Comparison of the Minimal Model and the Hyperglycemic Clamp for Measuring Insulin Sensitivity and Acute Insulin Response to Glucose

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Glucose clamp techniques are established methods for assessment of insulin sensitivity and secretion. The minimal model technique (MMT) has been proposed as an alternative approach to the hyperinsulinemic-euglycemic clamp technique for determination of an insulin sensitivity index (ISI), but has not been directly compared with the hyperglycemic clamp for measurement of ISI or insulin secretion. To address this issue, the present study was undertaken to compare determinations of ISI and the acute insulin response to glucose (AIRg) obtained using the MMT with similar measures obtained from a hyperglycemic clamp. Measures for ISI and AIRg obtained from MMT analysis of a tolbutamide-modified frequently sampled intravenous glucose tolerance test (FSIGT) were compared with similar measures obtained from a 3-hour hyperglycemic clamp performed at a plasma glucose level of 10 mmol/L (180 mg/dL). Paired comparisons were performed in 14 women with normal glucose tolerance. Significant positive correlation coefficients were obtained for both ISI ($r = .88$, $P < .001$) and AIRg ($r = .75$, $P < .005$) between the MMT and clamp studies. We conclude that indices for ISI and AIRg obtained with the MMT are highly correlated with those obtained using the hyperglycemic clamp. The MMT is a valid alternative to the hyperglycemic clamp for assessing insulin sensitivity and AIRg.

VARIOUS METHODS are currently used to assess pancreatic β-cell function and sensitivity of peripheral tissues to insulin.[1] Of these methods, glucose clamp studies are considered the "gold standard" against which all other techniques are measured.[2] The euglycemic-hyperinsulinemic clamp is a widely accepted method of measuring an insulin sensitivity index (ISI). Since insulin is infused during the euglycemic clamp study, assessment of pancreatic β-cell function is not possible. However, the hyperglycemic clamp allows for the measurement of both acute and steady-state glucose-stimulated insulin secretion, as well as ISI.[2,3] Values for insulin sensitivity obtained with a hyperglycemic clamp correlate highly with values obtained during a euglycemic clamp ($r = .84$, $P < .0001$).[3]

The minimal model technique (MMT) proposed by Bergman[4] can also be used to assess both insulin sensitivity and secretion. The MMT calculates an ISI from computerized modeling of insulin and glucose responses during a frequently sampled intravenous glucose tolerance test (FSIGT). This method has been proposed as a less labor-intensive alternative to the euglycemic clamp for assessing insulin sensitivity. Strong correlations ($r = .87$, .84, and .78) have been demonstrated for measures of ISI from the MMT as compared with similar measures from euglycemic clamp studies.[5-7] The present study was undertaken to compare measures of ISI and insulin secretion obtained from MMT analysis of data obtained during an FSIGT with those obtained using a hyperglycemic clamp study.

SUBJECTS AND METHODS

Subjects

The study protocol was approved by the Institutional Review Board for Biomedical Research at the University of Pittsburgh. Informed consent was obtained from each subject before study participation. Fourteen women with normal glucose tolerance participating in a study of oral contraceptives were studied before initiation of hormonal therapy. All hormonal therapy was discontinued for at least 3 months before study, and no participant was taking any medication known to interfere with glucose tolerance. Three participants smoked one to 10 cigarettes per day. Pregnancy tests were verified as negative before study in all women.

Nine subjects were lean, and five were obese (defined as body mass index [BMI] > 27 kg/m^2). Seven subjects (four lean and three obese) had clinical evidence of hyperandrogenism (ie, oligomenorrhea, acne, and/or hirsutism).

Comparison studies were performed in random sequence within 30 days of each other. All studies were performed during the follicular phase (days 1 to 10) of the menstrual cycle in seven eumenorrheic women and two hyperandrogenic women who reported regular menstrual cycles. Clamp and FSIGT studies were performed within 6 days of each other in five oligomenorrheic hyperandrogenic women, reducing the likelihood that these studies were conducted at different points in the menstrual cycle.

Anthropometric Measurements

Body fat was measured by bioelectrical impedance analysis Model BIA-103; (R.J.L. Systems, Mt. Clemens, MI).[8] Waist to hip ratios (WHRs) were determined as the ratio of the smallest waist circumference and the largest gluteal circumference.[9]

Experimental Procedures

All studies were performed at the General Clinical Research Center of the University of Pittsburgh Medical Center. Subjects were instructed to follow a weight-maintenance diet containing at least 200 g carbohydrate per day for 2 days before each study. Subjects were admitted to the Research Center at 6:30 AM of the study day after a 12- to 14-hour overnight fast.

FSIGT. An intravenous catheter was placed in an antecubital vein at 7:00 AM and maintained with an infusion of normal saline. A contralateral hand vein was cannulated in a retrograde fashion and placed in a Plexiglas (Rohm & Haas, Philadelphia, PA) thermoregu-

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lated (60° to 65°C) "hot box" for arterialization of venous blood for 30 minutes before initiation of the study. The warmed retrograde hand vein is not usually used for the FSIGT, but was used for this investigation because of the intended comparison with the hyperglycemic clamp, which uses glucose and insulin values from arterialized plasma samples.[2]

Baseline samples for glucose and insulin determinations were obtained at 15-minute intervals for the first 30 minutes. At 8:00 AM, a 50% dextrose infusion (0.3 g/kg) was administered over 1 minute. At 20 minutes after completion of the glucose bolus, tolbutamide (Orinase; Upjohn, Kalamazoo, MI) 5 mg/kg with a maximal dose of 300 mg was administered intravenously. This provocation of endogenous insulin secretion using tolbutamide facilitates measurement of insulin sensitivity using the MMT.[4] Samples for glucose and insulin determinations were obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 120, 150, and 180 minutes after administration of glucose.

Hyperglycemic clamp. Patients were prepared in a similar manner as for the FSIGT, with insertion of an antecubital catheter for the bolus and continuous infusions of glucose during the 3-hour study and retrograde cannulation of a hand vein warmed to 60° to 65°C for repeated drawing of arterialized blood samples. Three baseline samples for glucose and insulin determinations were obtained over a 30-minute period. At 8:00 AM, a bolus infusion of glucose (1.5 mg/kg of 50% dextrose for each desired 1-mg/dL increment in plasma glucose) was administered over 1 minute for rapid achievement of a plasma glucose concentration of 10 mmol/L (180 mg/dL). This level of hyperglycemia was previously used in a comparison study of the hyperglycemic and euglycemic clamps.[3] A variable infusion of 50% glucose was given over the next 3 hours using a Harvard infusion pump (South Natick, MA). Adjustments in the glucose infusion rate (GIR) were made according to determinations of plasma glucose every 5 minutes to maintain the desired concentration of 10 mmol/L (180 mg/dL). Blood samples for glucose and insulin determinations were drawn at 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 minutes after the glucose bolus.

Plasma samples for insulin assay were stored at −20°C until analysis by radioimmunoassay.

Analytic Determinations

Hemoglobin A_{1c} concentrations were determined by high-performance liquid chromatography (BioRad, Hercules, CA). Plasma glucose level was determined using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Insulin concentrations were measured by radioimmunoassay using the double-antibody method (Pharmacia, Fairfield, NJ). Intraassay and interassay coefficients of variation for this assay are 4.8% and 6.5%, respectively. GIR was calculated at 10- to 20-minute intervals for the duration of the clamp.

Acute insulin response to glucose. The acute insulin response to glucose (AIRg) was calculated as the mean incremental insulin response during the first 10 minutes after the glucose bolus minus the mean basal insulin value for both the FSIGT and the hyperglycemic clamp.[10]

Insulin sensitivity. For the FSIGT, an ISI was calculated for each subject using a computerized program (MINMOD, copyright R.N. Bergman). Detailed information including mathematic equations used for MMT determination of ISI have been previously described.[4,11] Briefly, MINMOD uses an iterative computer algorithm to estimate the variables that account for observed glucose values when insulin values are supplied as input.

For the clamp, an ISI was calculated by dividing the average GIR (milligrams per kilogram per minute) by the mean plasma insulin concentration (microunits per milliliter) during the last hour of the clamp and then multiplying by 100.

Statistics

Linear correlation analyses were performed on measurements of AIRg and ISI from the MMT and hyperglycemic clamp. P more than .05 was considered significant. All results are reported as the mean ± SEM unless otherwise indicated.

RESULTS

Subjects

Clinical characteristics of the subjects are listed in Table 1. All subjects demonstrated normal glucose tolerance using criteria established by the National Diabetes Data Group.[12]

FSIGT

Plasma glucose and insulin concentrations measured during the FSIGT are shown in Fig 1. Plasma glucose concentrations increased by 10.7 ± 0.6 mmol/L (193 ± 10 mg/dL) after the bolus of 50% dextrose (range, 6.4 to 14.8 mmol/L [116 to 268 mg/dL]). The mean increment in plasma insulin concentrations after acute induction of hyperglycemia was 540 ± 54 pmol/L, or 90 ± 9 μU/mL (range, 204 to 888 [34 to 148 μU/mL]), and it was 726 ± 73 pmol/L (121 ± 12 μU/mL) after injection of tolbutamide. The mean calculated AIRg was 318 ± 48 pmol/L, or 53 ± 8 μU/mL (range, 108 to 672 [18 to 112 μU/mL]).

The mean calculated ISI from MMT analysis of FSIGTs was $3.4 \pm 0.4 \times 10^{-4} \cdot \text{min}^{-1} \cdot$ μU/mL (range, 1.7 to 7.4).

Hyperglycemic Clamp

Plasma glucose and insulin concentrations during the hyperglycemic clamp are shown in Fig 2. After the glucose bolus at 0 minutes, the mean incremental insulin response was 420 ± 48 pmol/L, or 70 ± 8 μU/mL (range, 156 to 828 [26 to 138 μU/mL]). The calculated AIRg was 300 ± 54 pmol/L, or 50 ± 9 μU/mL (range, 102 to 708 [17 to 118 μU/mL]). The coefficient of variation for plasma glucose values during the last 60 minutes of clamp studies was 3.5% ± 0.2% with a mean glucose concentration of 9.9 ± 0.05 mmol/L (178 ± 1 mg/dL). The mean GIR obtained during this period was 58.3 ± 4.9 μmol/kg/min (10.6 ± 0.9 mg/kg/min). The mean plasma insulin concentration at steady state (Fig 2) was 570 ± 72 pmol/L, or 95 ± 12 μU/mL (range, 174 to 1,020 [29 to 170 μU/mL]). Using a repeated-measures ANOVA, no significant time point

Table 1. Clinical Characteristics of Subjects

Characteristic	Mean ± SEM	Range
Age (yr)	28.9 ± 1.2	21-35
BMI (kg/m²)	26.1 ± 1.9	16.3-42.0
WHR	0.78 ± 0.02	0.70-0.94
Hemoglobin A_{1c} (%)	5.0 ± 0.1	4.5-5.6
Body fat (%)	31.6 ± 1.9	18.8-44.8
Fasting PG (mg/dL)	90 ± 1	82-102
Fasting insulin (μU/mL)	10.8 ± 3	3-38
2-hour PG (mg/dL)	112 ± 6	68-154
2-hour insulin (μU/mL)	73 ± 8	40-128

Abbreviation: PG, plasma glucose.

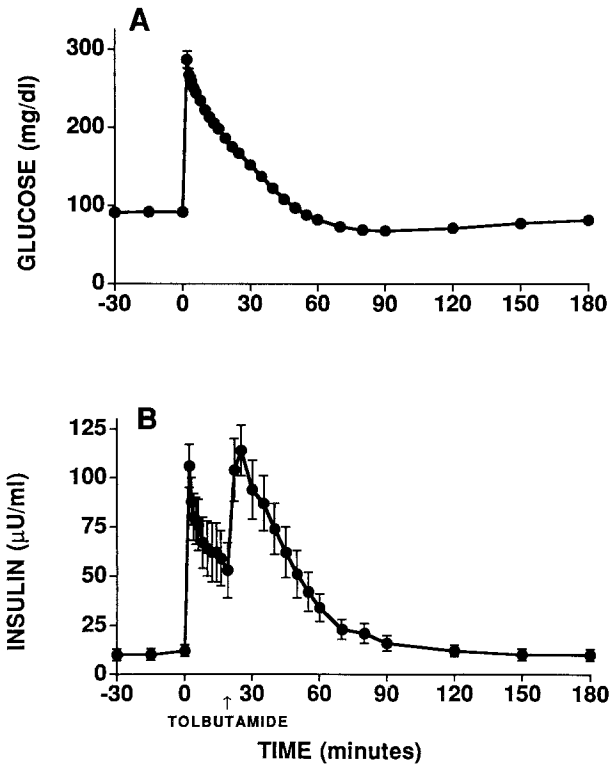

Fig 1. Plasma glucose (A) and insulin (B) responses (mean ± SEM) during a tolbutamide-modified FSIGT.

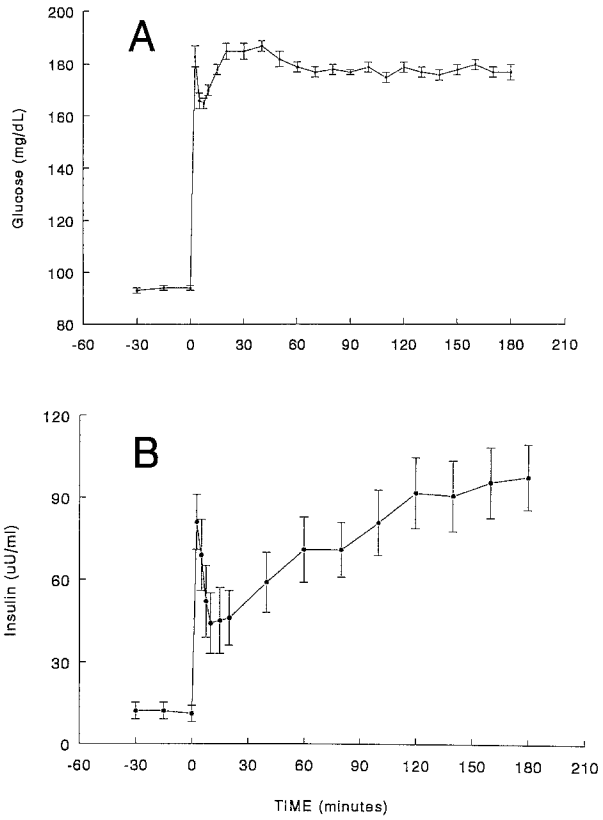

Fig 2. Plasma glucose (A) and insulin (B) levels (mean ± SEM) obtained during hyperglycemic clamp study.

differences were observed in plasma insulin concentrations during the final hour of the clamp study. The mean calculated ISI was 13.6 ± 1.8 mg · kg^{-1} · min^{-1} per μU/mL (range, 4.4 to 23.3).

Clamp v MMT

Measures of AIRg between the MMT and hyperglycemic clamp were highly ($r = .75$) and significantly ($P < .005$) correlated, as were measurements of ISI ($r = .88, P < .001$) (Fig 3). AIRg was inversely correlated with ISI from the MMT ($r = -.47$, $P < .05$) and hyperglycemic clamp ($r = -.85$, $P < .001$). Inverse correlations were noted for BMI, percent body fat, and WHR with both FSIGT- and clamp-derived measures of ISI (Table 2).

DISCUSSION

The present study demonstrates that measurement of insulin sensitivity and first-phase insulin secretion, here defined as AIRg, derived from the MMT are highly and significantly correlated with results obtained from a hyperglycemic clamp. Reductions in peripheral insulin sensitivity and first-phase insulin secretion are early markers for later development of abnormal glucose tolerance in first-degree relatives of individuals with non–insulin-dependent diabetes mellitus (NIDDM).[13] Both the MMT and hyperglycemic clamp allow measurement of insulin action and β-cell function, making these procedures more suitable for studying individuals at high risk for NIDDM than techniques such as the euglycemic clamp that measure only insulin sensitivity. Measurements of ISI from a euglycemic clamp have been demonstrated to be highly correlated with similar measurements from both the MMT[5] and a hyperglycemic clamp.[3]

There are several advantages to the use of the MMT as compared with a clamp study. Personnel conducting the study require less specialized skill in that frequent adjustments of a continuous glucose infusion are not necessary.

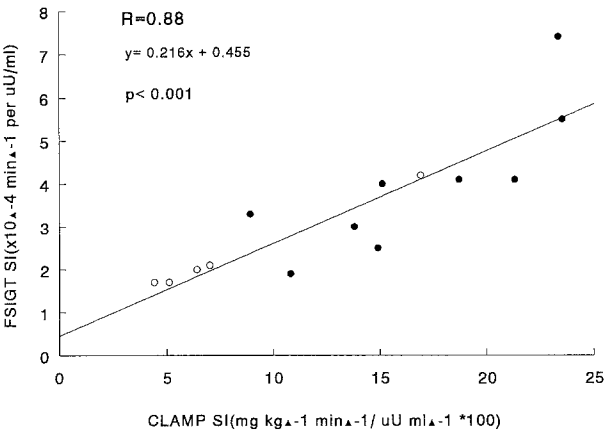

Fig 3. Correlation between ISI determined by MMT and hyperglycemic clamp. (○) Obese subjects; (●) lean subjects (ie, BMI < 27 kg/m²).

Table 2. Correlation Coefficients Between Measures of ISI From FSIGT and Hyperglycemic Clamp and BMI, Percent Body Fat, and WHR

	FSIGT		Clamp	
Parameter	*r*	*P*	*r*	*P*
ISI *v* BMI	−.56	<.01	−.69	<.005
ISI *v* % body fat	−.52	<.05	−.59	<.01
ISI *v* WHR	−.59	<.01	−.81	<.005

Also, glucose measurements can be performed after the study, obviating the need for the presence of a laboratory technician for automated glucose measurements during a clamp. Thus, the MMT represents a more easily applied method of identifying individuals at high risk of developing abnormal glucose tolerance.

An additional advantage of the MMT is the ability to measure the contribution of glucose-mediated glucose disposal, or glucose effectiveness (S_G), to overall glucose tolerance.[4] The mean S_G (0.020 ± 0.01 min^{-1}) in the present study was similar to that previously reported for healthy women[14] and obese nondiabetic individuals.[4] The importance of S_G as a contributor to glucose tolerance was illustrated by a study using MMT analysis of intravenous glucose tolerance tests performed in the offspring of diabetic parents. Subjects with a reduced S_G and low ISI were identified as being at greatest risk for later development of NIDDM.[15-16] Because the hyperglycemic clamp requires glucose concentrations to be held at a fixed concentration while the prevailing insulin concentration changes, there is no specific method of determining the contribution of glucose-mediated glucose disposal using this technique.

To reliably predict individuals who are at risk for abnormal glucose tolerance, the method chosen must be both accurate and reproducible. After introduction of the MMT in 1979 by Bergman et al,[11] several modifications have been introduced to improve both the reproducibility and accuracy of measures of ISI.[5-6,17] These modifications were introduced because of initially observed weak correlations with other accepted methods for measuring ISI, such as the euglycemic clamp procedure.[18,19] Because the MMT relies heavily on the glucose disappearance rate in response to insulin, adequate endogenous insulin secretion is necessary for accurate measurement of an ISI.[4] For this reason, regimens involving augmentation of insulin secretion with intravenous tolbutamide, such as in the present study, were introduced.[5,7] Measures of ISI calculated from tolbutamide-modified protocols correlate to a high degree with values obtained from a euglycemic clamp, resulting in an increased acceptance of the MMT.[5,7] Insulin-modified MMTs have improved the measurement of insulin sensitivity in individuals with relative or absolute insulin deficiency, such as those with NIDDM[20] or insulin-dependent diabetes mellitus.[17] Strong correlations for ISI and S_G have been demonstrated between insulin-modified and tolbutamide-modified FSIGT protocols.[20]

A major limitation of these tolbutamide- and insulin-modified protocols is the inability to measure second-phase insulin secretion or β-cell responsiveness. This limitation has stimulated a renewed interest in the original unmodified FSIGT that involves only an acute bolus administration of glucose.[11] One report suggests that evaluation of two FSIGT data sets from one individual greatly improves the measurement of ISI while still allowing full measurement of first- and second-phase insulin secretion.[21] Although this is helpful, it may not be reasonable to expect large groups of individuals to appear for two separate 3-hour FSIGTs. In one report, a low second-phase insulin response was significantly related to glucose intolerance in lean but not in obese subjects.[22] Because metabolic perturbations leading to NIDDM may differ between lean and obese individuals, the importance of accurately determining second-phase or steady-state insulin secretion as a predictor of NIDDM requires further clarification.

An additional modification to the MMT used in this study was the use of the "hot-hand" technique for arterialization of venous blood. It is possible that the use of this technique altered our results favorably toward a measurement of ISI that was highly correlated with that from the hyperglycemic clamp. However, it is unlikely that our results were altered in either direction, given the fact that measures for AIRg, posttolbutamide incremental insulin response, and ISI from the MMT were similar to those reported by others for nondiabetic individuals.[20] Our measures for ISI using the hyperglycemic clamp are also similar to previously reported values for lean and obese subjects[3]; however, mean steady-state insulin concentrations in this report are greater than those previously reported for normally menstruating women.[23] This is most likely a reflection of the greater degree of obesity in the subjects of this report, in which three subjects were included with a BMI greater than 32 kg/m^2. Also, seven subjects in this study were oligomenorrheic with evidence of hyperandrogenism, a group previously described as hyperinsulinemic.[24]

In summary, we have demonstrated that MMT analysis of a tolbutamide-modified FSIGT permits quantification of an ISI and the acute insulin response to glucose that is highly and significantly correlated with similar measures from a hyperglycemic clamp study.

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