Modeled Insulin Sensitivity and Interstitial Insulin Action from a Pilot Study of Dynamic Insulin Sensitivity Tests

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Abstract: An accurate test for insulin resistance can delay or prevent the development of Type 2 diabetes and its complications. The current gold standard test, CLAMP, is too labor intensive to be used in general practice. A recently developed dynamic insulin sensitivity test, DIST, uses a glucose-insulin-C-peptide model to calculate model-based insulin sensitivity, S_I. Preliminary results show good correlation to CLAMP. However both CLAMP and DIST ignore saturation in insulin-mediated glucose removal. This study uses the data from 17 patients who underwent multiple DISTs to investigate interstitial insulin action and its influence on modeled insulin sensitivity. The critical parameters influencing interstitial insulin action are saturation in insulin receptor binding, α_G, and plasma-interstitial diffusion rate, n_I. Very low values of α_G and very low values of n_I produced the most intra-patient variability in S_I. Repeatability in S_I is enhanced with modeled insulin receptor saturation. Future parameter study on subjects with varying degree of insulin resistance may provide a better understanding of different contributing factors of insulin resistance.

Keywords: insulin sensitivity, insulin resistance, dynamic insulin sensitivity test, intra-patient repeatability, insulin-mediated glucose removal saturation, interstitial insulin

1. INTRODUCTION

The prevalence of Type 2 diabetes has reached epidemic proportions and is still on the rise (Wild et al., 2004; Hossain et al., 2007; PriceWaterhouseCoopers, 2001). Type 2 diabetes is usually not diagnosed until complications start to reveal themselves and irreversible damage has happened (ADA, 1998; Gastaldelli et al., 2004; Kleinfield, 2006). However, insulin resistance (IR) has been found to be decreased by 60% up to 10 years before a diagnosis is made (Martin et al., 1992). If IR can be identified early, the onset of Type 2 diabetes can be significantly delayed or avoided by lifestyle and diet changes (Duncan et al., 2003; McAuley et al., 2002; Nishida et al., 2002; O'Gorman et al., 2006; Tuomilehto et al., 2001). As Type 2 diabetes is a "slow disease", early intervention and prevention would significantly reduce the social and economic costs currently associated with Type 2 diabetes, which mainly consist of chronic treatments (ADA, 2006).

Unfortunately, the "gold standard" test for IR– CLAMP, is too complex and labor intensive to be feasible in a wider clinical setting (DeFronzo et al., 1979). Other shorter clinical tests or surrogate indicators are often too crude to be truly useful (ADA, 1998). A simple test, DIST, providing a model-based insulin sensitivity marker was developed in recent years and correlates well to CLAMP results (Lotz, 2007).

DIST is a dynamic insulin sensitivity test using a low dose of insulin bolus with the addition of a low dose glucose bolus (Lotz, 2007). Measurements are taken for blood glucose, plasma insulin and C-peptide. Insulin sensitivity S_I, and other patient specific parameters are then calculated from a physiological model of C-peptide-insulin-glucose kinetics and dynamics. In a Monte Carlo study of DIST, it achieves a correlation of $r = 0.98$ (90% CI: 0.97-0.98) in S_I to CLAMP ISI (Lotz et al., 2008). The intra-patient variability between S_I in different DISTs is reported to be generally between 0–25%.

Due to measurements only available for blood glucose, plasma insulin and C-peptide, model parameters in Lotz (2007) are mostly determined *a priori* to limit the number of patient specific parameters to be identified. The kinetics of insulin and C-peptide had been extensively studied and well understood (Duckworth et al., 1988; Duckworth and Kitabchi, 1981; Van Cauter et al., 1992). However, what happens in the interstitium and at the receptor level and beyond still presents a lot of unknowns (Duckworth et al., 1998). In particular, dysfunctions at the cellular level are largely speculated to contribute to insulin resistance

(Duckworth et al., 1998; Barrett et al., 2009; Black et al., 1982; Brownlee, 2001; Bryant et al., 2002).

Saturation in insulin-mediated glucose removal has been observed at varying levels (Natali et al., 2000; Rizza et al., 1981; Prigeon et al., 1996; Nestler et al., 1988; Transberg et al., 1981; Docherty et al., 2010). However, this effect is not taken into account by CLAMP. Therefore, underestimation in insulin sensitivity can happen when performing a hyperinsulinaemic CLAMP, where super-physiological levels of plasma insulin is induced beyond regions of linear relationship between glucose disposal and plasma insulin level (Rizza et al., 1981; Prigeon et al., 1996). The model developed for DIST also ignores insulin effect saturation, though efforts were made to avoid reaching saturation levels during DIST (Lotz, 2007).

This study uses the data from the clinical pilot study of Lotz (2007) to investigate interstitial insulin action and its influence on modeled insulin sensitivity. It attempts to find a modeled interstitial insulin dose-response that best links insulin action in plasma to response in blood glucose levels. The critical parameters influencing the modeled shape of interstitial insulin action are saturation in insulin receptor binding and plasma-interstitial diffusion rate.

2. GLUCOSE-INSULIN-C-PEPTIDE MODEL

This study uses the C-peptide model from Lotz et al. (2008) and a glucose-insulin model, ICING, from Lin et al. (2010). The ICING model is an improved model revised from the glucose-insulin models of Lotz et al. (2008) and Chase et al. (2007). The ICING model addresses insulin receptor saturation, which is ignored in Lotz et al. (2008). Therefore this model is used in this study to investigate the level of insulin receptor saturation.

The C-peptide model has a plasma compartment and a interstitial compartment. It is defined:

$$\dot{C} = -(k_1 + k_3)C(t) + k_2 Y(t) + u_{en} \quad (1)$$

$$\dot{Y} = k_1 C(t) - k_2 Y(t) \quad (2)$$

The ICING Model has three compartments for plasma glucose, plasma insulin and interstitial insulin. The model is defined:

$$\dot{G} = -p_G G(t) - S_I G(t)\frac{Q(t)}{1+\alpha_G Q(t)} + \frac{P(t) + EGP_b - CNS}{V_G} \quad (3)$$

$$\dot{Q} = n_I(I(t) - Q(t)) - n_C \frac{Q(t)}{1+\alpha_G Q(t)} \quad (4)$$

$$\dot{I} = -n_K I(t) - \frac{n_L I(t)}{1+\alpha_I I(t)} - n_I(I(t) - Q(t)) + \frac{u_{ex}(t)}{V_I} + (1 - x_L)\frac{u_{en}}{V_I} \quad (5)$$

Table 1 lists the nomenclature for Equations (1)–(5).

This study focuses on Equations (4), which defines the insulin action in interstitium. Saturation parameter for insulin receptor binding is α_G. The receptor-bound insulin is $Q/(1 + \alpha_G Q)$, coupled with S_I for glucose removal to cells. Insulin degrading enzyme then degrades the receptor bound insulin interstitium at a rate n_C (Duckworth et al., 1998). The value of n_C is linked to n_I by the steady state ratio between I and Q (Lotz, 2007).

Table 1. Nomenclature

G	Blood glucose level	[mmol/L]
Q	Interstitial insulin level	[mU/L]
I	Plasma insulin level	[mU/L]
C	Plasma C-peptide concentrations	[pmol/L]
Y	Interstitial compartment C-peptide concentrations	[pmol/L]
EGP	Endogenous glucose production	[mmol/min]
EGP_b	Basal endogenous glucose production	[mmol/min]
CNS	Central nervous system glucose uptake	[mmol/min]
p_G	Insulin independent glucose removal (excluding CNS) and the suppression of EGP from EGP_b with respect to G	[min^{-1}]
S_I	Insulin mediated glucose removal and the suppression of EGP from EGP_b with respect to G and Q	[L/mU/min]
α_G	Saturation parameter for insulin mediated glucose removal	[L/mU]
V_G	Plasma glucose distribution volume	[L]
$P(t)$	Glucose injection	[mmol/min]
n_I	Plasma-interstitium insulin diffusion rate	[min^{-1}]
n_C	receptor-bound insulin degradation	[min^{-1}]
n_K	insulin clearance through kidneys	[min^{-1}]
n_L	insulin clearance through liver	[min^{-1}]
α_I	Saturation parameter for insulin clearance through liver	[L/mU]
$u_{ex}(t)$	Exogenous insulin	[mU/min]
$u_{en}(t)$	Endogenous insulin	[mU/min]
V_I	Insulin distribution volume	[L]
x_L	First pass hepatic clearance	
$k1, k2, k3$	C-peptide transport rates	[min]

3. METHODS

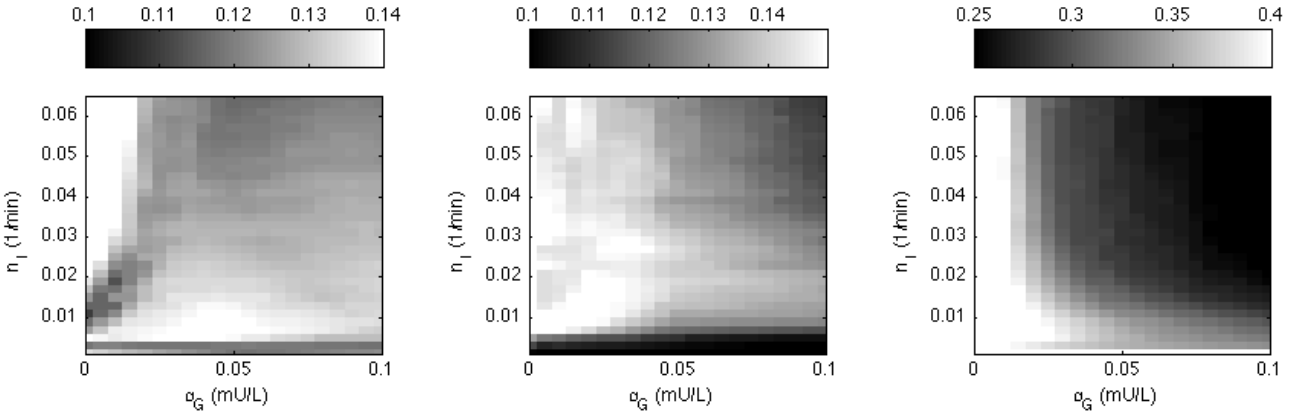
3.1 Study Cohort

Data from 17 patients were used in this study. These patients were recruited for the pilot study of DIST Lotz (2007). Each patient under went at least two DISTs at different times. Three different doses of insulin DISTs are performed for the pilot study. A low dose test involves an intravenous glucose injection of 5g followed by a intravenous insulin injection of 0.5U. A median dose test uses 10g of glucose and 1U insulin. A high dose test uses 20g glucose and 2U insulin. Table 2 summarizes the tests these patients underwent. More details on the patient cohort and the pilot study can be found in Lotz (2007).

Table 2. Participant Details Summary

Group	Number (M/F)	BMI (SD) (kg/m^2)	Test Dose		
			low	medium	high
NGT[#]	14 (5/9)	27.2 (6.7)	7	22	5
T2DM/IFG[#]	4 (1/3)	31.2 (4.1)	4	4	2

[#] NGT = normal glucose tolerance. T2DM = Type 2 diabetes mellitus. IFG = impaired fasting glucose.



(a) Median variability from all subjects

(b) Inter-quartile range of variability amongst subjects

(c) 90% confidence interval in variability amongst subjects

Fig. 1. Variation of modeled S_I. Color bars are the level of variations. Darker regions are areas of least intra-patient S_I variability from different DIST tests.

3.2 Patient Specific Parameter Identification

Measurements in blood glucose, $G(t)$, plasma insulin, $I(t)$, and C-peptide, $C(t)$, were taken during the tests. Patient parameter identification is performed in three stages using these measurements.

In the first stage, endogenous insulin secretion, u_{en}, is calculated using the C-peptide model in Equations (1)–(2).

In the second stage, patient specific first pass hepatic clearance, x_L, and liver insulin clearance, n_L, are fitted to plasma insulin measurements using insulin and glucose injections, $u_{ex}(t)$ and $P(t), and u_{en}(t)$ calculated in the first stage. Equations (4) and (5) are used in this stage, and a good fit will have modeled $I(t)$ in good agreement with plasma insulin measurements. An integral based fitting method (Hann et al., 2005) is used for the identification of x_L and n_L as a pair.

In the third stage, patient specific insulin sensitivity, S_I is solved by fitting Equation (3) to measurements in blood glucose levels. The same integral fitting method is used for the identification.

In the original model of Lotz (2007), V_G is also fitted, while n_K, k_1,k_2 and k_3 are calculated to be patient specific using formulas from Van Cauter et al. (1992). Lotz (2007) also used different volumes for plasma and interstitial insulin distribution. However these values do not vary significantly between patients, and are therefore fixed at generic population values for this study.

3.3 Grid Analysis of α_G and n_I

Because α_G and n_I are coupled to compartment Q, linking compartments I and G, these two parameters cannot be uniquely identified without measurements being available in Q. In reality, dynamic response in Q is more or less unmeasurable. This study analyse a grid of α_G and n_I values to study their influence on S_I. The analysis range for n_I is [0.001, 0.065] , and for α_G is [0 0.1]. These ranges cover the physiological ranges reported in literature, where the boundaries are super- or supra-physiological levels (Nestler et al., 1988; Natali et al., 2000; Prigeon et al., 1996; Transberg et al., 1981; Duckworth and Kitabchi, 1981).

Specifically, the variations in model fitted S_I are examined across the grid space. The common metric in evaluating the accuracy of an insulin sensitivity test is its intra-patient repeatability. An insulin sensitivity test producing the least variation in an individual over multiple tests is usually considered to be more accurate. The patient data used for this analysis comprised of 44 DISTs in 17 patients. Each DIST generates a patient specific S_I using the method described in Section 3.2. The intra-patient variability in S_I is calculated as:

$$variability = \frac{\sum abs(S_{I_{1..n}} - \bar{S_I})}{\sum S_{I_{1...n}}} \tag{6}$$

where n is the number of S_I from a single patient.

4. RESULTS

The fitted S_I for each subject in the parameter space of $\alpha_G = 0 \rightarrow 0.1$ and $n_I = 0.001 \rightarrow 0.065$ generally decrease with increasing n_I, and to a lesser degree, decreasing α_G. The variation in S_I in the parameter space across the 17 subjects can be seen in Figure 1

The intra-patient variation in S_I is generally low within the parameter space studied, as shown in Figure 1(a). The degree of variation is comparable to the modeled S_I from the original pilot study of (Lotz, 2007), which reported variation generally between 0–25%. The darker regions in Figure 1(b) are parameter values producing tighter inter-quartile spread of S_I variability across all subjects. The darker areas are similar to areas of low median variability from all patients in Figure 1(a). Figure 1(c) effectively shows that low $alpha_G$ and low n_I results in more cohort outliers of large intra-patient variability.

The spread of S_I variability across 17 patients can be seen in Figure 2. The combination [α_G, n_I] = [0, 0.049] represents the population values from the original DIST

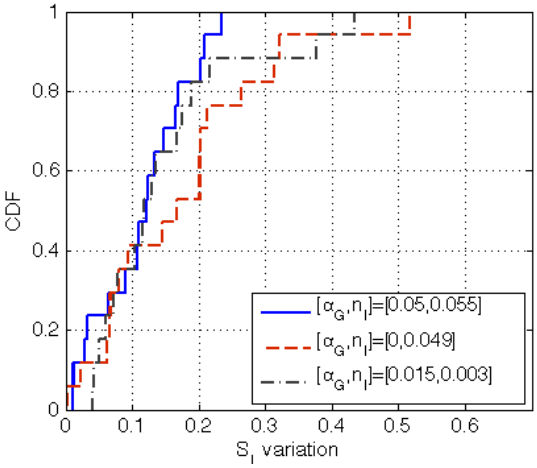


Fig. 2. Cumulative distribution of patient S_I variability at different α_G and n_I values

model Lotz et al. (2008). The modeled S_I using $[\alpha_G, n_I] = [0, 0.049]$ in this study correlates well to the S_I calculated using the original DIST model where more parameters are patient specific Lotz et al. (2008). The r value of 0.93 suggests that model accuracy is not compromised by adapting population values in parameters which involved patient specific calculation in the original DIST model. The correlation decreases for the other two combinations of parameter values shown in Figure 2, where $r = 0.85$ and 0.7 when $[\alpha_G, n_I] = [0.05, 0.055]$ and [0.015,0.003].

Although the combination of $[\alpha_G, n_I] = [0.015,0.003]$ limited the variability of intra-patient S_I, the resulting S_I is almost equally high for all patients, losing its diagnostic value in insulin resistance screening. The correlation to the original DIST S_I dropped significantly to $r = 0.70$. The same results are found when extreme values of α_G is used. The correlation dropped to $r = 0.78$ when $[\alpha_G, n_I] = [0.1,0.065]$. The decrease in r value is however not as significant as lowing n_I. The combination $[\alpha_G, n_I] = [0.05, 0.055]$ appears to deliver good intra-patient variability while maintaining good diagnostic accuracy. This set of parameter values produced low median S_I variation amongst all subjects where the inter-quartile range is also tight, as seen in Figure 1. The correlation to the original DIST S_I is $r = 0.85$. The identified S_I follows the same trend as the original DIST S_I and identified patients with impaired glucose tolerance with similar accuracy.

The combinations of α_G and n_I producing the lowest intra-patient S_I variability for each patient can be seen in Figure 3. The best combinations from each patient are scatter over the parameter space. This may be an indication that these parameters have significant inter-patient variability.

A typical DIST test response from a patient is shown in Figure 4. The model fits to plasma insulin measurements using different parameter values of α_G and n_I are effectively equally good across the physiological parameter space. Therefore, patient specific α_G and n_I cannot be solved simultaneously with n_L and x_L given that plasma insulin levels are the only measurements available. The effect of α_G and n_I on the shape of insulin at the receptor

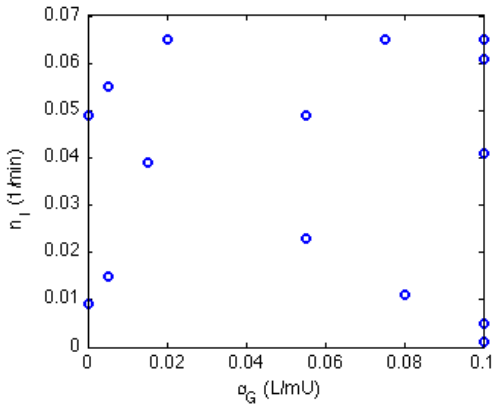


Fig. 3. α_G and n_I values for the lowest S_I variability in each patient

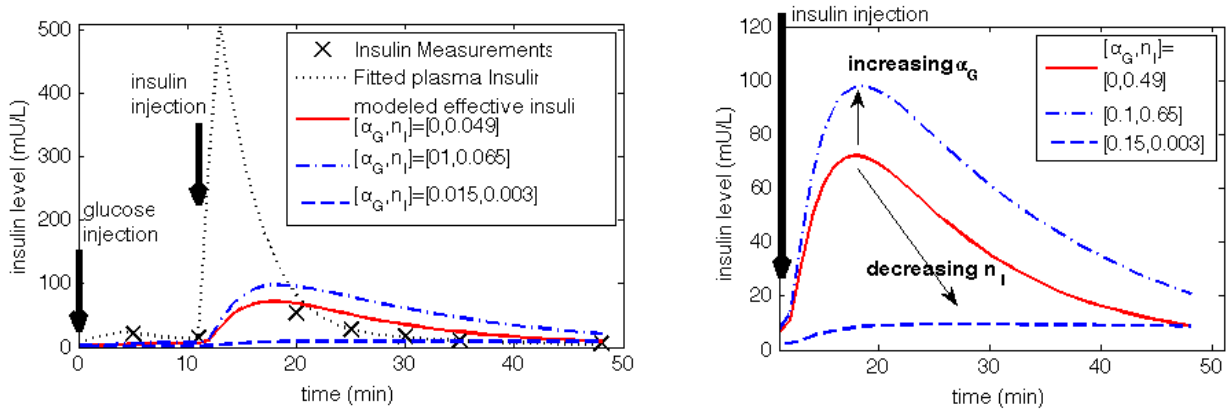
level (i.e. the *effective* insulin for glucose removal) can be seen in Figure 4. Within the physiological range, larger α_G results in a near uniform shift in the level of receptor bound insulin, whereas smaller n_I causes the shape of receptor bound insulin to be flatter with a delay in peak time.

5. DISCUSSION

This study attempted to investigate the relationship between insulin sensitivity and the shape of time-course receptor bound insulin. With the available plasma insulin and C-peptide data, the kinetics of insulin is well understood in the plasma compartment. However, what happens in the interstitium and at the receptor level and beyond still presents a lot of unknowns. In this study, the critical parameters influencing the shape of receptor bound insulin levels are the plasma-interstitium diffusion rate, n_I, and receptor binding saturation parameter, α_G.

The level of saturation in insulin-mediated glucose removal has been reported across a wide range. The plasma insulin level at half maximal action of glucose removal has been reported to be between 50–1000 mU/L. This is effectively equivalent to an α_G between 0.04 and near zero (Natali et al., 2000; Rizza et al., 1981; Prigeon et al., 1996; Nestler et al., 1988; Transberg et al., 1981; Docherty et al., 2010). The saturation in insulin-mediated glucose removal may not simply be due to the number of available receptors. A delay in insulin transportation to the skeletal muscle, common in insulin resistant individuals, would also been seen as saturation in insulin-mediated glucose removal (Barrett et al., 2009; Prigeon et al., 1996). In addition, the dynamic response of endogenous glucose production to the insulin injection is not accounted for in this study, due to limited available data. The underestimation of endogenous glucose production will cause glucose removal to appear slower, effectively adding to the saturation effect.

The level of modeled α_G has been found to have a magnification effect in insulin sensitivity in data from critically ill patient receiving intensive insulin therapy Chase et al. (2004). It can be seen in Figure 4(b), varying α_G shifts the magnitude of the modeled "effective" insulin without influencing the time of peak action. Therefore α_G does not impact on S_I as much as n_I. In the 17 patients evaluated in this study, including the saturation



(a) Plasma and effective insulin

(b) Effective insulin

Fig. 4. Typical DIST test response. Subfigure (b) focuses on the shape of effective (receptor bound) insulin with different α_G and n_I.

parameter α_G definitely enhanced the repeatability of the the modeled S_I within a patient, as this eliminates the outlying large variability seen in the low α_G region in Figure 1(c).

The level of n_I has both a magnification effect as well as phasing effect on the shape of effective insulin. When the level of n_I decreases, less insulin is able to reach interstitium before being cleared from plasma by the liver and kidneys. The value for n_I in the original DIST model was calculated from formulas developed using C-peptide data (Van Cauter et al., 1992). Using these formulas, the values of n_I amongst the 17 subjects in this study range between 0.048–0.05. Details for this calculation can be found in Van Cauter et al. (1992) and Lotz (2007). A population n_I value of 0.055 is found to provide good intra-patient repeatability in S_I.

In the validation study of the glucose-insulin model, ICING, using clinical data from critically ill patients receiving intensive insulin therapy, n_I was found to be very low at 0.003 min^{-1} (Lin et al., 2010). This may indicate significantly impaired trans-capillary transport for patients who are critically ill. In particular, sepsis causes a dysfunction in micro-circulation as well as cell metabolism, and is a condition that is prevalent in critical care (Träger and Radermacher, 2003).

Overall, this study presents a method that independently examines α_G and n_I under the limitation in available data. Cutting down the number of patient specific parameters did not seem to compromise the model accuracy, as very low fitting error in plasma insulin measurements is always achieved. More test data from larger cohorts will enable a more in-depth study of saturation in mediated glucose removal and plasma-interstitium insulin diffusion, or the action of insulin in the interstitum in general. A cohort of patients with mixed levels of insulin resistance will also further validate the accuracy of the modeled S_I. "Customising" patient α_G and n_I by finding the parameter values providing the best repeatability in S_I may reveal further information in the underlying factors of an individual's insulin resistance.

6. CONCLUSIONS

The intra-patient repeatability of S_I and its link to interstitial insulin action is studied in 17 patients. Very low values of insulin receptor saturation α_G and very low values of plasma-interstitial insulin diffusion n_I are found to produce the most intra-patient variability in S_I. A model accounting for insulin receptor saturation enhanced the repeatability in S_I. A larger cohort will enable a more in-depth investigation into the relationship between interstitial insulin action and insulin sensitivity. A parameter study on subjects with varying degrees of insulin resistance may provide a better understanding of the contributing factors of insulin resistance.

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