Independent Cohort Cross-validation of the Real-time DISTq Estimation of Insulin Sensitivity.

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Abstract

Insulin sensitivity (*SI*) is useful in the diagnosis, screening and treatment of diabetes. However, most current tests cannot provide an accurate, immediate or real-time estimate. The DISTq method does not require insulin or C-peptide assays like most *SI* tests, thus enabling real-time, low-cost *SI* estimation. The method uses a-posteriori parameter estimations in the absence of insulin or C-peptide assays to simulate accurate, patient-specific, insulin concentrations that enable *SI* identification.

Mathematical functions for the a-posteriori parameter estimates were generated using data from 46 fully sampled DIST tests (glucose, insulin and C-peptide). *SI* values found using the DISTq from the 46 test pilot cohort and a second independent 218 test cohort correlated R=0.890 and R=0.825, respectively, to the fully sampled (including insulin and C-peptide assays) DIST *SI* metrics. When the a-posteriori insulin estimation functions were derived using the second cohort, correlations for the pilot and second cohorts reduced to 0.765 and 0.818, respectively.

These results show accurate *SI* estimation is possible in the absence of insulin or C-peptide assays using the proposed method. Such estimates may only need to be generated once and then used repeatedly in the future for isolated cohorts. The reduced correlation using the second cohort was due to this cohort's bias towards low *SI* insulin resistant subjects, limiting the dataset's ability to generalise over a wider range. All the correlations remain high enough for the DISTq to be a useful test for a number of clinical applications. The unique real-time results can be generated within minutes of testing as no insulin and C-peptide assays are required and may enable new clinical applications.

Introduction

Insulin resistance (IR) has been widely accepted as a strong indicator of an individual's risk of type 2 diabetes (T2DM) [1; 2]. A longitudinal study of the pathogenesis of T2DM has shown that those subjects who were diagnosed with T2DM had a 60% higher IR than average when assessed 10 years earlier [3]. IR is thus a strong predictor of T2DM risk and cardiovascular disease [4]. Therefore, low-cost, accurate estimation of IR could be used to screen patients, monitor interventional lifestyle changes, and to guide other therapies that could drastically reduce the incidence and cost associated with T2DM [5].

The various tests used to estimate insulin sensitivity (*SI*, $SI=IR^{-1}$) use various methods to provoke and measure the subject's glycaemic responses [6; 7]. The euglycemic hyperinsulinaemic clamp (EIC) aims to suppress endogenous glucose production (EGP) and significantly suppress endogenous insulin production (*Uen*) to assess tissue sensitivity to exogenous insulin [8]. In contrast, the intravenous glucose tolerance test (IVGTT) stimulates *Uen* to measure insulin sensitivity [9]. Hence, while the metrics obtained by these tests are similar, they are not equivalent. An ideal metric for clinical or diagnostic use would measure the efficiency of insulin to dispose of glucose to the periphery at physiologically relevant glucose and insulin concentrations.

The gold standard for *SI* testing is the EIC. It measures the rate of glucose disposal at basal glucose, driven by hyper-physiological insulin concentrations designed to suppress *Uen*. The EIC is accurate and repeatable [8], but takes 4-5 hours and approximately 6 clinician hours to perform. The time, intensity and cost of the EIC

prohibits its use in many clinical situations. A reliable result is not necessarily guaranteed with an inexperienced clinician.

The IVGTT measures the subject's response to a 20-25g intravenous (IV) glucose bolus with very frequent 1-3 minute sampling. Some protocols modify the response with a 2-3U IV bolus of insulin following the glucose bolus (IM-IVGTT) [7]. *SI* is then typically obtained by fitting the minimal model [10] to the sampled data. The boluses in this test tend to be supra-physiological and the trial generally runs for 2-3 hours requiring significant clinical effort due to the frequent sampling. Model parameters are often unidentifiable, in particular in subjects with low SI values [11; 12].

Lower cost and lower intensity surrogate tests include fasting glucose, 2-hour oral glucose tolerance (2hr OGTT) and the homeostasis model assessment (HOMA). Fasting glucose allows a diagnosis of T2DM [13], but does not offer an estimate of *SI*. Elevated fasting glucose is a resulting symptom of significant IR and the inability to maintain glycaemic homeostasis. Once elevated fasting glucose is detected, significant, and often irreversible beta-cell damage has already occurred [14]. Hence, fasting glucose is not an effective screening tool for early risk diagnosis to prevent further disease development.

The 2hr OGTT measures the subject's ability to dispose a 75g oral glucose load. Two hours after ingestion of the glucose load the blood glucose concentration is measured for a T2DM diagnostic. The accuracy of the 2hr-OGTT is questionable, with studies finding intra-subject reclassification of diagnosis rates of 50-60% [15-17]. Similarly to a fasting glucose level, early diagnosis of risk factors prior to the development of T2DM is difficult with the OGTT.

HOMA multiplies the fasting insulin and glucose assay values from a single blood test to produce a surrogate estimation of IR. The underlying assumption is that subjects with low sensitivity will require more insulin to maintain glycaemic homeostasis, elevating fasting levels of glucose and/or insulin. This test has an inconsistent correlation with the clamp (R=-0.19 → R=-0.82) [18; 19], does not track changes from intervention well [20], and does not fully represent insulin-glucose dynamics at physiologically relevant concentrations.

The dynamic insulin sensitivity test (DIST) is a short, infrequently sampled, low dose intravenous glucose tolerance test. The test takes 30-45 minutes to administer. Glucose, insulin and C-peptide data is used with a clinically validated physiological model [20; 21] to provide accurate estimates for *Uen*, insulin clearance rate, and *SI*. The DIST has shown good correlation to the EIC in virtual trials (R=0.93) [20], and high repeatability in a clinical pilot study (Δ= 6%) [21], with a validation study ongoing.

This study presents the DISTq (quick DIST) which is an alternative method for solving DIST data using only glucose samples and the subjects' physical attributes (height, weight, sex, and age). Glucose samples can be assayed at the test station during sampling, enabling low cost test analysis. As no insulin or C-peptide assays are required, the DISTq can effectively provide *SI* immediately in "real-time". To remove the need for insulin and C-peptide assays, the insulin concentrations in plasma and interstitium must be estimated using knowledge available at testing. Parameter relationships derived from the fully sampled clinical DIST pilot study data [21] can be used to generate the required estimates [22]. These parameter estimates are used with

the physiological model shown in Figure 1 to simulate an interstitial insulin profile with sufficient accuracy to identify *SI* (R=0.86 to the fully sampled DIST) [22].

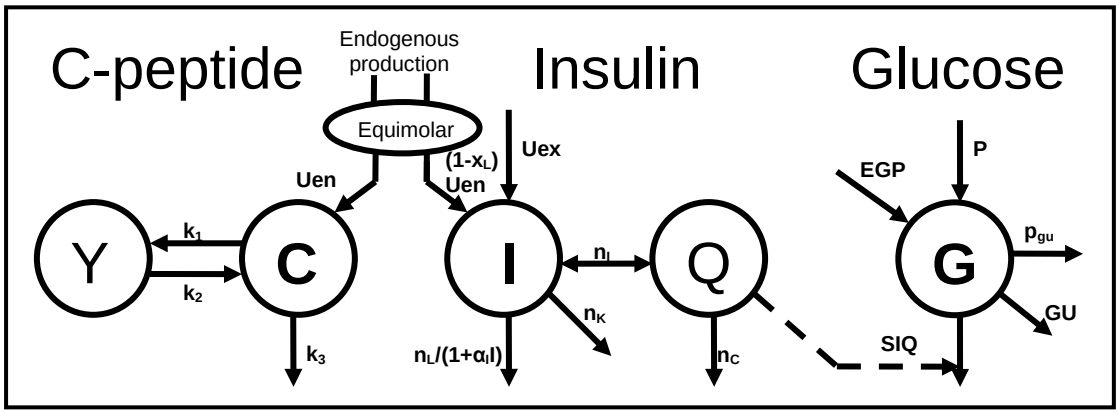

Figure 1: The physiological compartment model used to match the DIST test data (symbols are fully defined in the Methods section)

In this research study, the validity of the DISTq assumptions are tested on two separate cohorts. One cohort is used to generate the insulin estimation functions, which are then tested on both cohorts. The goal is to assess how applicable and valid these estimations are across cohorts, and thus estimate or identify any additional errors in using this.

Method

Model

The DISTq method utilises only the glucose and anatomical data (height, weight, sex and age) from each subject as used in the previously published DIST protocol [20; 21] to identify model based insulin sensitivity (*SI*). The model is defined:

$$\dot{C} = -(k_1 + k_3)C + k_2 Y + \xi \frac{Uen}{Vp} \quad 1$$

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

$$\dot{I} = -n_K I - n_L \frac{I}{1 + \alpha_I I} - \frac{n_I}{Vp}(I - Q) + \frac{Uex}{Vp} + (1 - x_L)\frac{Uen}{Vp} \quad 3$$

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

$$\dot{G} = -p_{gu}(G - G_e) - SI(GQ - G_e Q_b) + \frac{P}{Vg} \quad 5$$

where: k_1, k_2, k_3, n_K, n_L, and n_C are transport rate parameters [min^{-1}]; n_I, is the transport rate between plasma and interstitium [$L \cdot min^{-1}$]; α_I is the saturation coefficient of liver clearance [$L \cdot mU^{-1}$]; C and Y are plasma and interstitial compartment C-peptide concentrations [$pmol \cdot L^{-1}$]; ξ is a conversion factor [$6.94 mU \cdot pmol^{-1}$]; *Uen* is the rate of endogenous insulin and (equi-molar) C-peptide production [$mU \cdot min^{-1}$]; I and Q are plasma and interstitial compartment insulin concentrations [$mU \cdot L^{-1}$]; *Uex* and *P* are the insulin and glucose bolus inputs [mU and mmol]; *Vp* and *Vq* are volumes of distribution of plasma and interstitium, respectively [L]; x_L is the fractional first pass liver extraction [$mU \cdot mU^{-1}$]; G is the glucose concentration in plasma [$mmol \cdot L^{-1}$]; G_e and Q_b are equilibrium or basal levels of the

respective analytes [mmol·L^{-1} and mU·L^{-1}]; *Vg* is the volume of distribution of glucose [L]; and p_{gu} is the non-insulin mediated glucose disposal rate [min^{-1}].

The lack of insulin and C-peptide data allows estimation of *SI*, but unique estimation of *Uen* and insulin clearance is not possible.

DIST protocol

The DIST is a low dose insulin-modified intravenous glucose tolerance test that utilises a short protocol and infrequent sampling. Participants provided signed informed consent prior to testing, and reported to the place of testing after an overnight fast. Height, weight, age and sex were recorded prior to each test. A cannula was placed in a large vein on the inner elbow for the purposes of bolus administration and taking blood samples. Blood samples were taken at t = 0, 10, 15, 20, 25, 30, 35, 40, 45, and 55 minutes, with a boluses of glucose (50% dextrose) and insulin (actrapid) administered immediately after the t=10 and t=20 minute samples respectively. Participant's received a low dose (5g glucose and 0.5U insulin), medium dose (10g, 1U) or high dose (20g, 2U) test dose.

Insulin and C-peptide population-based estimation functions

The absence of insulin or C-peptide data requires a reasonable estimation of the participant's insulin response to accurately determine *SI*. These estimations can be made with information about the participant's anatomical data (height, weight, age and sex), protocol and predictions of the pharmaco-kinetic parameters typically used to simulate these profiles. Figure 2 shows ten characteristics from a DIST test that must be estimated in the absence of insulin and C-peptide assays to enable unique, patient-specific insulin production and concentration profiles. Although these

characteristics varied in magnitude between tests, each one was observed in each trial of the pilot investigation of the DIST [21].

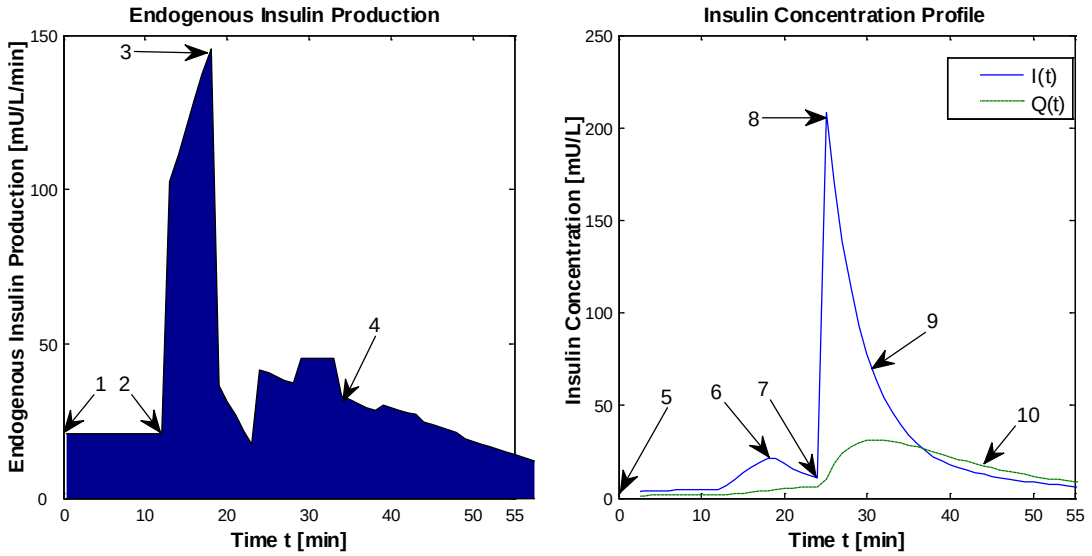

Figure 2: The ten features that can fully define the endogenous insulin production rate (*Uen*) and the plasma and interstitial insulin concentration responses (*I(t)* and *Q(t)* respectively) to a DIST protocol test [21].

Some characteristics are identified using knowledge of the protocol, or using published results. Hence, 5 of the 10 characteristics in Figure 2 can be identified robustly and are listed (with numbering as in Figure 2):

2. The first phase pancreatic insulin release occurs immediately after the glucose bolus [7].

6. The effect of the first phase pancreatic response on insulin concentrations in plasma is known once the first phase response is defined. First pass hepatic extraction x_L is set at 70% based on prior results [23-25] and is not a variable in this method, as it is in the full DIST method.

7. The time of the insulin bolus is recorded per protocol.

8. The maximum insulin concentration in plasma is estimated by dividing the known bolus mass by the volume of distribution of plasma as estimated by Van Cauter et al [26].

10. The insulin transport rate to interstitial fluid is defined using published population kinetic parameters [26].

The five other characteristics in Figure 2 cannot be estimated easily:

1. Basal endogenous insulin production rate (U_B).

3. The magnitude of the first phase response (U_{max}).

4. The magnitude of the second phase response (U_{ave}).

5. Basal insulin concentration in plasma (I_b), which is measured in the fully sampled DIST, but not in the DISTq

9. Liver clearance rate of insulin in plasma (n_L).

To estimate these 5 parameters, a relationship to attributes known at the time of the test must be derived. No a-priori relationships existed between these parameters and any function of anatomical data (height, weight, sex and age) that provided consistent estimates. However, strong relationships were evident between these five parameters and the *SI* value identified with the iterative integral method and full data. The iterative integral method uses 10 iterations to define *SI* by which time the parameter variation is in the order of 1e-3%.

Parameter estimation graphs are developed using the full DIST data set (including insulin and C-peptide assays) from the pilot cohort to identify the endogenous insulin production profile (*Uen*) and the liver clearance rate (n_L) as a function of insulin sensitivity (*SI*). These parameters were identified using an iterative integral method [22; 27].

Figure 3 shows how the basal insulin (I_b and **#5** in Figure 2) and liver clearance (n_L and **#9** in Figure 2) parameters are related to *SI*. The relationship between I_b and *SI* is identified using linear regression of the logarithms of *SI* and I_b:

$$I_b = 45.18 * (SI)^{-1.039} \qquad 6$$

The mathematical relationship between n_L and *SI* is identified with linear regression:

$$n_L = 0.0924 + 0.0041 * SI \qquad 7$$

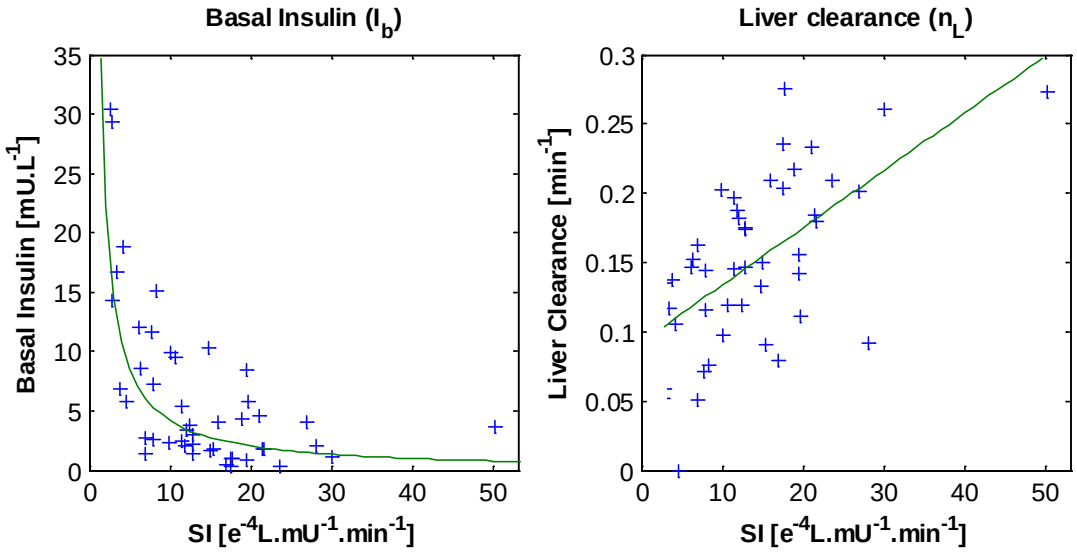

Figure 3: Basal insulin and the hepatic clearance rates from the pilot cohort presented as relationships to *SI* with the mathematical estimation equation lines.

The endogenous insulin production profile (*Uen* and Figure 2 (left)) is predicted using minute-wise equations based on *SI*. The *Uen* profiles from the pilot cohort are synchronised so the first phase response occurs at 10 minutes. Each minute of these 55-minute tests is then solved as an exponential function of *SI* using the linear regression of the logarithms of *SI* and *Uen*. This approach generates a matrix of 56 coefficients and 56 exponents to enable a 56 minute simulation of *Uen* using a single *SI* value. For clarity the t = 0, 15 and 35 minute equations, which represent the basal (#1 in Figure 2), maximum (#3 in Figure 2) and second phase production (#4 in Figure 2) rates are given in Equations 8-10. These equations are 3 of the 56 equations in the *Uen* estimation process and are not used in isolation by the DISTq method. Figure 4 shows the estimation profiles overlaid on the identified profiles of the 46 trials in the pilot study [21].

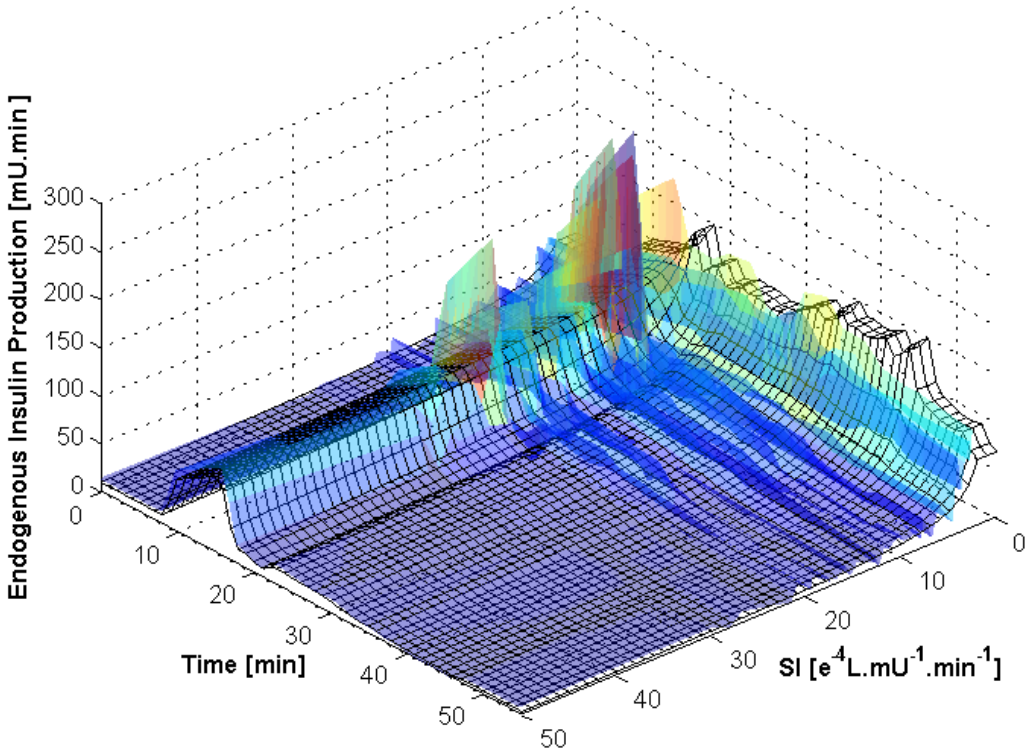

Figure 4: Endogenous insulin production rates identified from the 46 tests from the pilot cohort (semi transparent colour-map) and the resulting estimated *Uen* profiles over all of the *SI* range used (wire-grid). Note that the full DIST pilot results only existed in the range of the colour-map shown, and thus parameter estimation is restricted to the values within this range.

$$Uen(0) = 95.63 * (SI)^{-0.609} \quad (8)$$

$$Uen(15) = 122.7 * (SI)^{-0.116} \quad (9)$$

$$Uen(30) = 238.8 * (SI)^{-0.892} \quad (10)$$

The DISTq identification method uses an initial pilot cohort average value of *SI* of $10e^{-4}L \cdot mU^{-1} \cdot min^{-1}$ to make an initial guess of the *Uen* profile using the 56 *Uen* equations. Parameters I_b and n_L are subsequently estimated using this *SI* value in Equations 6-7. The *Uen* profile and I_b and n_L parameters are used with the a-priori parameter estimations of Van Cauter et al. [26] and the physiological model of Equations 1-5 to generate an interstitial insulin concentration profile (*Q(t)*).

The iterative integral method [22; 27] is then used with the (real) glucose test data and the estimated *Q(t)* profile to identify a new revised *SI*. This newly identified *SI* is

dependent on the population average insulin pharmaco-kinetics and *SI* estimate, and may not be truly representative of the participant's actual *SI*. Thus, *Uen*, n_L and I_b are re-identified using the population based parameter equations with this new *SI* value. A new *Q(t)* profile is identified that is more representative of the participant's actual *SI*, and a subsequent new, more accurate *SI* is found. This cycle is repeated no less than seven times, until *SI* converges to within 0.1% between iterations. Figure 5 shows this DISTq method schematically.

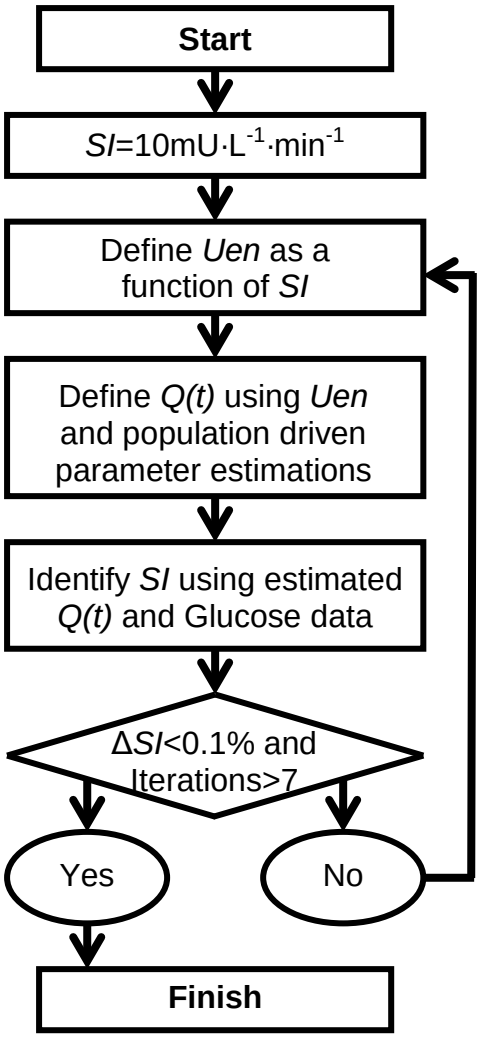

Figure 5: The DISTq method presented diagrammatically. The generation of the parameter estimation equations is not specifically part of the DISTq method. Instead, it is prepared previous to the use of the DISTq method from prior full test data.

Previously presented DISTq methods [22] have included an additional subsequent n_L variation sub-cycle to further refine the *SI* result, which has not been used in this analysis.

Pilot study and participants

For the DIST pilot study, eighteen subjects from the Canterbury and Otago regions of New Zealand were recruited. Forty-six individual trials were performed on these participants during a two-part study that sought to define the inter-dose and intra-dose repeatability. Seven high dose, 28 medium, and 11 low dose tests were completed. Further participant and test details are given in Lotz et al. [20; 21].

Second cohort and participants

Eighty-one female participants were recruited from the Otago region to take part in a dietary intervention study. Inclusion was conditional upon an increased risk of T2DM. Participants either had a BMI>25 at the time of the first test, a BMI>23 with a family history of T2DM, or had an ethnicity with an increased risk of T2DM and a BMI>23. Participants attended three tests, each at the medium dose, at week 0, week 4 and week 10 of the intervention. Seven participants did not return for either week 4 or week 10 tests, and the samples from their first tests were not assayed. Three participants did not attend the week 4 test, and one did not attend the week 10 test. The assays for these participants were completed making 218 full data sets available from this cohort.

Table 1 outlines the key attributes of the cohort participants.

	Trials	Status	BMI	Sex	Age	HOMA-IR	DIST-SI
Cohort	N	NGT/IFG/T2D	Mean(SD)	M/F	Mean(SD)	Q_1 Q_2 Q_3	Q_1 Q_2 Q_3
Pilot	46	14/2/2	27.9(6.2)	6/12	43.0(13.4)	0.34 0.70 2.18	6.83 13.5 19.9
2nd Cohort	218	63/11*/0	32.4(5.4)	0/74	42.6(11.5)	1.37 2.15 3.11	5.79 7.83 10.9
Combined	264	77/13/2	31.5(5.8)	6/86	42.7(11.9)	1.08 1.91 2.89	6.00 8.24 11.8

Table X: participant characteristics and sensitivity results according to cohorts. NGT is normal glucose tolerance, IFG is impaired fasting glucose (>5.6 mmol·L^{-1}), and T2D is type 2 diabetic. HOMA-IR is calculated: IR=$G_b I_b$/22.5. DIST-SI is identified with the iterative integral method and the full data set. * Only one participant was diagnosed with IFG in all three tests, two were diagnosed in two of the three tests and eight had IFG once.

Method assessment

The DISTq method will be evaluated in a three step process, involving the DIST pilot study cohort, a second independent study cohort and a third cohort combining the first two. In the first step, the data obtained during the pilot investigation of the DIST test is used to generate n_L, I_b and minute-wise *Uen* estimation equations. Using only the glucose data, *SI* is identified using the DISTq method and compared to the fully-sampled (including insulin and C-peptide assays) DIST *SI* of the pilot study [20]. This first comparison allows a self validation of the DISTq.

The parameter estimates from the pilot data are then used to define *SI* metrics for the second 218 trial cohort. The *SI* values found using DISTq for the second cohort are compared to the DIST results for that cohort. This comparison provides a form of cross validation.

Finally, the gradient of the fully sampled *SI* to DISTq *SI* will be identified to show if there is equivalence in terms of magnitude for the two cohorts and their respective fully sampled results (ie grad=1.0). The gradient is defined as:

$$\text{grad} = \left\| SI_{DISTq} \right\|_2 / \left\| SI_{DIST} \right\|_2 \qquad 11$$

In the second step, the process is reversed, where the parameter estimation equations are generated using the fully sampled second cohort data. These parameter estimation graphs are overlaid upon the pilot graphs of Figure 3 to analyse any disparity between the cohorts. Finally, as above, the pilot and second cohort data are used to identify *SI* using the DISTq method and results compared to the full DIST results for each cohort, providing a second, reversed self- and cross-validation result.

In the third step, a combined cohort of both the pilot data and the second cohort are used to define the parameter estimation equations jointly. This overall approach allows a 3x3 table to be constructed, showing the correlations and gradients between the fully sampled and DISTq *SI* values for each combination of pilot, second and combined cohort generated estimation equations and analysis set. Hence, variations induced due to using different cohorts to create the insulin parameter estimations for the DISTq can be assessed and analysed.

Results

Pilot cohort derived parameter estimates

The pilot data yielded parameter estimation graphs sufficiently accurate to enable a correlation of R=0.890 between the fully sampled DIST method and the DISTq method. Furthermore, the correlation of DIST and DISTq *SI* for the second cohort using these same estimations was R=0.825. The pilot cohort showed good equivalence with fully sampled datasets with a gradient of 1.049, whereas the second cohort gradient showed a significant shift or bias in magnitude (grad=1.507). Figure 6 shows the relationship between *SI* solved using the DISTq and fully sampled DIST methods for both the pilot data and the second cohort data. The bias seen in the gradient for the second cohort is evident in the rotated slope seen in Figure 6 (right). Similarly, the small bias for the pilot data set is evident in Figure 6 (left).

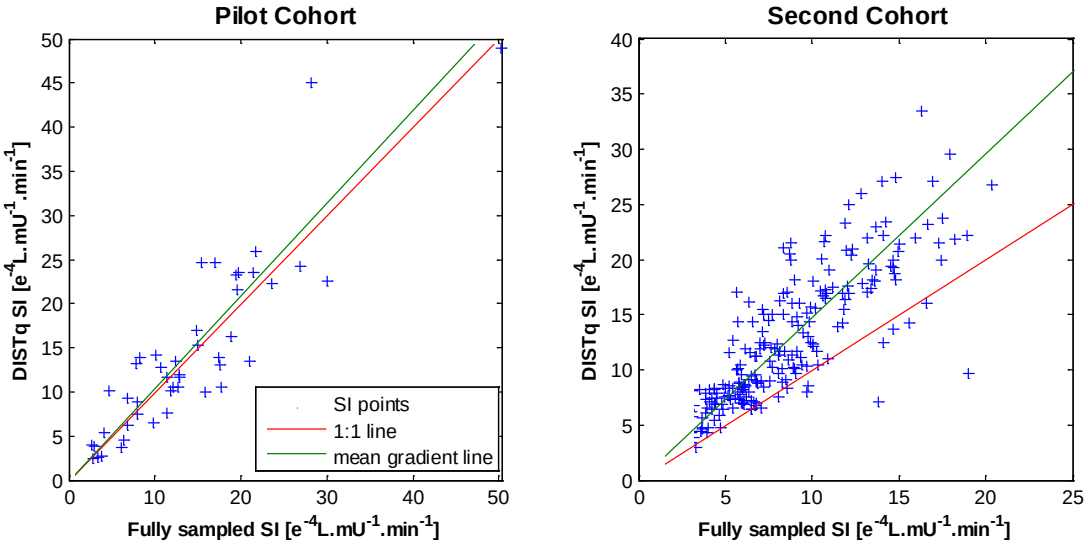

Figure 6: The relationships between *SI* derived by the DISTq method and the fully sampled method from both the pilot cohort and the second cohort when the parameter estimation equations derived using the pilot data are used.

Second cohort derived parameter estimates

The second cohort parameter estimation graphs generally showed consistency with the pilot estimation graphs regarding the form of the relationships. However, in contrast to the pilot study, which sought to find a cohort representative of insulin sensitivity across the wider community, the second cohort was targeted at participants with high risk of T2DM. Thus, the DIST *SI* distribution was different to the pilot cohort ($p<0.000001$) as were the I_b and n_L values ($p<0.001$ and $p<0.000001$ respectively). Figure 7 shows the second cohort parameter relationships overlaid upon the corresponding relationships from the pilot investigation. The three lines are representative of the equations generated using the pilot, second, and (forthcoming) combined cohorts.

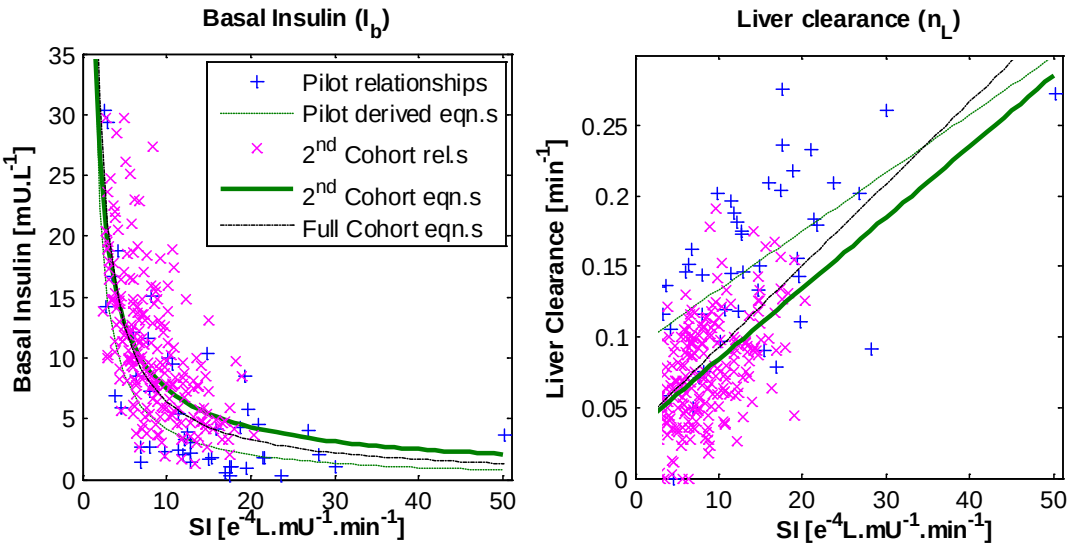

Figure 7: Parameter relationships between *SI* and the basal insulin and liver clearance rate defined using the fully sampled DIST test. The second cohort is shown with the pilot cohort to show that there is regional consistency in parameter behaviour between cohorts, but a global tendency toward lower *SI* in the second cohort.

Utilising the second cohort for the development of the parameter estimation equations reduced the pilot data correlation ($R=0.765$) and even caused a small reduction in the correlation of the second cohort ($R=0.818$). However, the gradient of the second

cohort showed a significant improvement in this self-validation (grad=1.106). The pilot cohort gradient was still strong, but reduced (grad=0.918) in this cross-validation.

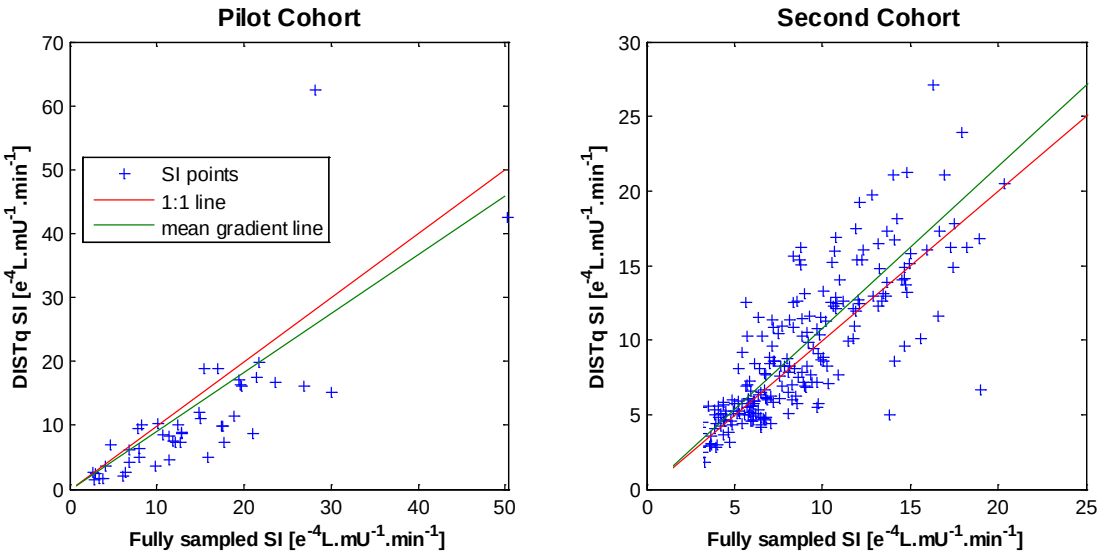

Figure 8: The relationships between the *SI* derived by the DISTq method and the fully sampled method from both the pilot cohort and the second cohort when the parameter estimations equations from the second cohort are used.

Comparing DISTq *SI* values identified using the second cohort derived parameter equations to those identified using the pilot cohort derived parameter estimations, the correlation remains high (R=0.962). This result indicates some measure of robustness regarding the cohort used to create insulin estimates. However, the overall results using the second cohort indicate it is less representative than the pilot cohort.

Combined cohort derived parameter estimates

Combining full data from both cohorts to generate the parameter estimates improved upon the second cohort parameter estimations when used to identify the pilot cohort in a partial self-validation (R=0.883). However, this result did not hold when they were used in the second cohort (R=0.783). The gradients of the pilot and second

cohort (Table 1) showed that the combined cohort parameter estimations caused the DISTq to under-estimate *SI* in the pilot cohort, and over estimate *SI* in the second cohort, effectively splitting the biases seen in Figures 6 and 8. Figure 9 shows the differing behaviour of the contributing cohorts when the combined cohort parameter estimation equations are used.

Table 1 illustrates the effects of the derivation cohorts on the correlation between the fully sampled and DISTq *SI* values. It is clear that the best correlation results come from using only the pilot cohort with its broad range of subjects and doses. However, the gradients are closest to 1 when the identified cohort is used to generate the parameter estimations, as expected for a self-validation test.

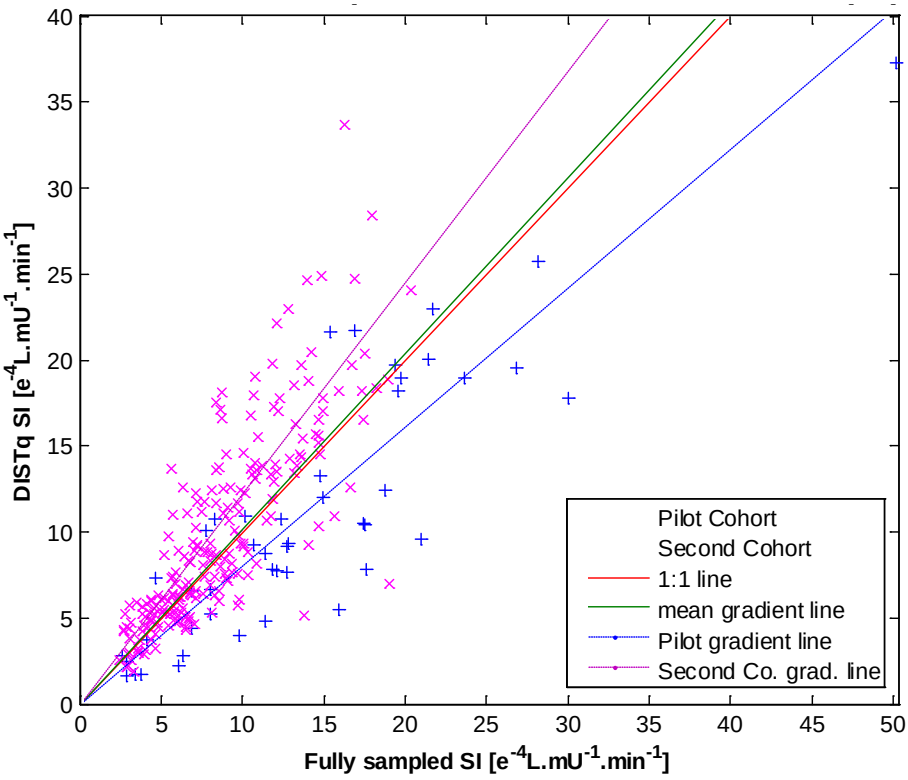

Figure 9: The total cohort relationships between *SI* solved with the DISTq method using population estimation equations derived with the total cohort data and *SI* solved with the fully sampled DIST method. The distinguishing symbols show the differing behaviour of the two contributing cohorts.

Correlation (gradient)		Derivation set		
		Pilot	2nd Cohort	Combined
Identification set	Pilot	0.890 (1.049)	0.765 (0.918)	0.883 (0.808)
	2nd Cohort	0.825 (1.507)	0.818 (1.106)	0.813 (1.228)
	Combined	0.815 (1.316)	0.774 (1.023)	0.783 (1.056)

Table 1: table of the Pearson's correlation factors and (gradient) values between the fully sampled and the DISTq *SI* values for the various subgroups when the parameter generation equations derived from the various subgroups are used.

Discussion

The pilot-derived correlation (R=0.890) confirms the primary assertion that a physiologically relevant *SI* metric can be identified using only anatomical data and glucose samples, without requiring insulin and C-peptide measurements. This approach enables relatively low-cost, immediate or real-time identification of *SI*. Furthermore, the correlation between DIST SI and DISTq SI improved compared to the previously presented DISTq method [22] which used an ad-hoc method of *Uen* simulation. Overall, the high correlation ensures good accuracy relative to the fully sampled and high resolution DIST [20; 21]. However, this type of validation can only be used to identify the robustness of the identification method; it is not particularly well suited to identifying the predictive value of the method. In order to properly identify the predictive value of the test a comparison to a well-established or gold standard test must be made.

The self-validation correlations for the second and combined cohorts, where they are used to create the insulin estimates, were lower (R=0.818 and R=0.783 respectively). However, these values still represent a good overall result when considering that no insulin or C-peptide samples were assayed. Hence, testing these correlations on the data from which the estimate is derived, or self-validating, shows a good result. However, a self-validation is not clinically useful if tests are performed on a different cohort. Thus, the cross-validations performed in this study are more meaningful in evaluating clinical efficacy of the DISTq method. Best performance was achieved using the pilot cohort. A larger cohort of DIST results over an even wider range of SI values could potentially improve the quality of this approach.

The parameter estimation equations derived from the combined cohort enabled relatively high *SI* correlations for both the pilot and second cohort individually, but not as a combined cohort. This difference can be attributed to the differing DISTq to fully sampled *SI* gradients of the contributing cohorts, as shown in Figure 9. Both cohorts show good adherence to their respective regression lines, as exhibited in their individual correlations for this derivation set. However, when the results are combined, the differing gradients cause a reduction in correlation. Thus, as individual cohorts using the combined parameter estimation equations, there is a high correlation, but this correlation is not persistent when the results are combined.

Correlation coefficients measure the spread of a comparison, as well as the equivalence [28]. Thus, a targeted cohort on a reduced range of a wider population will deliver a correlation parameter less than what would be appropriate for the performance of the test in a wider context. The reduced cross-validation correlations of the second cohort show this effect as the distribution of *SI* in the second cohort was significantly reduced compared to the pilot cohort.

Finally, a high correlation does not explicitly imply a good predictive value for a test. Hence, equivalence is necessary as measured by the gradient (grad=1). The second cohort correlated well when the pilot parameter estimations were used, but the high gradient would mean that many false negative risk assessments could be made if using a fixed scale due to the bias (grad=1.507), as seen in Figure 6 (right).

Both the pilot and the second cohort performed best (in terms of correlation and adherence to the 1:1 line) using parameter estimations derived from their own full data sets, as should be expected. Some of this self-validation result can potentially be explained by the differing anatomical and physiological makeup between the two

cohorts. The pilot cohort was representative of the wider population but limited in numbers, whereas the second cohort was restricted to overweight (BMI>25) women or those who had an increased risk of developing T2DM. Thus, the DISTq method more accurately captures the lower average n_L values and higher first phase insulin secretion rate of the second cohort when using parameter estimations derived from the second cohort. The broader pilot cohort based estimates did better, even for the second cohort, than the second cohort estimates in terms of correlation, but the gradient of the second cohort reduced the method's predictive and diagnostic power.

If the DISTq were to be used in another unique, highly specific population, no such population specific parameter estimations would be available. Thus, a set of population driven parameter estimations must be selected from those previously available or reported. The pilot data is sparse compared to the second cohort, but generally allowed better correlations across all possible cohorts. Either set is capable of confidently predicting *SI* metrics with correlations in excess of 0.75. Overall, the results of this study indicate that the best results can be obtained using a broad cohort with a relatively even *SI* distribution as the basis for creating the parameter estimates for the DISTq. However, a denser broad cohort or estimates from a prior more specific cohort would, in the long term, be ideal.

More specifically, further fully sampled data sets may allow a more refined result and may point to the adoption of cohort specific parameter estimation equations. If sufficient fully sampled data sets become available, future DISTq methods may incorporate numerous, cohort specific equation sets, which could be selected by the subject's sex, age, diet, exercise regime, or family history of T2DM. However, such a

system would remove the operator independence of the current method. This study did not have the large number of data sets required to achieve this level of precision.

The DISTq is capable of high accuracy in *SI* estimation due to the insulin administration in the protocol. The exogenous insulin given during the test generally has a much greater effect on plasma insulin concentration than the participant's first phase insulin response (which is diminished by first pass hepatic extraction). As a result, the majority of the insulin in the participant's interstitium during the active period of the test can be attributable to the exogenous insulin bolus, which is a known amount given at a known time. The effect of the uncertainty in the population-derived parameter-estimation equations on the final *SI* metric is thus diminished. In addition, most kinetic parameters can be defined by a-priori published population parameter estimations [26]. Thus, much of the pharmaco-kinetics of insulin can be defined prior to testing. Further robustness is achieved with the relatively low-dose protocol, which avoids saturation effects.

Figures 3 and 7 show that the parameter relationship equations are not particularly accurate when considering the fit to all data points. However, these mathematical relationships are sufficient to, in the overall result, accurately predict *SI* in an isolated cohort (Table 1). Instead of using smooth and simple equations to estimate these relationships, multiple-term equations could have been used that could fit the noise in the relationships, and would likely provide higher intra-cohort correlations. However, the applicability of these multiple-term relationships in an isolated cohort would be limited, and could potentially reduce the performance of the test significantly.

A potential and significant strength of the DISTq is the ability to obtain a result for the fully sampled DIST using the stored blood samples, if desired. If a participant's *SI*

result lies close to a particular clinically specified *SI* threshold value, stored blood samples taken during the test can be assayed for insulin and/or C-peptide and a full DIST analysis can be performed. Thus, a high accuracy classification study could be performed wherein 90% of the participants could have results from the DISTq and the remaining 10% (for example) could have a high resolution fully sampled DIST without the need for a new test. This approach reduces cost over 90% as insulin and C-peptide assays are significantly more costly than glucose assays, and thus ensure the extra difference is only spent where there may be doubt on the resolution.

Regardless of these possibilities for improvement, the DISTq method currently allows relatively high resolution *SI* estimation in its own right. The cost of the DISTq in terms of assay cost and clinician time is comparable to the 2hr OGTT and HOMA, but the DISTq has a higher correlation to fully sampled DIST and similar *SI* tests. In contrast, to these other tests, the DISTq also measures a true insulin sensitivity at physiologically relevant doses, and does not rely on surrogate metrics. The accuracy of DISTq is lesser than that of fully sampled tests such as the EIC and the fully sampled DIST test, but reduces costs by over 90-95%. More importantly, unlike all of these tests, it allows real-time *SI* testing with a result immediately available before a subject leaves the clinic, as glucose can be assayed at the “bedside”, whereas insulin and C-peptide must be assayed in a laboratory, which typically requires 1-4 days to process.

This unique real-time capability of the DISTq may enhance treatment for individuals who require glycaemic stabilisation treatment. Newly diagnosed type 2 diabetic individuals often have a highly transient *SI*, and would benefit significantly from real-time assessment to enable more accurate insulin treatment regimes to be developed.

Many surrogate metrics tests have been endorsed for low-cost assessment of insulin sensitivity [29-32]. However, none of these tests have been incorporated into routine care. These surrogate metrics generally require both glucose and insulin samples from the 2-hour glucose tolerance test. Thus, the resultant metrics are not available in real-time. The time required for assays may be sufficient for changes in *SI* to render the identified metrics obsolete. In contrast, the presented protocol and assay schedule allow a result immediately after a 50 minute protocol. The presented method is an important advance in this field that should prompt re-evaluation of sensitivity testing for this particular application.

The unique real-time capability of this method may enable further possible applications. In particular, intensive care or high dependency patients could use accurate *SI* values to aid sepsis identification [33] or prior to beginning model-based or other forms of tight glycaemic control [34].

Finally the low-cost attribute of the method enables the obvious application in low-cost screening for early detection of type 2 diabetes risk. Tests of this nature have been used in such studies and found significant relationships [3; 35; 36], and it would be reasonable (in the absence of direct evidence) to expect that the proposed method would be appropriate for this type of application.

Conclusion

The DISTq method allows real-time, low-cost *SI* prediction using the DIST protocol, a series of population based parameter estimation equations, and the iterative integral identification method. The method produces *SI* metrics that highly correlate with the fully sampled DIST test.

The addition of the second cohort confirmed the applicability of the test in cohorts isolated from the development cohort via cross-validation. Cross-validation correlations were slightly reduced, but still relatively high given the large differences in the cohorts.

The low cost of the DISTq and its unique real-time capabilities ensure that it can be considered for a place amongst the myriad of available *SI* tests. The cost of the DISTq is comparable to the HOMA or 2hr OGTT while still measuring a physiologically relevant *SI*, and not relying on surrogate metrics. The ability to get a higher resolution DIST result with a different analysis of the same blood samples already obtained creates a simple, single test hierarchy of SI estimates, useful for both low cost screening and higher resolution monitoring of at risk individuals. Hence, it is potentially suitable for a wide range of clinical applications.

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