Monte Carlo analysis of a new model-based method for insulin sensitivity testing

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**A B S T R A C T**

Insulin resistance (IR), or low insulin sensitivity, is a major risk factor in the pathogenesis of type 2 diabetes and cardiovascular disease. A simple, high resolution assessment of IR would enable earlier diagnosis and more accurate monitoring of intervention effects. Current assessments are either too intensive for clinical settings (Euglycaemic Clamp, IVGTT) or have too low resolution (HOMA, fasting glucose/insulin). Based on high correlation of a model-based measure of insulin sensitivity and the clamp, a novel, clinically useful test protocol is designed with: physiological dosing, short duration (<1 h), simple protocol, low cost and high repeatability. Accuracy and repeatability are assessed with Monte Carlo analysis on a virtual clamp cohort (N = 146). Insulin sensitivity as measured by this test has a coefficient of variation (CV) of CVSI = 4.5% (90% CI: 3.8–5.7%), slightly higher than clamp ISI (CVSI = 3.3% (90% CI: 3.0–4.0%)) and significantly lower than HOMA (CVHOMA = 10.0% (90% CI: 9.1–10.8%)). Correlation to glucose and unit normalised ISI is r = 0.98 (90% CI: 0.97–0.98). The proposed protocol is simple, cost effective, repeatable and highly correlated to the gold-standard clamp.

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**1. Introduction**

A major risk factor in the pathogenesis of type 2 diabetes and cardiovascular disease is insulin resistance (IR) [1]. IR, or low insulin sensitivity, is defined as a reduced ability of the cells to utilise insulin to take up glucose as energy. The pancreas tries to compensate for increasing IR by increasing its insulin production, often leading to an exhaustion of the insulin producing beta-cells, resulting in impaired fasting glucose (IFG). An early diagnosis of IR can enable early intervention and delay the onset of diabetes, thus greatly reducing the effects and cost of further complications.

The ability to sensitively and accurately identify individuals with IR is critical for the implementation and assessment of intervention programmes in high risk groups. To diagnose IR in population studies and to be applicable in clinical settings, a test has to be simple and cost effective. However, it must also be accurate enough to assess small changes in IR or the progression of treatment.

The euglycaemic-hyperinsulinaemic clamp (clamp) [2] is the gold-standard to assess insulin sensitivity. However, it is clinically very intense and thus not practicable for large populations [3,4] or typical clinical use. Hence, simpler methods have emerged, such as the insulin tolerance test (ITT)
[5], the intravenous glucose tolerance test (IVGTT) [6–8] and the oral glucose tolerance test (OGTT) [9–11]. The intravenous tests mostly use the minimal model of glucose kinetics to quantify insulin resistance [12], whereas the OGTT has been analysed with a variety of approaches [10,13]. All these tests rely on a steady state glucose concentration in the end to avoid unmodelled feedback dynamics in insulin and glucose after a perturbation, either through insulin and glucose infusions, bolus injections or oral glucose loads. They require 2–4 h to perform. The model-based methods require frequent sampling of glucose and insulin.

Simple, fasting assessments include Homeostasis Model Assessment (HOMA) [14] and Quantitative Insulin Sensitivity Check Index (QUICKI) [15]. These metrics require only one fasting sample of glucose and insulin, but are less accurate and repeatable due to varying fasting conditions, i.e. a pulsatile insulin secretion pattern [16], natural variability in fasting glucose or simply assay errors [17,18]. To be a useful clinical tool for repeated and easy use, these measures must correlate well with the gold-standard clamp test. To date, they have not achieved enough resolution to accurately assess a wide range in insulin sensitivity, limiting their application to a rough initial estimation of at-risk populations.

However, they are the primary avenue for diagnosis of type 2 diabetes and IFG. As a result of their low resolution the diagnosis can occur very late in the aetiology of the disease, and after some physiological damage has occurred [19,20]. Combined with incomplete compliance with regular medical checks, many individuals in at-risk groups can go undiagnosed for several years [21,20].

This research engineers a new model-based insulin sensitivity test, relying on the dynamic state after a low-dose glucose and insulin input. During this transient state, the metabolic system model employed has been shown to be highly correlated ($r = 0.97$) to the clamp test [22,23]. The goal is a highly accurate, short and cost effective test that is useful in a clinical setting and overcomes the limitations of currently used metrics.

The main performance criteria for this test are thus accuracy and repeatability. To assess these criteria, a Monte Carlo analysis is performed on test simulations, taking into account significant errors. These errors include: errors in laboratory assays, dilution of input solutions, timing of samples, and errors due to unmodelled dynamics. These latter errors could include poorly or unmodelled endogenous glucose production (EGP) and first pass hepatic insulin extraction. The simulations are based on model-based insulin sensitivity values obtained from fitting a cohort of 146 euglycaemic-hyperinsulinaemic clamp tests, covering a range of metabolic responses. Rather than assessing clinical/physiological validity of the estimated insulin sensitivity, this study aims at validating the robustness of the proposed test in a noisy clinical test environment.

2. Methodology

2.1. Procedure

The simulation procedure used in this study is shown in the schematic in Fig. 1, and explained in more detail in the following sections:

1. Insulin sensitivity from 146 euglycaemic-hyperinsulinaemic clamp tests [24] is calculated from the test data (ISI).
2. A virtual cohort is created by fitting the metabolic model to the clamp tests, resulting in a model-based insulin sensitivity for each subject ($S_i$).
3. Monte Carlo simulations on the proposed test protocol are run on the virtual cohort by adding random noise from published error levels to measurements and inputs, and accounting for potentially unmodelled regulatory dynamics.
4. The metabolic model is fit to the simulated test profiles (glucose, insulin and C-peptide concentrations), resulting in insulin sensitivity from the proposed low intensity test ($S_{MC}$).
5. Performance of the method is assessed by the coefficient of variation ($CV = S.D./\text{mean}$) of $S_{MC}$ and by correlating $S_{MC}$ with ISI and $S_i$. Additional comparisons are made to HOMA.

2.2. Test protocol

The test is designed to be a short dynamic test to assess insulin sensitivity from a metabolic system model fit to the transient plasma glucose and insulin curves after intravenous (IV) bolus injections of glucose and insulin. The model then relates interstitial insulin to plasma glucose to determine the subject’s sensitivity to insulin. The protocol has to account for a wide variety of individuals (lean, obese, insulin resistant, diabetic) and be short, robust and simple enough to be applicable in a clinical setting. The dosing should be lower than in an IVGTT to assess a more physiological state and to minimise regulatory responses, such as suppression of endogenous glucose production (EGP) and pancreatic insulin secretion.

The protocol used in this development study results in metabolic dose–response curves as shown for glucose and insulin in the example in Fig. 2. It is 55 min long and includes:

1. Inject a fixed dose of glucose (5, 10 or 20 g) at 0 min.
2. Inject a fixed dose of insulin (0.5, 1 or 2 U) at 10 min.
2.3. System model

A simpler form of the glucose–insulin metabolic system model shown in Fig. 3 has been presented previously and validated on a wide range of subjects, namely on retrospective intensive care unit (ICU) data [26], in glycaemic control trials in critical care [27–30] and on euglycaemic-hyperinsulinaemic clamp and IVGTT data on healthy, insulin resistant and type 2 diabetes subjects [22,31]. To account for metabolic differences between critically ill and healthy subjects, a time-varying insulin sensitivity parameter was employed in validations on critically ill subjects. On non-ICU populations, this correction was not necessary and the model was able to accurately account for all dynamics [22,31]

The glucose–insulin pharmacodynamic model is derived from the Minimal Model by Bergman et al. [6]. This model is further enhanced by glucose clearance saturation dynamics in the form of a Michaelis–Menten equation [32,33].

The two compartment insulin pharmacokinetics model used here is the primary enhancement from the original system model. It is derived from earlier studies by Sherwin et al. [34]. The accessible central compartment can be understood as plasma plus fast exchanging tissues. The peripheral compartment represents interstitial fluid. The model accounts for the major losses of insulin from the central compartment by the liver and the kidneys and the loss out of the peripheral compartment, mainly insulin binding and eventual degradation by the cells. Transport between the compartments is assumed to be bi-directional diffusion. The resulting system model is defined by the following equations [31]:

\[
\dot{G} = -p_c G - S_i(G + G_e) + \frac{Q}{1 + \alpha_e Q} + \frac{P}{V_G} + EGP, \quad G(0) = 0
\]

\[
\dot{Q} = -n_c Q + \frac{m}{V_Q} (I - Q), \quad Q(0) = \frac{3}{5} I_E
\]

\[
I = -n_k I - \frac{n_l I}{1 + \alpha_I} - \frac{n_l I}{V_P} (I - Q) + \frac{u_{ex}}{V_P} + (1 - x) \frac{u_{en}}{V_P}, \quad I(0) = I_E
\]

where \(G\) is the concentration of plasma glucose above equilibrium level \(G_e\) (mmol\(1^{-1}\)); \(I\) the concentration of plasma insulin (mU\(1^{-1}\)); \(Q\) the concentration of insulin in interstitial fluid (mU\(1^{-1}\)); \(G_e\) the equilibrium (fasting) plasma glucose concentration (mmol\(1^{-1}\)); \(I_E\) the equilibrium (fasting) plasma insulin concentration (mU\(1^{-1}\)); \(u_{ex}\), \(u_{en}\) the exogenous, endogenous
insulin input rate (mU min\(^{-1}\)); \(P\) the exogenous glucose input rate (mmol min\(^{-1}\)); \(EGP\) the endogenous glucose production rate (mmol l\(^{-1}\) min\(^{-1}\)); \(P_0\) the clearance rate of plasma glucose at basal insulin (min\(^{-1}\)); \(S_I\) the insulin sensitivity (l mU\(^{-1}\) min\(^{-1}\)); \(V_p\) the plasma volume (+Fast exchanging tissues) (l); \(V_Q\) the interstitial fluid volume (l); \(V_G\) the glucose distribution volume (l); \(x\) the fractional first pass hepatic insulin extraction (decimal % value); \(n_k\) the kidney clearance rate of insulin from plasma (min\(^{-1}\)); \(n_l\) the liver clearance rate of insulin from plasma (min\(^{-1}\)); \(n_i\) the diffusion constant of insulin between compartments (l min\(^{-1}\)); \(n_c\) the cellular insulin clearance rate from interstitium (min\(^{-1}\)); \(\alpha_l\) the Michaelis–Menten parameter for liver clearance rate saturation (l mU\(^{-1}\)); \(\alpha_G\) is the Michaelis–Menten parameter for insulin-stimulated glucose clearance saturation (l mU\(^{-1}\)).

### 2.4. Parameter fitting and identification

The parameters are identified a priori where possible and using an integral based fitting method for patient specific parameters, as described in refs. [26,22]. In the insulin model parameters \(V_p\), \(V_Q\), \(n_k\), \(n_c\) are assumed to be identical to corresponding values for C-peptide, due to the similar molecular weight of insulin (5800 Da [35]) and C-peptide (3600 Da [35]) and their similar passive properties. The parameters are taken from a well validated population model of C-peptide kinetics [36]. Variable \(n_c\) is calculated to achieve a steady state concentration ratio of \(I/Q = 5/3\) [37–39], and \(\alpha_l = 0.0017\) is a mean population value from [40,26].

In the glucose model, parameter \(P_0 = 0.01\) is fixed to an approximate population value [40,26]. Note that \(P_0\) can be estimated on some data sets, as in clinical glycaemic control trials [41,42], but the data in this study does not have the resolution to uniquely identify it. In addition, it is not a dominant dynamic in the presence of low doses and exogenous insulin [32,33]. Equilibrium glucose concentration \(G_0\) is set to the fasting glucose level of each subject, as shown in the cohort description in Table 1. Glucose clearance saturation is set to \(\alpha_G = 0\) in this study, as the subjects are fasted and with the low dose injection, saturation is not very likely. This value also better matches the assumptions used in calculating ISI for the supra-physiological clamp test [2].

The remaining parameters, \(n_l\) and \(x\) for insulin and \(S_I\) and \(V_G\) for glucose are identified using the integral based fitting method described in more detail by Hann et al. [26]. Briefly, the differential equations are integrated in different time-steps by interpolating between discrete measurements, transforming the non-convex non-linear problem into a set of linear equations that can be easily solved using linear least squares (LS). The method is convex and not starting point dependent, unlike commonly used non-linear recursive least squares (NRLS). Errors in the integration of equations, i.e. due to noise, are not critical, as the method minimises areas under the curve, not absolute differences, thus effectively filtering noisy data [26,43]. The errors have been shown to be, in the limit, on the order of model error [26]. Integrating Eq. (1) in the interval \([t_0, t_1]\) yields:

\[
G(t_1) - G(t_0) = -P_0 \int_{t_0}^{t_1} G(t) dt - S_I \int_{t_0}^{t_1} (G(t) + G_0) \frac{Q(t)}{I + \alpha_G Q(t)} dt + \int_{t_0}^{t_1} P(t) dt + \int_{t_0}^{t_1} EGP(t) dt
\]

This step can be repeated for different time intervals, resulting in a set of linear equations that can be readily solved.

\[
A \begin{bmatrix} S_I & \frac{1}{V_G} 
\end{bmatrix}^T = \bar{b}
\]

The same method is applied to the measured plasma insulin profile \(i(t)\) to estimate parameters \(n_k\) and \(x\), using the analytical solution for \(Q(t)\) in integrating Eq. (3).

\[
Q(t) = \frac{n_1}{V_Q} \int_0^t I(x) e^{-\frac{n_B}{n_k/V_Q}}(t-x) dx
\]

The result is a set of linear equations:

\[
\bar{b}[n_L, x]^T = \bar{c}
\]

The time intervals used in the integrations can be chosen to suit the available data density, as long as the minimum number of intervals required are used to ensure an optimal LS solution [26]. In this study, two sets of measured data are available \((i(t)\) and \(G(t)\)) to estimate two parameters in each. The optimal interval length was identified as 2 min in this study, resulting in 28 integral equations for each data set. No additional weighting or normalisation was performed with the equations.

### Table 1 – Description of the intervention study population from McAuley et al. [24], used to create the virtual simulation cohort in this study

<table>
<thead>
<tr>
<th></th>
<th>Pre-intervention ((N = 73))</th>
<th>Post-intervention ((N = 73))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (all (N = 146))</strong></td>
<td>Mean (S.D.)</td>
<td>Range</td>
</tr>
<tr>
<td>Fasting glucose (mmol l(^{-1}))</td>
<td>4.9 (0.6)</td>
<td>4.0–6.8</td>
</tr>
<tr>
<td>Fasting insulin (mU l(^{-1}))</td>
<td>19.9 (12.1)</td>
<td>6.6–84.3</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>34.4 (4.9)</td>
<td>24.5–45.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.7 (15.3)</td>
<td>67.9–140.8</td>
</tr>
<tr>
<td>ISI (mg/kg/min) (mU/l)(^{-1}))</td>
<td>3.03 (0.9)</td>
<td>1.36–5.15</td>
</tr>
<tr>
<td>HOMA (mU mmol(^{-1}))</td>
<td>4.4 (3.2)</td>
<td>1.4–24.4</td>
</tr>
</tbody>
</table>

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2.5 Test simulation on clamp cohort

To simulate the proposed test and make it comparable to the clamp, a simulation cohort was created using metabolic information estimated from a set of clamp trials performed by McAuley et al. [24] to study the effects of lifestyle interventions on insulin resistance. The data consist of 146 trials performed on 73 individuals, once before and once after a 16 week intervention. Details of the clamp study population are given in Table 1.

The clamp is a test to determine insulin sensitivity in a research setting. It is currently the most accurate method to measure insulin sensitivity, but too intensive and expensive to be used in a clinical setting. A constant insulin infusion is paired with a varying glucose infusion to achieve a steady state glucose concentration. Blood is sampled every 10 min for 2-4h. Due to the supra-physiological dosing, it is assumed that endogenous glucose and insulin are fully suppressed after 60 min. The insulin sensitivity index (ISI) derived from this test is the ratio of the average glucose infusion rate to the mean plasma insulin concentration during steady state ($ISI = \frac{P_{ss}}{I_{ss}}$).

The clamp trials were fitted by the model described in Eqs. (1)-(3) by estimating parameters $S_i$ and $n_i$. Mean absolute errors of the fits were 5.9% (S.D. 6.6%) for glucose and 6.2% (S.D. 6.4%) for insulin [22].

Insulin sensitivity, $S_i$, was estimated as time-varying, piecewise constant during transient and steady state [31]. The steady state value was taken for the subsequent simulations. Mean $n_i$ estimated from the clamp was very low compared to that seen on dose–response tests. This can be caused by various factors, i.e. heavy saturation of the liver being exposed to such large supra-physiological concentrations [44], reduced clearance in obesity [45] or incomplete suppression of pancreatic insulin during the clamp, which is not accounted for in the model fit and results in underestimated $n_i$. Incomplete suppression of pancreatic insulin secretion is particularly likely, given the shorter and lower dose method used in this clamp study [3].

To achieve a more realistic insulin profile, $n_i$ was thus increased by 0.1 min$^{-1}$ for all individuals based on empirical testing on various dose–response data (unpublished). This increase results in a more realistic simulated insulin profile and does not change the insulin sensitivity. Thus, the outcome of the test and the performance of the simulations are not affected. Due to the low resolution of the clamp data, further parameters had to be identified a priori, as described in Section 2.4. In addition, $V_G$ was set to $V_G = 1.2(V_P + V_Q)$, as the clamp data is not dense enough to allow a unique identification. A 20% larger volume than the total insulin distribution volume was chosen, as glucose distribution volume has been found to be larger than for insulin, due to fast hepatic storage and non-insulin dependent uptake by the brain [46,47]. This choice does not affect the outcome of the study, as $S_i$ and $V_G$ are subsequently identified from the test profiles as described in Section 2.4.

Model simulation parameters determined from the clamp population as described in Sections 2.4 and 2.5 are given in Table 2. These parameter values are used to create the virtual cohort on which the test protocol is simulated.

Pancreatic insulin secretion is not known for this cohort, as C-peptide data is not available. A healthy pancreas responds to a glucose input by secreting insulin in two phases. The first phase consists of an insulin burst, lasting approximately 10 min, followed by a second phase of lower dose, but longer duration [35]. Insulin secretion can be suppressed or reduced by exogenous insulin, with a full suppression only achievable by a prolonged infusion of large amounts of insulin [3]. In the protocol for this study, an insulin bolus is injected 10 min after glucose, thus not affecting first phase burst, but suppressing second phase insulin secretion. Simulated total insulin secretion rate is thus reduced back to its basal rate after the bolus injection of exogenous insulin [23,48,49].

Pre-hepatic endogenous insulin secretion can be simulated by a basal secretion rate, superimposed by a first-phase burst. The burst peaks at a rate of 72 mU min$^{-1}$ m$^{-2}$BSA [50,51], which is dependent on body surface area (BSA), and is followed by an exponential decay lasting 10 min. For the lower and higher dose protocol, this first-phase burst is halved and doubled, respectively [52]. Basal endogenous secretion $u_b$ is calculated from the steady state fasting insulin balance using Eq. (3) with insulin concentrations $I_b$ and $Q_b = (3/5)I_b$, and a

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Table 2 – Simulation model parameters calculated and estimated as described in Sections 2.4 and 2.5 to generate the virtual simulation cohort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Pre-intervention ($N = 73$)</th>
<th>Range</th>
<th>Post-intervention ($N = 73$)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_P$($l$)</td>
<td></td>
<td>4.52 (0.37)</td>
<td>3.98-5.93</td>
<td>4.46 (0.36)</td>
<td>3.90-5.96</td>
</tr>
<tr>
<td>$V_Q$($l$)</td>
<td></td>
<td>5.67 (0.54)</td>
<td>4.52-7.47</td>
<td>5.54 (0.57)</td>
<td>4.44-7.26</td>
</tr>
<tr>
<td>$V_G$($l$)</td>
<td></td>
<td>12.22 (1.06)</td>
<td>10.20-15.67</td>
<td>12.00 (1.08)</td>
<td>10.00-15.75</td>
</tr>
<tr>
<td>$n_i$(min$^{-1}$)</td>
<td></td>
<td>0.28 (0.027)</td>
<td>0.22-0.36</td>
<td>0.27 (0.029)</td>
<td>0.21-0.36</td>
</tr>
<tr>
<td>$n_b$(min$^{-1}$)</td>
<td></td>
<td>0.060 (0.0024)</td>
<td>0.053-0.064</td>
<td>0.060 (0.0028)</td>
<td>0.053-0.064</td>
</tr>
<tr>
<td>$n_c$(min$^{-1}$)</td>
<td></td>
<td>0.15 (0.027)</td>
<td>0.10-0.21</td>
<td>0.16 (0.022)</td>
<td>0.10-0.20</td>
</tr>
<tr>
<td>$S_i$(10$^{-1}$min$^{-1}$)</td>
<td></td>
<td>0.032 (0.0037)</td>
<td>0.032-0.033</td>
<td>0.032 (0.0038)</td>
<td>0.032-0.033</td>
</tr>
<tr>
<td>$P_i$(min$^{-1}$)</td>
<td></td>
<td>4.91 (1.54)</td>
<td>2.08-8.29</td>
<td>5.18 (2.13)</td>
<td>3.07-13.0</td>
</tr>
<tr>
<td>$a_0$(lU$^{-1}$)</td>
<td></td>
<td>0.01 (fixed)</td>
<td></td>
<td>0.0017 (fixed)</td>
<td></td>
</tr>
<tr>
<td>$a_1$(lU$^{-1}$)</td>
<td></td>
<td>0 (fixed)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
randomly generated first pass hepatic extraction \( x \):

\[
\bar{u}_b = \frac{V_P b}{1 - x} \left( n_K + \frac{n_L}{1 + \alpha x} b + \frac{2 n_I}{5 V_P} \right)
\]

Total pre-hepatic endogenous insulin secretion is thus modeled as:

\[
u_{\text{en}}(t) = \left\{ \begin{array}{ll}
\bar{u}_b + (72\text{BSA}) e^{-0.3 t} & 0 \leq t < 10 \text{ min} \\
\bar{u}_b & t < 0 \text{ and } t \geq 10 \text{ min}
\end{array} \right.
\]

First pass hepatic extraction is often approximated around 50%, but is generally higher in fasting state [53–55], often reaching values of over 90% [54]. As a conservative choice, \( x \) is thus determined from a uniform distribution of values between 0.5 and 0.95 (almost complete extraction). Using this model, the total insulin secreted and the peak during the first phase match values reported in the literature [50,51].

Basal endogenous glucose production \( \text{EGP}_b \) is calculated from the fasting steady state glucose balance in Eq. (1), where \( Q_b = (3/5) b \) and \( G(0) = 0 \):

\[
\text{EGP}_b = \left( \frac{3}{5} b \right) \frac{(3/5)b}{1 + \alpha_G(3/5)b}
\]

### 2.6. Monte Carlo analysis

The Monte Carlo analysis simulates test result accuracy in the presence of assay, timing, insulin and glucose dilution errors, and unmodeled suppression of endogenous glucose production. The assay errors are assumed normally distributed with inter- and intra-batch coefficients of variation \( \text{CV}_{\text{inter}}, \text{CV}_{\text{intra}} \) reported by the assay manufacturers. Random intra-batch errors are generated for each sample of a test and added to an inter-batch error, equal for all samples of a given test. As \( \text{CV}_{\text{intra}} \) is assumed to be included in the reported \( \text{CV}_{\text{inter}} \), the CV to be superimposed on \( \text{CV}_{\text{intra}} \) \( \text{CV}_{\text{add}} \) is calculated:

\[
\text{CV}_{\text{add}} = \sqrt{\text{CV}_{\text{inter}}^2 - \text{CV}_{\text{intra}}^2}
\]

Errors in timing of samples are caused by variations in blood sampling procedure and are assumed to be normally distributed between ±30 s around the sampling time. Due to anticipation of these small complications, the sampling procedure is usually initiated early, thus sometimes resulting in early sampling. Dilution errors can occur when drawing up glucose in a syringe or when diluting insulin, which is typically distributed in highly concentrated form (e.g. 100 U/ml). Insulin has also been reported to bind to inner walls of syringes and tubes when being administered, causing a loss of insulin during the dilution process [56]. As these are well known problems and usually taken into account by the investigator and the choice of equipment, the errors are assumed to be normally distributed around the mean.

Suppression of EGP is caused by increases in plasma insulin or glucose [49]. The amount and efficiency of suppression is dependent on the administered dose [57]. With the low dose this test aims at, the suppression is likely not as large as during an IVGTT (75–100%) [58,59]), but cannot be neglected. Since this level of suppression cannot be easily measured, a linear reduction of EGP is assumed from the time of insulin input, reaching a randomly generated maximal suppression \( \text{EGP}_{\text{sup}} \) at the end of the test. It is defined:

\[
\text{EGP}(t) = \left\{ \begin{array}{ll}
\text{EGP}_b (1 - \text{EGP}_{\text{sup}} t/\text{t}_{\text{end}}) & t_{\text{end}} \geq t > 0 \text{ min} \\
\text{EGP}_b & t \leq 0 \text{ min}
\end{array} \right.
\]

The maximal suppression at the 10 g/1 U dose was chosen randomly from a normal distribution between 25 and 75%. For the lower (5 g/0.5 U) and higher (20 g/2 U) dose variants, \( \text{EGP}_{\text{sup}} \) was shifted to 0–50 and 50–100%, respectively. Studies have shown a direct dose-dependent relationship between glucose concentration and suppression of EGP [57], validating this basic approach.

The random disturbances thus assumed in this Monte Carlo analysis are:

- Glucose assay errors: \( \text{CV}_{\text{intra}} = 1\%; \text{CV}_{\text{inter}} = 2\% [18] \)
- Insulin assay errors: \( \text{CV}_{\text{intra}} = 2\%; \text{CV}_{\text{inter}} = 2.8\% [18,60] \)
- C-peptide assay errors: \( \text{CV}_{\text{intra}} = 3\%; \text{CV}_{\text{inter}} = 3.4\% [61] \)
- Glucose input error: \( \text{CV} = 1.67\% \)
- Insulin input error (dilution): \( \text{CV} = 3.33\% \)
- Sample timing error: S.D. 10 s
- First pass hepatic insulin extraction: \( x \in [0.50, 0.95] \)
- Maximal suppression of EGP: \( \text{EGP}_{\text{sup}} = 50\% (10 \text{ g/1 U}); 25\% (5 \text{ g/0.5 U}); 75\% (20 \text{ g/2 U}) \text{ (S.D. 8.3\%)} \)

The required number of Monte Carlo simulations was identified to be 500 in a convergence test, as the variability in the standard deviation (S.D.) of the resulting \( S_I \) value identified did not change significantly with more runs.

An example of the resulting simulated profiles of glucose and insulin responses during the proposed test, employing all three dosing options on one virtual subject, is shown in Fig. 2.

### 2.7. Performance metrics and statistics

Performance of the method was assessed by correlation (Pearson correlation) of the estimated insulin sensitivity \( S_I \) with the gold-standard clamp test. Accuracy of estimation of \( S_I \) is given as its coefficient of variation \( \text{CV} = \text{S.D.}/\text{mean} \). The distribution of \( S_I \) can be assumed to be normal, as assessed by the single sample Kolmogorov–Smirnov (KS) test.

Accuracy of ISI was assessed by Monte Carlo analysis with assay errors as described above and a glucose infusion error of 10%. Accuracy of HOMA is affected by assay errors and pulsatile basal insulin secretion. It is estimated through Monte Carlo analysis with a CV of 10%, as reported by Wallace et al. [17].

### 3. Results

The model parameter for insulin sensitivity \( n_I \) from clamp trials is \( S_I = 5.55/\text{S.D.1.95} \times 10^{-4} \text{ mU}^{-1} \text{ min}^{-1} \). This value is higher than clamp ISI normalised by steady state glucose and corrected for units (ISI_G = ISI/G × weight/V_Q) ISI_G = 3.23/\text{S.D.1.16} \times 10^{-4} \text{ mU}^{-1} \text{ min}^{-1} \). This difference is due to the different compartmental insulin concentrations used in the respective calculations. The clamp uses plasma insulin (I)
and the modeled \( S_I \) uses interstitial insulin (Q). Clamp fitted \( S_I \) and measured ISI correlate \( r = 0.93 \). However, \( S_I \) and \( \text{ISIG} \) correlate much better \( r = 0.99 \). The higher correlation with \( \text{ISIG} \) is a result of the unit correction, which reduces variability introduced by other parameters and imperfect clamping to a basal glucose level [9].

Mean insulin sensitivity resulting from Monte Carlo analysis is \( S_{I-MC} = 5.56(\text{S.D.} 1.96) \times 10^{-4} \text{mU}^{-1} \text{min}^{-1} \) and thus identical to \( S_I \). Correlations with ISI and \( \text{ISIG} \) are slightly lower, at \( r = 0.91 \) (90% CI: 0.90–0.92) and \( r = 0.98 \) (90% CI: 0.97–0.98), respectively. Fig. 4 shows the correlation plot of \( S_{I-MC} \) and \( \text{ISIG} \) with the 90% CI’s of each metric.

Intra-individual CV in \( S_{I-MC} \) using the proposed low-intensity test method is \( \text{CVSI} = 4.5\% \) (90% CI: 3.8–5.7%). This value is larger than the CV for ISI, \( \text{CVISI} = 3.3\% \) (90% CI: 3.0–4.0%), but significantly lower than the CV for HOMA, \( \text{CVHOMA} = 10.0\% \) (90% CI: 9.1–10.8%). The three intra-individual CV’s are shown in Fig. 5 for all \( N = 146 \) subjects.

The increase in insulin sensitivity after lifestyle intervention [24] was captured by the model, with \( S_I \) increasing from \( S_{I-MC(BEFORE)} = 4.99 \) (S.D. 1.47) \times 10^{-4} \text{mU}^{-1} \text{min}^{-1} \) to \( S_{I-MC(AFTER)} = 6.19 \) (S.D. 2.02) \times 10^{-4} \text{mU}^{-1} \text{min}^{-1} \). This value matches the increase in ISI shown in Table 1. Correlation between the change in \( S_{I-MC} \) and \( \Delta \text{ISI-MC} \) and \( \Delta \text{ISIG} \) is \( r = 0.96 \) (90% CI: 0.96–0.97) with a mean regression line of \( \text{ISIG} = 0 + 0.58 \text{S}_{I-MC} \). Note that the ~60% slope is due to the fixed 3/5 ratio of insulin concentration in interstitium (Q) and plasma (I) during steady state. Specifically, ISI is calculated using I and \( S_I \) is identified using Q.

The intra-individual CV of \( S_{I-MC} \) decreased slightly from \( \text{CV}_{S_{I-MC(BEFORE)}} = 4.6\% \) (90% CI: 3.8–5.9%) to \( \text{CV}_{S_{I-MC(AFTER)}} = 4.3\% \) (90% CI: 3.7–5.2%). A strong correlation of \( r = 0.83 \) could be seen between a decrease in insulin sensitivity ISI and intra-individual CV in \( S_{I-MC} \). Fig. 6 shows the linear relationships between ISIG and CVSI before and after intervention. A clear reduction in accuracy of estimated \( S_I \) can be seen in subjects with very low insulin sensitivities.

Re-simulating the low intensity test protocol with different doses of glucose and insulin showed a clear dependence of accuracy of the method on the dose employed, as can be seen in Fig. 7. Administering 5 g glucose and 0.5 U insulin resulted in \( \text{CV}_{S_I} = 6.9\% \) (90% CI: 4.9–9.9%). The high dose variant with 20 g glucose and 2 U insulin resulted in a more accurate measure with \( \text{CV}_{S_I} = 3.6\% \) (90% CI: 3.0–4.5%), which is very close to the accuracy of ISI. Correlation of \( \text{CV}_{S_I} \) with \( \text{ISIG} \) was stronger in the low dose protocol (\( r = 0.90 \)) but showed a weaker linear relationship in the high dose variant (\( r = 0.46 \)).

Simulated hepatic insulin clearance \( n_L \) and simulated first pass hepatic insulin extraction \( x \) were underestimated slightly in the Monte Carlo analysis, by \( -4.4\% \) (90% CI: \(-16.5–8.1\% \)) and \(-2.2\% \) (90% CI: \(-12.4–7.3\% \)), respectively. Simulated glucose distribution volume \( V_G \) was overestimated by 1.7% (90% CI: 0.7–3.5%).

4. Discussion

The model-based protocol presented was developed with the main goal to provide a clinically useful, highly accurate

Fig. 5 – Intra-individual coefficients of variation for \( S_{I-MC} \) (CVSI), \( \text{ISIG} \) (CVISI) and HOMA (CVHOMA).

Fig. 6 – Relationship between ISIG and intra-individual coefficients of variation CVSI before and after intervention.


IVGTT is considered the best clamp-correlated method, with correlation values of up to \( r = 0.89 \) being reported [64]. However, lower results as low as \( r = 0.44 \) have also been reported [62].

Other popular methods, widely used due to their simplicity, are surrogate measures such as the OGTT (measuring the rate of glucose decay after an oral glucose load) and HOMA (based on one fasting glucose and insulin sample). These methods are less correlated to the clamp, as they too measure different effects. In particular, HOMA can be very variable due to a pulsatile secretion of insulin [16] and assay inaccuracies, leading to a CV > 10% [17].

The proposed low intensity protocol presented was designed to specifically measure the same effects as the clamp in a much shorter and less intense transient test. Variability is constrained to insulin dependent effects in the periphery, controlled by the insulin sensitivity parameter \( S_i \). Modeled \( S_i \) is lower than clamp ISI, but it does not introduce additional variability. The difference is consistent across all individuals, due to the fixed ratio of steady state plasma (\( L \)) and interstitial (\( Q \)) insulin in the model. The model and fitting method employed have been well validated [26,22] and correlated to clamp data in transient and steady state [31], resulting in very high correlations (\( r = 0.97 \) in transient state, \( r = 0.99 \) in steady state) [31].

The proposed method was able to estimate \( S_i \) with high accuracy, given the assay errors and unmodeled suppression of EGP. CV_SI was slightly larger than CV_SI. This larger CV can be expected given the highly dynamic state of the proposed test. Accuracy decreased drastically by 53% in the lower dose test (5 g glucose, 0.5 U insulin), though accuracy was still better than HOMA. The higher dose test (20 g glucose, 2 U insulin) improved accuracy by 20%. As suppression of EGP was adjusted to the dose accordingly, being higher in the high dose test, the still improved accuracy suggests a strong dependence on the signal to noise ratio of the test, with EGP playing a minor role.

In spite of the improved accuracy at higher dose, it is not as practical for a simple clinical test, for a variety of reasons. As IV glucose is commonly available in 50% solution, 20 g requires a 40 ml injection of a very viscous solution, which could in reality worsen results. The 10 g glucose and 1 U insulin dose also increases risk of hypoglycaemia, particularly in lean subjects. Finally, an intravenous glucose bolus of 20 g is on the upper physiological range, possibly triggering other glucose regulatory effects not accounted for in this simulation, which could in reality worsen results. The 10 g glucose and 1 U insulin dose is only slightly less accurate, but a lot easier and safer to administer in clinical practice. Mean and range of CV_SI are greatly reduced in the step from low to medium dose, whereas the improvement from medium to high dose is not as pronounced any more. This decay is shown in Fig. 8, which illustrates that the medium dose of 10 g glucose and 1 U insulin appears to be the best compromise in practicability, safety and accuracy.

A strong negative correlation was seen between a decrease in insulin sensitivity ISI and CV_SI. This correlation was even stronger with the low dose test, but was markedly reduced in the high dose test. The origin of this effect is likely physiological, as insulin-dependent effects are less dominant in subjects with low insulin sensitivity, leading to a reduced sig-

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Fig. 7 – \( CV_{SI} \) compared to \( CV_{SI} \) and \( CV_{HOMA} \) simulating the protocol with 5 g glucose and 0.5 U insulin (above), and 20 g glucose and 2 U insulin (below).
Fig. 8 – CV_{SI} with the 90% confidence intervals for the low (5 g glucose, 0.5 U insulin), medium (10 g glucose, 1 U insulin) and high (20 g glucose, 2 U insulin) dose test variants.

Fig. 9 – Example of two pilot clinical tests of the proposed protocol on one subject. Test 1 is shown above and test 2 below. The left side shows plasma glucose concentrations and the right side plasma insulin concentrations (measurements and model fitted profiles). Modeled insulin profiles shown are in plasma (I(t), solid) and in interstitial fluid (Q(t), dashed).
5. Conclusions

The proposed method to diagnose insulin resistance proved to be very accurate in Monte Carlo simulation, and only slightly less accurate than the gold-standard clamp test. As a result of its design to measure the same effects as the clamp, it is highly correlated to the gold-standard clamp ISI metric. The physiological dosing, simple and robust protocol and high accuracy make it very attractive for early diagnosis and monitoring of interventions. Accuracy and correlation to gold-standard tests in a clinical setting must still be assessed. However, this study has indicated that the proposed test should possess the accuracy and robustness required, as compared to a large cohort of clamp results.

REFERENCES


