ISTITUTO DI ANALISI DEI SISTEMI ED INFORMATICA

CONSIGLIO NAZIONALE DELLE RICERCHE

Simona Panunzi, Andrea De Gaetano, Geltrude Mingrone

INSULIN SENSITIVITY DETERMINATION FROM THE DISCRETE SINGLE DELAY MODEL

R. 662, May 2007

Simona Panunzi - CNR-IASI BioMatLab, Largo A. Gemelli 8 – 00168 Rome, Italy
simona.panunzi@biomatematica.it

Geltrude Mingrone - Cattedra di Medicina Interna II, Facoltà di Medicina e Chirurgia, Università Cattolica del Sacro Cuore, Roma, Italia
gmingrone@rm.unicatt.it

Andrea De Gaetano - CNR-IASI BioMatLab, Largo A. Gemelli 8 – 00168 Rome, Italy.
andrea.degaetano@biomatematica.it

Collana dei Rapporti dell'Istituto di Analisi dei Sistemi ed Informatica, CNR
Viale Manzoni 30, 00185 ROMA, Italy

tel. ++39-06-77161
fax ++39-06-7716461
email: iasi@iasi.rm.cnr.it
URL: http://www.iasi.rm.cnr.it

Introduction

Insulin Resistance (IR) plays a central role in the development of Type 2 Diabetes Mellitus (T2DM). In fact, IR develops long before diabetes, as it has been described in the relatives of type 2 diabetic patients (1). Further, the metabolic consequences of elevated body mass index (BMI), such as IR, are the critical factors that confer risk for type 2 diabetes (2) or cardiovascular disease associated with fatness (3).

IR is present in a variety of diseases other than T2DM and obesity, including hypertension (4), coronary heart disease (5), chronic renal failure (6), liver cirrhosis (7). Due to the large prevalence of IR in the general population (8) and to its correlation and possibly causative role in many diseases (9), it has become of considerable interest to have an accurate measurement of the degree of IR by tests that are easy to perform and operator-independent. While the Euglycemic Hyperinsulinemic Clamp (EHC) has been long considered as the "golden standard" in clinical research (10), it requires careful training of the operator, and may be harmful for the subjects investigated due to the high levels of insulinemia reached during the test. Moreover, due to the intrinsic laboriousness of this test (the subjects must lie in bed, infusion pumps and continuous bedside measurements of glycemia are required), it cannot be applied to studies involving large patient samples. The Insulin Resistance Atherosclerosis Study (IRAS), for instance, performed on 398 black, 457 Hispanic, and 542 non-Hispanic white, evaluated insulin sensitivity (SI) by the frequently sampled intravenous glucose tolerance test (IVGTT), analyzed by means of the so-called Minimal Model (MM) (11), introduced in the late seventies. This historical approach, however, also suffers from some relevant biases: the same Authors who used the MM recognized that the frequent occurrence of "zero-S_I" values, i.e. of very low point estimates of the insulin sensitivity index, represents a problem, particularly in large clinical studies (12).

Recently, on a series of subjects with BMI < 30 and with fasting glycemia < 7 mM (13), it has been demonstrated that the S_I parameter from the MM was statistically unidentifiable (being estimated as not significantly different from zero) in 50% of the healthy subject population. The possibility to reliably estimate an index of IR is, of course, pivotal in any model aiming at being useful to diabetologists. Part of the problem of the lack of identifiability of the S_I from the MM may reside in the MM being actually overparametrized with respect to the information available from the 23-point IVGTT. Another important element determining this lack of identifiability resides in the parameter estimation strategy suggested by the Authors (14) and commonly followed in applications, i.e. to use interpolated observed insulinemias (obviously affected by experimental error) as the input function in the model for fitting glycemias. This 'decoupling' fitting strategy

produces parameter estimates which optimize the adherence of the model to observed glycemias taking chance variations of insulinemias as the true input: these estimates result, quite understandably, prone to error.

We have recently published a Single Delay Model (SDM) to assess insulin sensitivity after an IVGTT (13), have discussed in detail the effect of avoiding the above sources of error, and have demonstrated the appropriate mathematical behaviour of the model itself (15). The SDM was designed to fit simultaneously both glucose and insulin time courses with a reduced number of parameters (six free parameters overall instead of at least eight), and was shown to provide robust and precise estimates of insulin sensitivity in a sample of non-obese subjects with normal fasting glycemia.

The goal of the present study is to apply the same SDM to a heterogeneous population, composed of overweight, obese and morbidly obese subjects compared with lean individuals, in order to verify the performance of this model with respect to the MM over the entire spectrum of interest for diabetologists assessing insulin resistance.

Methods

Experimental protocol

Data from 74 volunteers (28 males and 46 females, BMI ranging from 18.51 and 62.46, average anthropometric characteristics reported in Table 1), who had been previously studied in several protocols at the Catholic University Department of Metabolic Diseases were analyzed. 19 subjects were lean individuals (BMI below or equal to 24, average 22.40 ± 1.68 SD), 22 were over-weighted (BMI between 24 and 30, average 25.78 ± 1.34), 22 were obese (BMI between 30 and 40, average 34.34 ± 2.74) and 11 were morbidly obese (BMI over 40, average 48.68 ± 6.68). All subjects had negative family and personal histories for Diabetes Mellitus and other endocrine diseases, were on no medications, had no current illness and had maintained a constant body weight for the six months preceding each study.

For the three days preceding the study each subject followed a standard composition diet (55% carbohydrate, 30% fat, 15% protein) ad libitum with at least 250g carbohydrates per day. Written informed consent was obtained in all cases; all original study protocols were conducted according to the Declaration of Helsinki and along the guidelines of the institutional review board of the Catholic University School of Medicine, Rome, Italy.

Each study was performed at 8:00 AM, after an overnight fast, with the subject supine in a quiet room with constant temperature of 22-24 °C. Bilateral polyethylene IV cannulas were inserted into antecubital veins. The standard IVGTT was employed (without either Tolbutamide or insulin injections) (11): at time 0 (0') a 33% glucose solution (0.33 g Glucose / kg Body Weight) was rapidly injected (less than 3 minutes) through one arm line. Blood samples (3 ml each, in lithium heparin) were obtained at -30', -15', 0', 2', 4', 6', 8', 10', 12', 15', 20', 25', 30', 35', 40', 50', 60', 80', 100', 120', 140', 160' and 180' through the contralateral arm vein. Each sample was immediately centrifuged and plasma was separated. Plasma glucose was measured by the glucose oxidase method (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, CA, USA). Plasma insulin was assayed by standard radio immunoassay technique. The plasma levels of glucose and insulin obtained at -30', -15' and 0' were averaged to yield the baseline values referred to 0'.

Seven out of the 74 subjects also underwent a Hyperinsulinemic-Euglycemic glucose Clamp study. They were admitted to the Department of Metabolic Diseases at 6.00 p.m. of the day before the study. At 7:00 a.m. on the following morning, indirect calorimetric monitoring was started; the infusion catheter was inserted into an antecubital vein; the sampling catheter was introduced in the contralateral dorsal hand vein and this hand was kept in a heated box (60°C) to obtain arterialized blood. The glycemia of diabetic patients was maintained below 100 mg/dl by small bolus doses of

short-acting human insulin (Actrapid HM, Novo Nordisk, Denmark) until the beginning of the study. At 9.00 a.m., after 12 to 14 hour overnight fast, the euglycemic hyperinsulinemic glucose clamp was performed as described by De Fronzo et al (16). A priming dose of short-acting human insulin was given during the initial 10 minutes in a logarithmically decreasing way, in order to acutely raise the serum insulin to the desired concentration. Insulin concentration was then maintained approximately constant with a continuous infusion of insulin at an infusion rate of 40 mIU/m^2/minute for 110 minutes.

The SDM model

The schematic diagram of the mathematical model is represented in Figure 1 and its equations are reported below:

$$(1)\quad \frac{dG(t)}{dt} = -K_{xgI}\, I(t) G(t) + \frac{T_{gh}}{V_g}$$

$$(1')\quad G(t) \equiv G_b \;\forall\; t \in (-\infty, 0)\;,\quad G(0) = G_b + G_\Delta,\quad \text{where}\quad G_\Delta = \frac{D_g}{V_g}$$

$$(2)\quad \frac{dI(t)}{dt} = -K_{xi}\, I(t) + \frac{T_{ig\,max}}{V_i} \frac{\left(\frac{G(t-\tau_g)}{G^*}\right)^\gamma}{1 + \left(\frac{G(t-\tau_g)}{G^*}\right)^\gamma}$$

$$(2')\quad I(0) = I_b + I_{\Delta G}\, G_\Delta\,,$$

The meaning of the structural parameters are reported in Table 2. The initial condition $G_b + G_\Delta$ expresses the glucose concentration as the variation with respect to the basal condition, as the consequence of the IV glucose bolus. In the second equation, the second term represents second-phase insulin delivery from the β-cells. Its functional form is consistent with the hypothesis that insulin production is limited, reaching a maximal rate of release T_{igmax}/V_i by way of a Michaelis-Menten dynamics or a sigmoidal shape according to whether the γ value is 1 or greater than 1 respectively. Situations where γ is equal to zero correspond to a lack of response of the pancreas to variations of circulating glucose, while for γ values between zero and 1 the shape of the response resembles a Michaelis-Menten, with a sharper curvature towards the asymptote. The parameter γ expresses therefore the capability of the pancreas to accelerate its insulin secretion in response to progressively increasing blood glucose concentrations. The initial condition $I_b + I_{\Delta G}\, G_\Delta$ represents instead the immediate first-phase response of the pancreas to the sudden increment in glucose plasma concentration. The model was discussed in detail in (13)

From the steady state condition at baseline it follows that:

$$T_{gh} = K_{xgI}\, I_b\, G_b\, V_g \text{ and } T_{ig\,max} = K_{xi}\, I_b\, V_i \left[1+\left(\frac{G_b}{G^*}\right)^{\gamma}\right] \Big/ \left(\frac{G_b}{G^*}\right)^{\gamma}$$

The index of insulin sensitivity is easily derived from this model by applying the same definition as for the Minimal Model (11), i.e.

$$(3)\quad \frac{\partial}{\partial I}\left[-\frac{\partial}{\partial G}\left(\frac{dG}{dt}\right)\right] = \frac{\partial}{\partial I}\left[-\frac{\partial}{\partial G}\left(-K_{xgI}G(t)I(t)+\frac{T_{gh}}{V_g}\right)\right] = K_{xgI}$$

Insulin Sensitivity from the SDM

For each subject the discrete Single Delay Model (13) (block diagram representation in Figure 1) was fitted to glucose and insulin plasma concentrations by Generalized Least Squares (17), in order to obtain individual regression parameters along with an estimate for the glucose and insulin coefficients of variation. All observations on glucose and insulin were considered in the estimation procedure except for the basal levels. Subject coefficients of variation (CV) for glucose and insulin were estimated with phase 2 of the GLS algorithm, whereas single-subject CVs for the model parameter estimates were derived from the corresponding variances, obtained from the diagonal elements of the estimated asymptotic variance-covariance matrix of the GLS estimators. The insulin sensitivity index from the SDM coincides with one of the model structural parameters to be estimated, the K_{xgI} parameter, expressed in the same units of measurement as the MM-derived S_I index (min^{-1} pM^{-1}) (13).

Insulin Sensitivity from the MM

For the MM, fitting was performed by means of a Weighted Least Squares (WLS) estimation procedure, considering as weights the inverses of the squares of the expectations and as coefficients of variation 1.5% for glucose and 7% for insulin (14). Observations on glucose before 8 minutes from the bolus injection, as well as observations on insulin before the first peak were disregarded, as suggested by the proposing Authors (11;18). A BFGS quasi-Newton algorithm was used for all optimizations (19). The insulin sensitivity index was computed as the ratio between the parameters p_3 and p_2 representing the scale factor governing the amplitude of insulin action and the elimination rate constant of the remote insulin compartment from which insulin action is emanated respectively.

Basal insulin sensitivity measurements and HOMA

The HOMA insulin resistance index was computed as the product of the fasting values of glucose, expressed as mM, and insulin, expressed as μIU/mL, divided by the constant 22.5) (20-22). Its reciprocal 1/HOMA-IR (23), represents an insulin sensitivity index.

Statistical analysis

Model fitting was performed using Matlab version 7 (The MathWorks, Inc) whereas statistical analyses were performed using R (version 2.6.1 Copyright (C) 2007 The R Foundation for Statistical Computing). The entire sample composed of 74 subjects was divided into four groups: lean subjects (BMI less or equal to 24), overweight subjects (BMI between 24 and 30), obese (BMI greater than 30 and less or equal to 40) and morbidly obese subjects (BMI greater than 40). For each parameter of the SDM and MM the a-posteriori model identifiability was determined by computing the asymptotic coefficients of variation for the free model parameters: a CV smaller than 52% translates into a standard error of the parameter smaller than 1/1.96 of its corresponding point estimate and into an asymptotic confidence region of the parameter not including zero.

ANOVA one-way analyses were performed to determine if a significant difference arose among the four groups for the variables K_{xgI}, S_I and 1/HOMA-IR.

The different insulin sensitivity indices were correlated using Pearson's r coefficient.

A further comparison was made between the insulin sensitivity (m index) assessed with Euglycemic Hyperinsulinemic Clamp and either of the two model-derived insulin sensitivity indices (K_{xgI} and S_I) on the 7 subjects who underwent both IVGTT and EHC. Given the small number of subjects, both the parametric Pearson's r correlation coefficient and the nonparametric Spearman coefficient were computed. The scatter diagram of the estimates is shown in Figure 7.

Results

SDM and MM fitting

The two models were both able to satisfactorily fit all the available data sets. Figure 2 shows the experimental data of glucose and insulin concentrations as well as the corresponding time course predictions from the SDM for four subjects coming from the four different BMI subgroups. Figure 3 shows the same four subjects fitted with the MM.

The sensitivity index K_{xgI} from the SDM was identifiable (CV < 52%) in 73 out of 74 subjects. For the single subject with the coefficient of variation greater than 52% the estimated K_{xgI} was 2.87 $\times 10^{-4}$ and its CV was equal to 68.83%.

The sensitivity index S_I from the MM was not identifiable (CV ≥ 52%) in 36 subjects out of 74, with coefficients of variation ranging from 52.76% to $2.36\times10^{+9}$ %. In 18 of these subjects the S_I estimates were either suspiciously large (from 3.99 to 890 in 11 subjects) or very small (less than or equal to 1.5×10^{-12}, the so called "zero-S_I", in 7 subjects) . Parameter estimates from the SDM over the 74 subjects are reported in the Appendix.

Comparison between K_{xgI}, S_I and 1/HOMA-IR

The relationship between the three indices was examined by means of the Pearson correlation coefficient. Two situations were examined, either considering the entire 74-subject sample, or considering a reduced sub-sample obtained eliminating those 18 subjects whose S_I values were extreme (11 very large, > 3 ; 7 very small, <= 1.5×10^{-12}).

The correlation between K_{xgI} and 1/HOMA-IR was positive and highly significant both in the whole sample (r=0.565, P<0.001) and in the reduced sub-sample (r=0.572, P<0.001).

The correlation between S_I and 1/HOMA-IR was positive and significant (r = 0.525, P<0.001) only when the reduced sub-sample was considered, whereas in the overall sample no correlation was apparent (r = -0.074, P=0.529).

In the reduced sub-sample, where the extreme-S_I subjects are not considered, correlation between K_{xgI} and S_I was clearly positive and significant (r = 0.864, P<0.001), see figure 4. In this sub-sample, the absolute values also agreed very well (mean $K_{xgI} = 1.07\times10^{-4}$ vs. mean $S_I = 1.01\times10^{-4}$).

The results of a Bland-Altman procedure on K_{xGI} and S_I are reported in Figure 5. Because of the non-uniformity of the variance (the differences between each pair of insulin sensitivity indices depend on the values of the computed indices) on the ordinates, instead of the absolute differences, the logarithms of the ratios are reported. The 95% interval around the average mean is reported along with the plotted values. From an inspection of the graph it can be noted that, in the sub-sample without extreme S_I values, the two methods are equivalent.

Comparison between the four BMI-classes

Table 3 reports the mean values over the two samples (the Full Sample and the Sub-Sample) of the three insulin sensitivity indices in the four BMI-identified classes. The ANOVA analysis resulted significant for 1/HOMA-IR and for K_{xgI} both in the Full Sample (P<0.001 for the K_{xgI} and P=0.002 for the 1/HOMA-IR) and in the Sub-Sample (P<0.001 for the K_{xgI} and P=0.005 for the 1/HOMA-IR), S_I was significantly different in the four groups only when the Sub-Sample is considered (P=0.006), and not significantly different among groups on the full sample (P=0.297). Figure 6

summarizes the comparison between the S_I and K_{xgI} in terms of average values in the four BMI-identified classes.

Comparison with the EHC results

Table 4 reports the values of the insulin sensitivity assessed with Euglycemic Hyperinsulinemic Clamp (M index) , along with the two insulin sensitivity indices, K_{xgI} and S_I, derived from the SDM and the MM respectively. It is first of all to be noticed that all of these seven subjects had good parameter estimates (CV < 52%) for the S_I, which is somewhat lucky for the MM. Figure 7 reports a scatter plot where the two model-derived insulin sensitivity indices (K_{xgI} and S_I on the ordinate) are plotted against the clamp-derived insulin sensitivity M index (on the abscissa). The points show a linear correlation between the two model-derived indices and the M. Given the small number of available subjects, the non parametric Spearman index was computed along with the parametric coefficient of correlation (Pearson's r). When the non parametric correlation is considered the P values are not significant, even if for the K_{xgI} the P value is border line (Spearman's rho = 0.75, P = 0.052 for the correlation K_{xgI}-m; Spearman's rho = 0.571, P = 0.181 for the S_I-m); when the Pearson's r coefficient is computed both correlations result positive and significant (Pearson's r = 0.918, P = 0.004 for the K_{xgI} and Pearson's r = 0.832, P = 0.020 for the S_I). A more thorough study is clearly necessary, studying a larger number of subjects.

Relationship between the AIR and the K_{xgI}.

In order to evaluate the capability of this new model to reproduce the known information and relationships, the Acute Insulin Response (AIR) was computed, as suggested by (24), as the ratio of the difference of estimated initial condition and observed basal insulin ($I_\Delta=I_0-I_b$), over the first order insulin disappearance rate ($AIR= I_\Delta /K_{xi}$); figure 8 reports the scatter plot between the two quantities. A one-way ANOVA test on AIR, with factor the BMI class, resulted significant (P=0.001). The average values in the four classes were: 5666 ± 4053 for BMI ≥ 24, 7519 ± 5077 for 24 < BMI ≤30, 17069 ± 19690 for 30 < BMI ≤ 40 and 22956 ± 15606 for BMI > 40. The disposition index DI (computed as the product between AIR and K_{xgI}) resulted instead not significantly different among the four BMI classes by one-way ANOVA (P=0.718, average values: 0.69 ± 0.32 for BMI ≥ 24, 0.68 ± 0.25 for 24 < BMI ≤30, 0.76 ± 0.44 for 30 < BMI ≤ 40 and 0.61 ± 0.39 for BMI > 40).

A linear regression was also performed in order to evaluate whether the increase in AIR is accompanied with an increase in BMI: the beta coefficient was positive (β=764) and significant (P<0.001).

Discussion

In the quest for simpler and more effective methods to evaluate the degree of sensitivity to insulin in a subject, the Intravenous Glucose Tolerance Test (IVGTT) has been proposed as an alternative to established, but undoubtedly more cumbersome, Euglycemic Hyperinsulinemic Clamp. The IVGTT data, however, need to be interpreted by fitting onto them a suitable mathematical model: in the choice of the model to be applied the possibility of reliable and precisely estimate an index of insulin sensitivity should be a major consideration, together with the physiological plausibility, if the model is to be really useful to the diabetological community.

The aim of the present work is to evaluate a recently published model (the Single Delay Model, SDM), interpreting glucose and insulin concentrations observed during a standard IVGTT, by applying it to a heterogeneous population composed of lean, overweight, obese and morbidly obese subjects. The final goal is to compare the SDM-derived insulin sensitivity index K_{xgI} with the well known S_I from the Minimal Model (MM).

As a general observation, we are aware that sex is unevenly distributed among groups, however it has been shown (Clausen et al JCI 1996) that the insulin sensitivity index does not differ between men and women.

In previous works (15) the SDM, designed to fit simultaneously glucose and insulin concentrations, had already been compared with the Minimal Model and shown to offer a closer approximation of the relevant physiology, more appropriate mathematical behaviour of the solutions and greater statistical robustness. These theoretical advantages over the already demonstrated shortcomings of the MM (25), translated, in a series of healthy volunteers, into a much better precision in estimating insulin sensitivity.

In the present series the comparison between the two indices has been extended to a 74-subjects sample with widely ranging BMI, comparing it with the 1/HOMA-IR and (over a subsample) with the clamp-derived "M" index of insulin sensitivity.

The first result of the present assessment is that while in 50% of the subjects, the MM-derived S_I does not result significantly different from zero, and while several subjects exhibit questionably large or small S_I values, the SDM-derived index of insulin sensitivity, K_{xgI}, exhibits estimates with coefficient of variation less than 52% in every subject except one, whose estimated CV results in any case equal to 69%.

This result points to a marked degree of variability in the estimation of the parameters of the Minimal Model, compared with a very good numerical stability in the corresponding estimation of the SDM parameters. Reasons for this different behaviour have been discussed elsewhere (13), and

can be summarised as a mathematical formulation more respectful of physiological understanding, of a smaller number of free parameters (the SDM is in fact more "minimal" than the Minimal Model) and in the avoidance of the statistically incorrect procedure of assuming interpolated noisy insulin concentrations as the true forcing function for glucose kinetics. The phenomenon of the "zero-SI" is in any case well known (12), but even more important, from a practical viewpoint, is the large fraction of extreme estimates of the SI (24.32% in the present series) and more generally of estimates of SI for which the confidence interval contains the zero, and which therefore no meaningful confidence region can be attributed (50% in the present series).

The second result of the present work is the physiological correctness of the obtained estimates. While in the principle, estimates could be precise but biased, this is in fact not the case for the SDM-derived K_{xgI} index. When excluding the extreme S_I values, the correlation between S_I and K_{xgI} is very high and significant, and the Bland and Altman procedure shows the two measures to be indeed equivalent.

While the SI suffers from the presence of questionable and extreme values, the K_{xgI} correlates uniformly and better than the SI with both the 1/HOMA-IR, and with the clamp-derived M-index (where this could be measured).

Even if this last result seems clear, the limited size of the available sample of subjects who underwent both clamp and IVGTT does represent a limitation of the present study, which should be addressed in the future by applying the SDM to other series of subjects simultaneously studied with both EHC and IVGTT.

The performance of the K_{xgI} has also been tested with regards to its capability of reproducing the well-known existing relationship between insulin resistance and body mass index. This is clearly visible in Table 3, where the considered population has been divided into four BMI subpopulations. Table 3 shows that increasing BMI is accompanied by a gradual decrease in insulin sensitivity, as estimated by either 1/HOMA-IR or K_{xgI} (in the full sample) or by S_I in the reduced sample only. The ANOVA performed on the K_{xgI} and on the inverse Homa index highlight a significant difference of insulin sensitivity among the four classes. This result is obtained both in the total considered population and in the subpopulation obtained eliminating those subjects with extreme estimates of the S_I index. For the latter indeed the ANOVA resulted significant only when the subsample is considered.

It is well known that there is a hyperbolic relationship between early insulin secretion, measured e.g. by the Acute Insulin Response (AIR) index, and insulin action, as expressed by an insulin sensitivity index, which, in the present case, is the model parameter K_{xgI}**.**

This hyperbolic relationship of AIR with insulin sensitivity is well reproduced starting from the obtained SDM parameter estimates. The corresponding graph based on the full sample of SI estimates is not shown given the extreme values which the SI index takes in some subjects. While not offering anything new from the physiological viewpoint, this graph shows that not only the insulin sensitivity index K_{xgI}, but the overall SDM model is stable and meaningful, the AIR index being obtained in this case by the model-estimated I_{Δ}.

The increase in AIR with increasing BMI confirm the current consensus. In nondiabetic subjects, fasting insulin secretion increases with BMI in an approximately linear fashion (26). Similar results are obtained after an oral load of 75 g of glucose, total insulin output over the 2 h following ingestion, in fact, increases in linear proportion to BMI (26).

The observation that no relationship exists between DI and BMI would indicate that in the present series no progression of disease is apparent, in the sense that all subjects, whatever their body composition, seemed adequately compensated.

Conclusion

The new insulin sensitivity index K_{xgI}, derived from a mathematical model that simultaneously fits glucose and insulin concentrations, has been reliably computed on a 74-subject heterogeneous sample with a wide BMI range and its performance has been found better than that of the MM-derived S_I index. The equivalence with the S_I in the sub-sample where extreme S_I values are disregarded, the high correlation with the 1/HOMA-IR in the full sample, and the positive correlation with the clamp-derived M index, demonstrate its inherent correctness. The fact that it can be computed on essentially every single subject with high precision points to its inherent stability. These two properties make the SDM model and its derived insulin sensitivity index KxgI a suitable mathematical model of the analysis of IVGTT.

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Appendix

Parameter Estimation from the SDM over the 74 Subjects.

Subject	Gb	Ib	Vg	Idelta	taug	Kxgl	Kxi	gamma	Tigmax	Tgh
1	4.39	62.47	0.19	57.14	23.00	5.30E-05	0.06	2.52	6.63	0.003
2	4.39	51.70	0.11	42.79	13.00	1.72E-04	0.16	2.17	11.75	0.004
3	4.11	29.37	0.13	20.87	52.00	1.67E-04	0.04	0.00	0.63	0.003
4	4.44	56.58	0.13	90.58	0.00	7.51E-05	0.08	0.00	2.12	0.003
5	4.33	47.39	0.12	47.59	40.00	1.07E-04	0.07	0.81	2.30	0.003
6	5.06	39.20	0.19	34.71	12.00	1.20E-04	0.08	2.40	3.91	0.004
7	4.89	68.64	0.19	37.55	10.00	9.46E-05	0.05	2.32	4.84	0.006
8	4.44	53.63	0.08	53.65	0.00	1.31E-04	0.09	0.00	2.34	0.003
9	4.89	41.07	0.16	39.87	18.00	7.00E-05	0.06	3.45	5.87	0.002
10	5.00	24.96	0.15	14.33	8.00	2.60E-04	0.13	2.74	4.90	0.005
11	5.22	82.80	0.14	38.87	22.00	9.08E-05	0.06	2.64	6.84	0.005
12	4.39	115.10	0.14	78.01	8.00	6.51E-05	0.07	1.86	9.62	0.005
13	4.11	24.00	0.29	38.00	13.00	1.23E-04	0.10	3.26	8.00	0.004
14	3.78	15.83	0.14	25.88	22.00	3.37E-04	0.15	1.43	2.66	0.003
15	3.94	24.00	0.19	45.28	2.00	2.31E-04	0.09	2.05	3.29	0.004
16	4.11	65.97	0.24	70.69	6.00	9.17E-05	0.10	1.72	7.70	0.006
17	4.33	14.62	0.20	30.11	45.00	1.48E-04	0.04	0.00	0.32	0.002
18	4.28	13.80	0.10	29.57	0.00	4.36E-04	0.24	1.53	3.48	0.003
19	4.17	22.64	0.14	86.52	12.00	4.34E-05	0.06	3.82	6.42	0.001
20	4.09	98.00	0.18	91.92	34.00	8.53E-05	0.17	1.94	23.21	0.006
21	4.83	49.98	0.22	76.95	21.00	5.08E-05	0.06	3.89	8.72	0.003
22	4.15	41.73	0.18	23.49	23.00	1.12E-04	0.04	1.97	2.29	0.003
23	4.44	35.94	0.14	46.50	12.00	1.40E-04	0.17	2.21	8.87	0.003
24	4.94	30.80	0.20	43.33	29.00	6.18E-05	0.06	3.65	4.36	0.002
25	4.83	29.40	0.19	48.62	26.00	8.09E-05	0.10	3.08	5.75	0.002
26	4.72	39.90	0.10	64.09	24.00	6.32E-05	0.08	3.15	7.18	0.001
27	4.56	19.25	0.18	44.40	21.00	8.39E-05	0.07	3.64	4.60	0.001
28	4.44	26.25	0.18	41.84	28.00	8.08E-05	0.07	3.03	4.19	0.002
29	4.50	28.70	0.20	49.83	25.00	8.08E-05	0.08	2.57	4.06	0.002
30	4.67	20.30	0.10	26.89	98.61	2.08E-04	0.07	0.00	0.71	0.002
31	5.22	37.80	0.08	26.72	16.00	1.31E-04	0.09	2.19	3.77	0.002

32	5.83	38.40	0.18	20.46	28.00	1.44E-04	0.05	2.82	2.07	0.006
33	5.33	40.20	0.07	11.43	30.00	2.86E-04	0.06	2.38	2.82	0.004
34	4.39	24.60	0.11	18.77	11.00	2.29E-04	0.07	2.20	2.37	0.003
35	4.83	36.00	0.10	19.40	22.00	1.71E-04	0.09	1.82	3.16	0.003
36	3.89	50.40	0.14	20.68	18.00	1.40E-04	0.08	1.24	3.91	0.004
37	3.78	39.60	0.10	22.85	16.00	1.65E-04	0.07	1.99	4.74	0.002
38	3.89	29.40	0.09	44.87	3.00	2.96E-04	0.38	1.63	13.65	0.003
39	3.99	35.00	0.23	23.77	10.00	1.10E-04	0.07	2.49	5.63	0.003
40	5.83	26.40	0.11	23.07	22.00	1.12E-04	0.05	3.49	1.84	0.002
41	4.22	50.78	0.18	46.93	10.00	1.93E-05	0.03	3.90	8.83	0.001
42	4.61	53.50	0.14	34.83	21.00	5.05E-05	0.03	3.14	4.19	0.002
43	4.33	24.36	0.16	51.79	18.00	7.28E-05	0.07	3.38	5.72	0.001
44	4.28	198.68	0.16	68.64	13.00	1.25E-05	0.02	2.75	7.62	0.002
45	4.44	73.28	0.18	52.65	6.00	2.35E-05	0.02	3.73	6.75	0.001
46	5.61	93.67	0.19	32.78	24.00	3.11E-05	0.01	3.21	1.57	0.003
47	4.61	68.63	0.18	39.64	15.00	2.74E-05	0.02	3.70	5.49	0.002
48	5.11	59.03	0.16	23.64	11.00	3.71E-05	0.03	3.37	2.89	0.002
49	4.78	173.54	0.18	44.10	29.00	2.66E-05	0.02	2.40	5.28	0.004
50	4.94	50.25	0.18	12.33	2.00	3.30E-05	0.02	4.79	3.74	0.002
51	5.03	65.14	0.15	60.43	35.00	2.70E-05	0.02	3.83	3.84	0.001
52	5.28	32.14	0.15	31.79	38.00	7.75E-05	0.02	0.00	0.38	0.002
53	4.69	29.70	0.15	77.08	20.00	4.19E-05	0.06	3.87	6.20	0.001
54	4.97	142.16	0.18	13.10	10.00	3.08E-05	0.04	2.15	6.89	0.004
55	4.61	30.29	0.20	60.92	16.00	5.73E-05	0.07	3.46	6.18	0.002
56	4.94	70.20	0.19	2.44	2.00	1.06E-04	0.12	2.36	10.52	0.007
57	4.17	73.80	0.18	53.80	27.00	3.48E-05	0.04	2.62	5.63	0.002
58	4.36	93.60	0.15	47.88	21.00	4.75E-05	0.07	2.03	8.46	0.003
59	3.39	66.00	0.12	92.03	22.00	4.02E-05	0.06	1.70	5.83	0.001
60	4.00	71.40	0.12	33.10	26.16	5.26E-05	0.02	0.00	0.70	0.002
61	4.44	22.20	0.15	34.89	13.00	1.12E-04	0.08	2.61	3.25	0.002
62	3.56	47.40	0.12	23.55	9.00	1.27E-04	0.12	1.30	6.11	0.003
63	4.22	110.60	0.13	58.49	13.00	6.31E-05	0.07	1.99	10.81	0.004
64	4.63	229.95	0.23	23.91	4.00	1.86E-05	0.01	3.27	6.17	0.005
65	4.81	142.10	0.20	162.92	18.44	2.16E-05	0.03	0.00	2.34	0.003
66	4.24	140.70	0.15	103.22	21.00	2.89E-05	0.03	1.83	5.50	0.003

67	4.67	103.25	0.19	38.35	31.00	5.15E-05	0.02	1.57	2.29	0.005
68	4.83	48.00	0.10	69.57	14.00	9.48E-05	0.13	2.96	11.11	0.002
69	3.89	48.00	0.13	19.06	26.00	1.37E-04	0.06	1.14	2.70	0.003
70	4.22	89.40	0.13	72.30	16.00	3.29E-05	0.05	2.15	6.90	0.002
71	4.67	24.60	0.15	13.57	0.01	2.87E-04	0.05	0.00	0.62	0.005
72	3.61	33.00	0.11	9.78	96.68	2.64E-04	0.07	0.00	1.13	0.003
73	3.81	39.90	0.25	24.76	10.00	7.34E-05	0.04	2.29	3.48	0.003
74	3.03	9.80	0.16	32.13	0.00	1.88E-04	0.14	3.78	21.52	0.001

Table 1: Anthropometric characteristics of the subjects studied (mean, standard deviation (SD) and sample size (N))

Class		Age	Height	BW	BMI	Gb	Ib	Gender
<=24	**Mean**	41.74	166.84	62.68	22.40	4.39	33.02	9 M
	SD	18.46	9.77	9.51	1.68	0.58	13.17	10 F
	N	19	19	19	19	19	19	19
>24 & <=30	**Mean**	47.23	165.95	71.27	25.78	4.59	46.11	9 M
	SD	14.84	7.86	8.77	1.34	0.52	26.50	13 F
	N	22	22	22	22	22	22	22
>30 & <=40	**Mean**	49.55	163.00	91.51	34.34	4.32	69.97	8 M
	SD	17.47	8.26	12.37	2.74	0.48	46.44	14 F
	N	22	22	22	22	22	22	22
>=40	**Mean**	40.36	162.00	127.41	48.68	4.81	96.36	2 M
	SD	9.75	8.45	16.22	6.68	0.38	59.68	9 F
	N	11	11	11	11	11	11	11
Total	**Mean**	45.49	164.72	83.43	30.86	4.49	57.31	28 M
	SD	16.18	8.62	24.34	9.35	0.53	42.69	46 F
	N	74	74	74	74	74	74	74

Table 2: Definition of the symbols in the discrete Single Delay Model

Symbol	Units	Definition
t	[min]	time
G(t)	[mM]	glucose plasma concentration at time t
G_b	[mM]	basal (preinjection) plasma glucose concentration
I(t)	[pM]	insulin plasma concentration at time t
I_b	[pM]	basal (preinjection) insulin plasma concentration
K_{xgI}	[min^{-1} pM^{-1}]	net rate of (insulin-dependent) glucose uptake by tissues per pM of plasma insulin concentration
T_{gh}	[mmol min^{-1} $kgBW^{-1}$] is the	net balance of the constant fraction of hepatic glucose output (HGO) and insulin-independent zero-order glucose tissue uptake
V_g	[L $kgBW^{-1}$]	apparent distribution volume for glucose
D_g	[mmol $kgBW^{-1}$]	administered intravenous dose of glucose at time 0
G_Δ	[mM]	theoretical increase in plasma glucose concentration over basal glucose concentration at time zero, after the instantaneous administration and distribution of the I.V. glucose bolus
K_{xi}	[min^{-1}]	apparent first-order disappearance rate constant for insulin
T_{igmax}	[pmol min^{-1} $kgBW^{-1}$]	maximal rate of second-phase insulin release; at a glycemia equal to G^* there corresponds an insulin secretion equal to $T_{igmax}/2$
V_i	[L $kgBW^{-1}$]	apparent distribution volume for insulin
τ_g	[min]	apparent delay with which the pancreas changes secondary insulin release in response to varying plasma glucose concentrations
γ	[#]	progressivity with which the pancreas reacts to circulating glucose concentrations. If γ were zero, the pancreas would not react to circulating glucose; if γ were 1, the pancreas would respond according to a Michaelis-Menten dynamics, with G^* mM as the glucose concentration of half-maximal insulin secretion; if γ were greater than 1, the pancreas would respond according to a sigmoidal function, more and more sharply increasing as γ grows larger and larger
$I_{\Delta G}$	[pM mM^{-1}]	first-phase insulin concentration increase per mM increase in glucose concentration at time zero due to the injected bolus
G^*	[mM]	glycemia at which the insulin secretion rate is half of its maximum

Table 3: Descriptives of the 1/HOMA-IR and of the two insulin-sensitivity indices K_{xgI} and S_I in the Full Sample and in the Sub-sample

		Full Sample			Sub-Sample		
		1\HOMA_IR	K_{xgI}	S_I	1\HOMA_IR	K_{xgI}	S_I
≤ 24	Mean	1.41	1.62E-04	47.211	1.50	1.56E-04	1.42E-04
	Std. Deviation	1.07	9.32E-05	205.786	1.14	9.57E-05	8.92E-05
	Std. Error of Mean	0.25	2.14E-05	47.211	0.29	2.39E-05	2.23E-05
	N	19	19	19	16	16	16
> 24 & ≤ 30	Mean	1.01	1.25E-04	13.77	1.04	1.27E-04	1.10E-04
	Std. Deviation	0.62	7.61E-05	64.60	0.63	7.76E-05	6.28E-05
	Std. Error of Mean	0.13	1.62E-05	13.77	0.14	1.69E-05	1.37E-05
	N	22	22	22	21	21	21
> 30 & ≤ 40	Mean	0.77	8.35E-05	101.31	0.63	5.31E-05	7.48E-05
	Std. Deviation	0.44	7.08E-05	246.92	0.44	2.83E-05	7.84E-05
	Std. Error of Mean	0.09	1.51E-05	52.64	0.13	8.53E-06	2.36E-05
	N	22	22	22	11	11	11
> 40	Mean	0.44	2.82E-05	139.80	0.42	2.78E-05	3.60E-05
	Std. Deviation	0.20	9.46E-06	270.87	0.19	1.02E-05	1.35E-05
	Std. Error of Mean	0.06	2.85E-06	81.67	0.07	3.61E-06	4.79E-06
	N	11	11	11	8	8	8
Total	Mean	0.96	1.08E-04	67.12	1.00	1.07E-04	1.01E-04
	Std. Deviation	0.75	8.52E-05	203.33	0.83	8.48E-05	7.75E-05
	Std. Error of Mean	0.09	9.90E-06	23.64	0.11	1.13E-05	1.04E-05
	N	74	74	74	56	56	56

Table 4: values of the insulin sensitivity (M index) assessed with Euglycemic Hyperinsulinemic Clamp (EHC), along with the two insulin sensitivity indices, K_{xgI} and S_I, derived from the SDM and the MM respectively.

Subject	m	K_{xgI}	S_I	BMI
1	2.76	6.31E-05	9.70E-05	35.55
2	1.81	1.86E-05	2.10E-05	49.33
3	3.28	2.16E-05	1.30E-05	39.79
4	1.41	2.89E-05	3.40E-05	34.89
5	4.74	7.34E-05	5.99E-05	33.32
6	4.19	5.15E-05	5.80E-05	38.57
7	7.89	1.88E-04	0.000201	18.51

Figure 1: Block diagram of the SDM model. The model consists of two compartment: the glucose plasma concentrations and the insulin plasma concentrations. Elimination of glucose from plasma occurs dependently from plasma insulin concentrations.

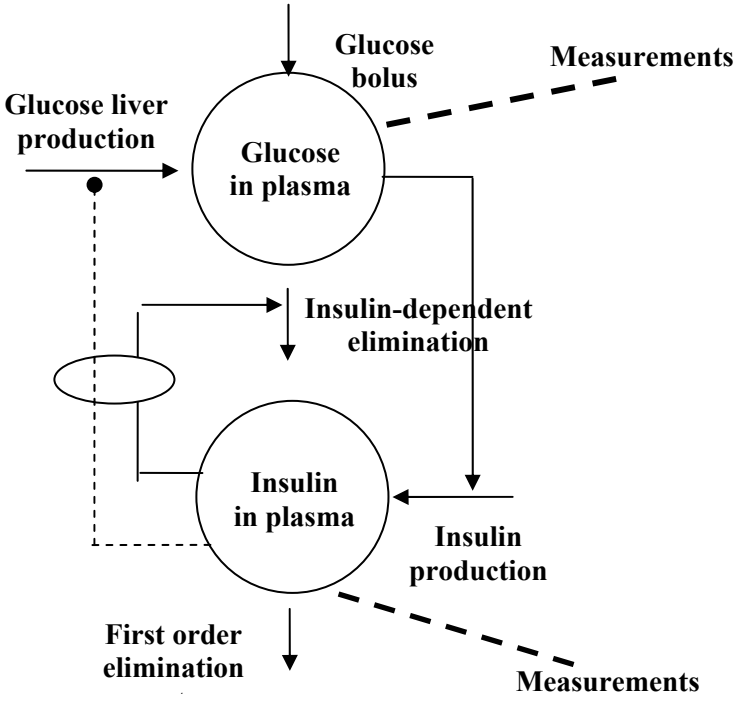

Figure 2: Glucose and Insulin observed concentrations (circles) along with their SDM time predictions (continuous line) for four subjects belonging to different BMI classes.

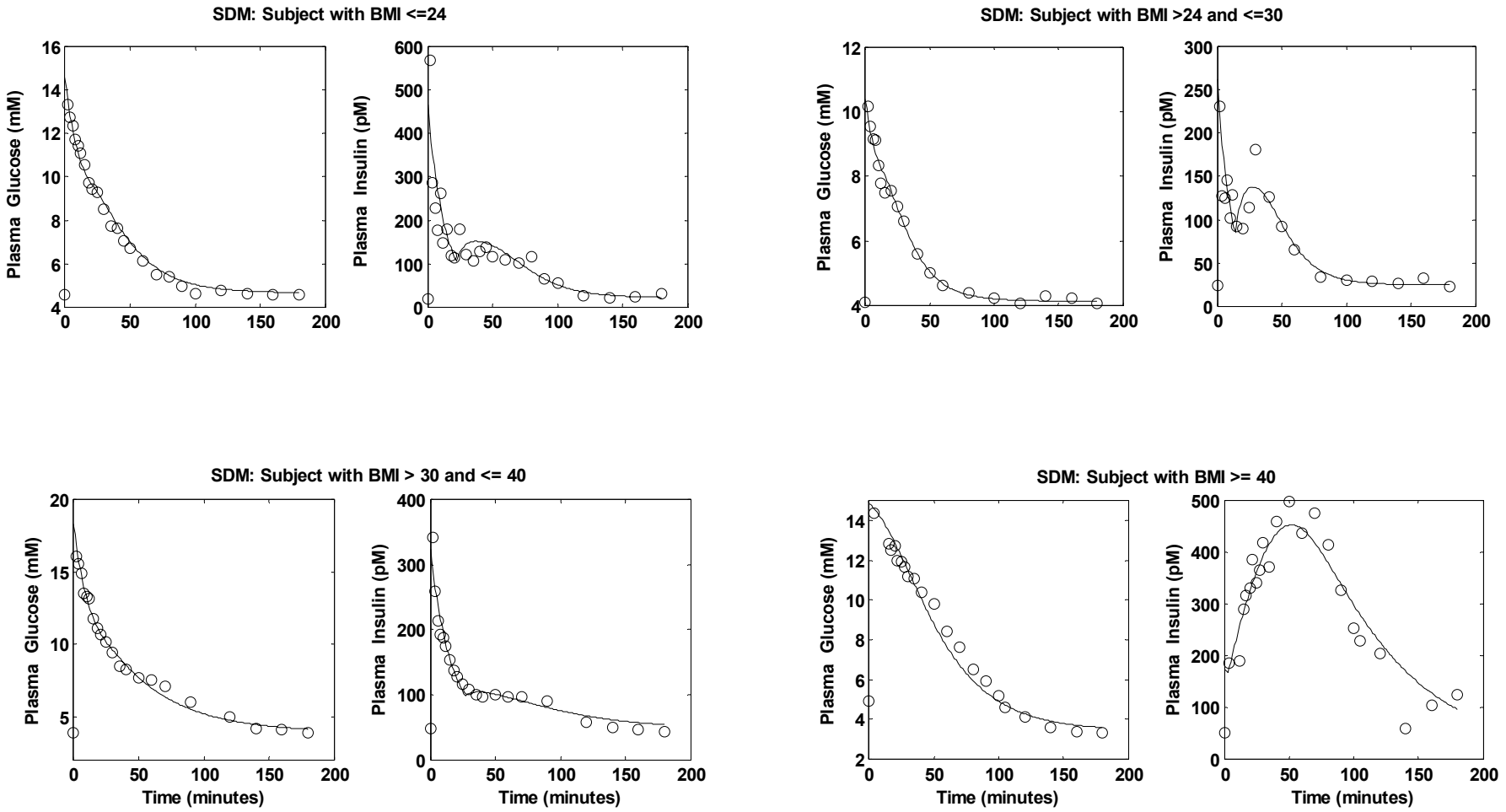

Figure 3. Glucose and Insulin observed concentrations (circles) along with the Minimal Model glucose time predictions and interpolated insulin observations (continuous line) for four subjects belonging to different BMI classes.

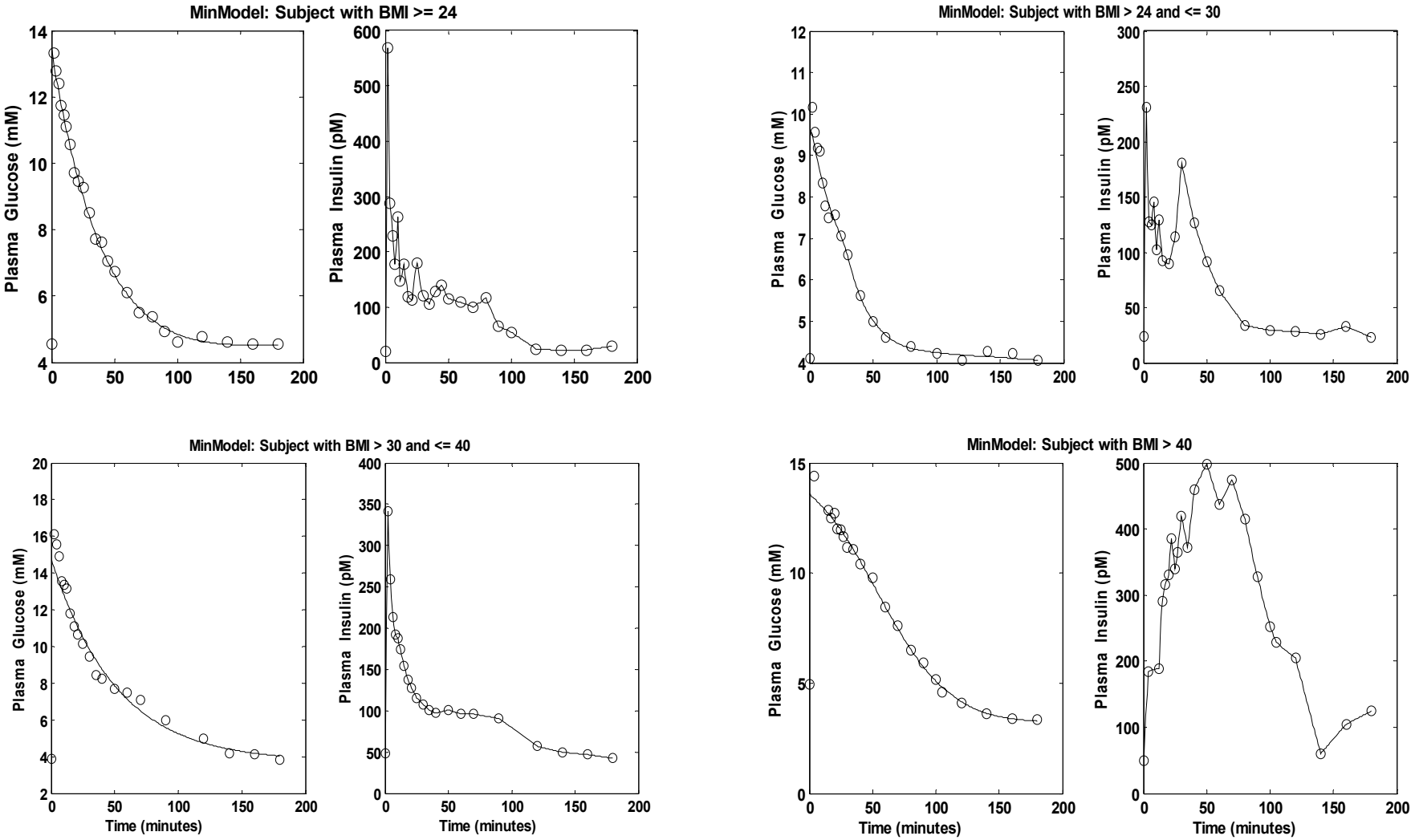

Figure 4: Scatter plot of the two Insulin Sensitivity Indices from the SDM (K_{xgl})and from the MM (S_I)on the sub-sample obtained eliminating the 18 extreme-S_I subjects.

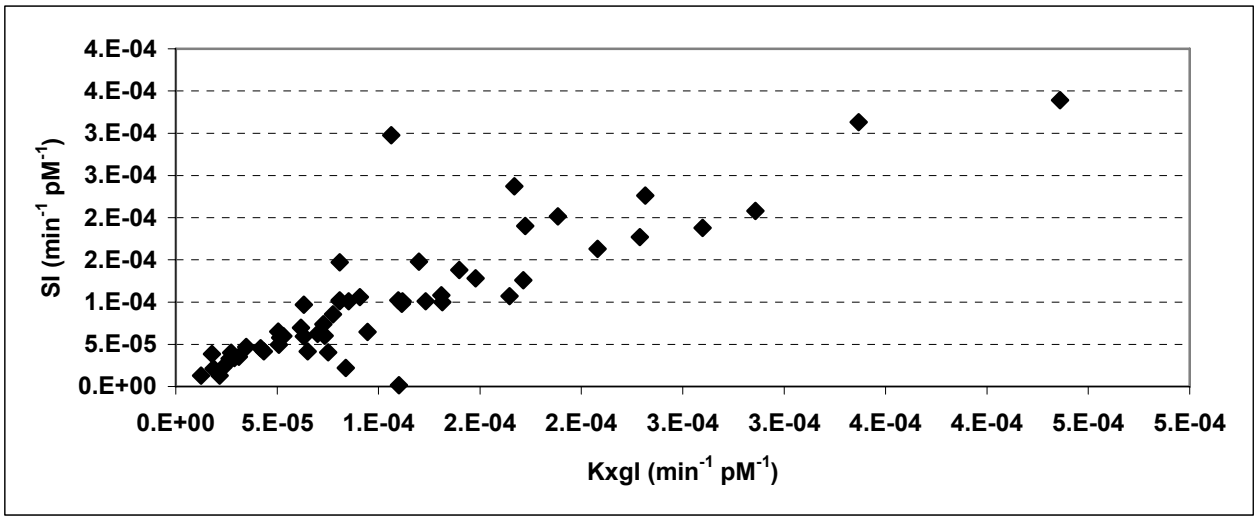

Figure 5: Bland-Altman Procedure: on the abscissas are reported the averages of each pair of Insulin Sensitivity Indices (one from the SDM K_{xgI} and one from the MM S_I) on the sub-sample obtained eliminating the 18 extreme-S_I subjects; on the ordinates the logarithms of the ratios between the K_{xgI} and the S_I are reported.

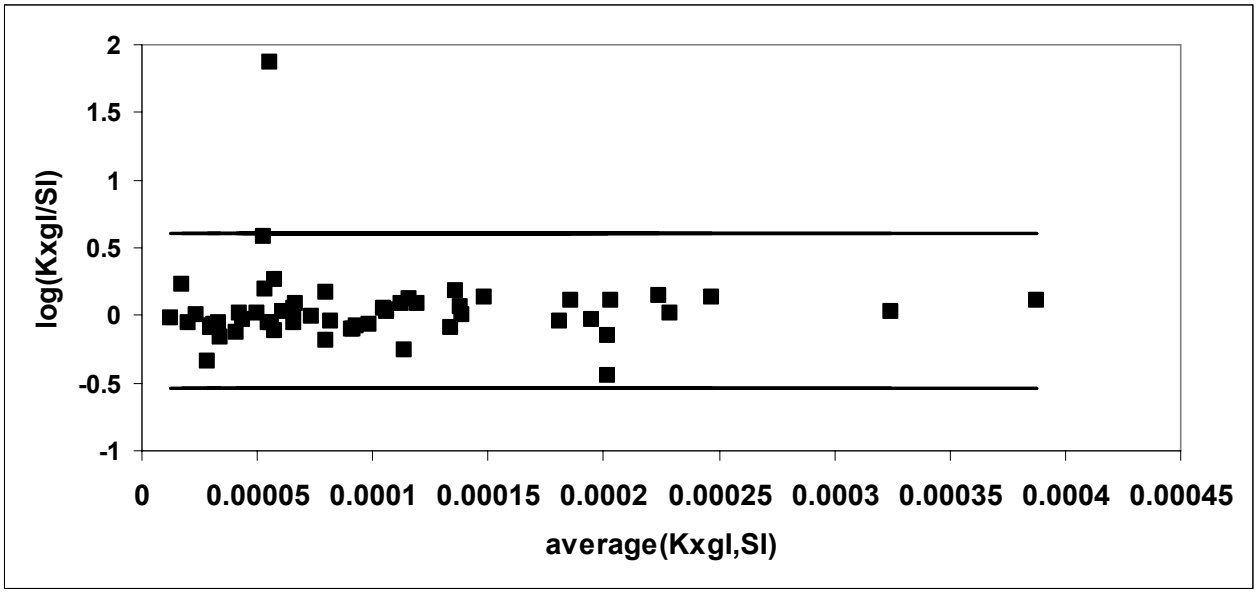

Figure 6: Mean values and Standard Errors for the Insulin Sensitivity Indices from the SDM (K_{xgI}(ALL) and K_{xgI}(SS) and from the MM S_I(SS)). The term SS refers to the sub-sample obtained eliminating the 18 extreme-S_I subjects. The average values of the S_I index over the full sample were out of scale for all four groups and could not be plotted.

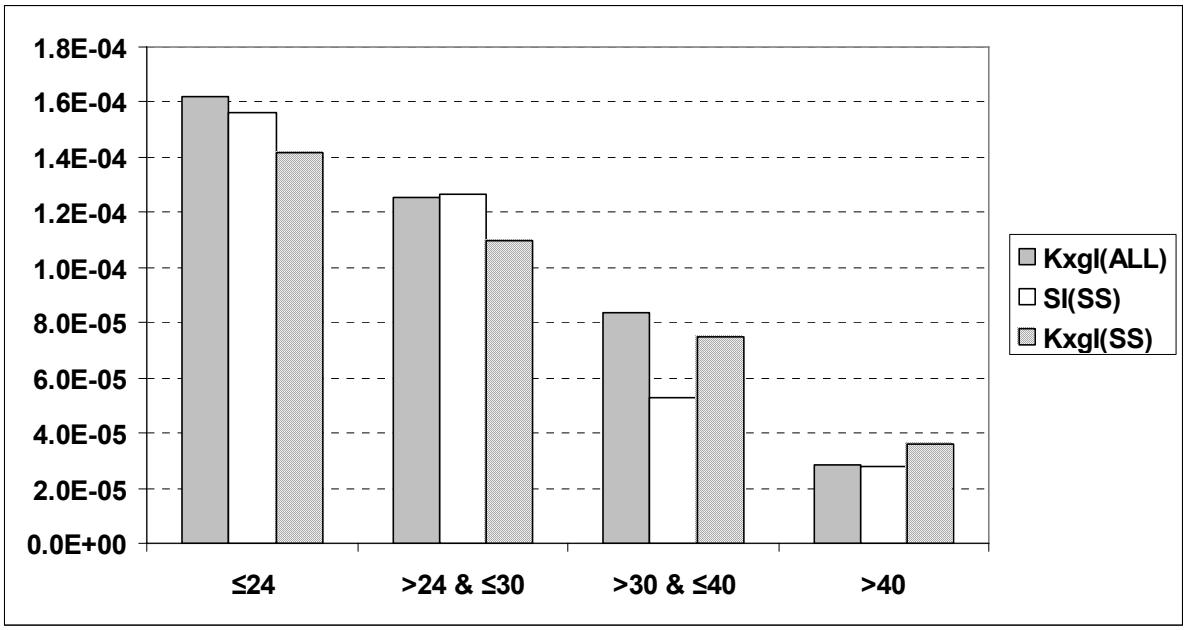

Figure 7. Scatter plot of the two Insulin Sensitivity Indices (K_{xgI} and S_I) versus the m clamp derived index of insulin sensitivity in seven subjects underwent both IVGTT and Clamp.

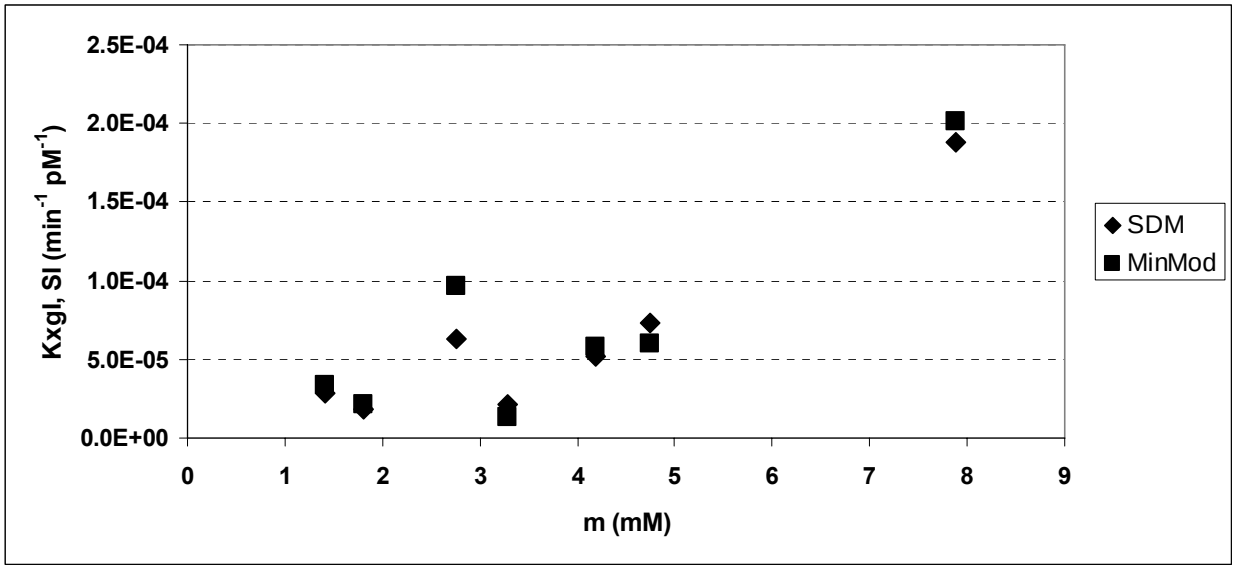

Figure 8. Relationship between the Insulin Sensitivity Indices (K_{xgl}) and the Acute Insulin Response (AIR) in the 74 subjects.