}I'_b G_b\right) \tag{A24}$$

A similar substitution is made with equation A11 in which Km_I is used as the Michaelis-Menten constant for the transport of insulin from the plasma to the interstitium:

$$\frac{\partial I'}{\partial t} = 0.6 \ p_2 \ I\frac{Km_I}{Km_I + I} - p_2 I' \tag{A25}$$

Equations A24 and A25 are the equations that are used for the current analysis.

Appendix B

The effect of saturation from both sites can be represented as the product of SF_I, the plasma to interstitium saturation factor, and SF_A, the insulin action saturation factor:

$$SF_{total} = SF_I \cdot SF_A \tag{A26}$$

To determine the proportion due to SF_I, compute the ratio of the natural logarithm of SF_I to SF_{total}:

$$ln(SF_{total}) = ln(SF_I) + ln(SF_A) \tag{A27}$$

$$\% \ due \ to \ SF_I = \%SF_I$$

$$= 100 \cdot \frac{ln(SF_I)}{ln(SF_{total})} = \frac{ln(SF_I)}{ln(SF_I) + ln(SF_A)} \tag{A28}$$

Introduce insulin level as a weighting factor and obtain the average percent of saturation effect due to SF_I over the duration of the IVGTT:

$$Average\%SF_I = \frac{\int \% \ SF_I \ I \ dt}{\int I \ dt} \tag{A29}$$

Equation A29 is evaluated using the trapezoidal rule.

Acknowledgments

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