Evaluation of the Performances and Costs of a Spectrum of DIST Protocols

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Abstract: The strategic design of most insulin sensitivity (*SI*) tests maximises either accuracy or economy, but not both. Hence, accurate, large-scale screening isn't feasible. The DIST was developed to better optimize both important metrics. The highly flexible DIST protocol samples insulin, glucose and C-peptide during a comparatively short test. Varying the sampling periods and assays, and utilising alternative computational methods enables a wide range of tests with different accuracy and economy tradeoffs. The result is a hierarchy of tests to facilitate low-cost screening.

Eight variations of the DIST are evaluated against the fully-sampled test by correlating the *SI* and endogenous insulin production (*Uen(t)*) metrics. Five variations include sample and assay reductions and three utilise DISTq parameter estimations. The DISTq identification methods only require glucose assays and thus enable real-time analysis. Three DISTq methods were tested; the fully-sampled, the Short, and the 30 minute two-sample protocol. 218 DIST tests were completed on 84 participants to provide the data for this study.

Methods that assayed insulin replicated the findings of the full DIST particularly well (R=0.89~0.92) while those that assayed C-peptide managed to best replicate endogenous insulin metrics (R=0.72~1.0). The three DISTq protocols correlated to the fully-sampled DIST at R=0.83, 0.77 and 0.71 respectively.

As expected, test resolution increased with rising protocol cost and intensity. The ability of significantly less expensive tests to replicate the values of the fully-sampled DIST was relatively high (R=0.92 with four glucose and two insulin assays and 0.71 with only two glucose assays). Thus, an *SI* screening programme could achieve high resolution at a low cost by using a lower resolution DIST test. When an individual's result is close to a diagnostic threshold stored test samples could be re-assayed for more species to allow a higher resolution analysis without the need for a second invasive clinical test. Hence, a single test can lead to several outcomes with this hierarchy approach, enabling large scale screening with high resolution only where required with minimal and feasible economic cost and only a single invasive clinical procedure.

Keywords: Physiological Models, Parameter Identification, Diagnostic Tests, Dynamic Tests.

1. INTRODUCTION

Numerous investigations have found that insulin sensitivity (*SI*) is an important metabolic marker (Hanley et al. 2005; McLaughlin et al. 2007; Santaguida et al. 2005; Zimmet et al. 1999) and type 2 diabetes risk evaluator (DeFronzo & Ferrannini 1991; Ferrannini 1997; Harris et al. 2003; Martin et al. 1992). Generally, *SI* tests have either intense high-cost protocols that enable high resolution identification of *SI*, or lower intensity protocols that provide lower accuracy and cost (Ferrannini & Mari 1998; Pacini & Mari 2003).

Our group has previously presented the dynamic insulin sensitivity test (DIST) that was designed to fill the space between these contrasting design strategies (Lotz 2007; Lotz et al. 2008; McAuley et al. 2007). The DIST is a low-dose, short duration insulin-modified intra-venous glucose tolerance test, with comparatively infrequent sampling of 5-10 minutes (N<10). A pharmaco-kinetic/dynamic physiological (*PK/PD*) model uses insulin, glucose and C-peptide assays obtained during the DIST to identify participant-specific metrics for *SI*, first-pass liver extraction of insulin (x_L), liver clearance of plasma insulin (n_L) and endogenous insulin production profiles (*Uen(t)*). The *Uen(t)* profile yields valuable insight to the participant's beta-cell health, which is a critical aspect of the pathogenesis of type 2 diabetes (Ferrannini & Mari 2004; Pacini & Mari 2003).

The DISTq method is an evolution of the DIST identification method that utilises novel population based parameter estimations of insulin secretion and kinetics to identify *SI* in the absence of insulin or C-peptide assays (Docherty et al. 2009). It enables real-time analysis of *SI* because it uses only anatomical and glucose data that can be rapidly assayed at the place of testing. It is a faster, much less costly, but also less accurate test for *SI* screening.

A range of DIST and DISTq protocols could be designed to occupy the space between the fully-sampled DIST and the DISTq. Investigation of these variations would identify how many samples are required for accurate identification of *SI* and *Uen(t)*. This article presents and evaluates a spectrum of tests between the fully-sampled DIST and the DISTq with further extrapolations to extremely sparsely sampled tests. The overall outcome is a hierarchy of DIST-derived screening tests. A major advantage is that this hierarchy requires only one test per participant. When a participants result from a less expensive test is close to a threshold, stored blood samples can be re-assayed for different species to provide sufficient data for higher resolution and cost DIST identification methods. Thus, this approach enables screening at a low cost, but with high resolution.

2. METHOD

2.1 Participants

Eighty-two female participants from the Otago region of New Zealand took part in a longitudinal intervention investigation (ref). All participants had characteristics associated with an increased risk of developing T2DM (BMI, family history and/or ethnicity). In total, 218 full DIST tests were performed at 0, 4 and 10 weeks of a macro-nutrient intervention. Full details on participant demographics and study design can be seen in (TeMorenga et al. 2010).

2.2 Full DIST test protocol

Participants attended the place of testing in the morning after an overnight fast. Age, weight and height were recorded and signed informed consent was obtained prior to the first test. Weight was recorded prior to each subsequent test. The University of Otago Human Ethics Committee granted approval for this study.

Participants reported to the place of testing in the morning after an overnight fast. A canulla was placed in the antecubital fossa (a large vein in the inner elbow) for sampling blood and delivering boluses. Blood was sampled at t=0, 10, 15, 20, 25, 30, 35, 40, and 50 minutes. 10g glucose (50% dextrose) and 1U insulin (Actrapid^{TM}) were administered immediately after the t=10 and 20 minute samples respectively. Blood samples were assayed for glucose immediately, then spun and frozen for later insulin and C-peptide assays.

2.3 Design strategy of the various proposed protocols

Eight (reduced) variations of the DIST test protocol are evaluated by their ability to re-identify the *SI* value identified by the fully-sampled DIST. Each variation has differing advantages in terms of sample and cost reduction, with resulting different test resolutions. The 5 test protocols that utilise the DISTq (Docherty et al. 2009) identification methods limit the availability of patient-specific x_L, n_L and *Uen(t)* values. The sampling protocols, assay schedules, relative costs, and diagnostic parameters of the various tests, including HOMA and the fully-sampled DIST protocol, are defined in Table 1:

Fully-sampled: The fully-sampled protocol was designed by our group and is detailed in Lotz et al. (Lotz 2007; Lotz et al. 2008). It utilises all of the assay species (C-peptide, insulin and glucose) for every available sample time.

Short: The Short protocol was designed to capture all major dynamics of the three species with reduced test time and samples.

DIST-E/SI: The DIST-E/SI protocol was designed to enable accurate identification of *SI* and participant-specific *Uen(t)* metrics, while minimising sample assay cost. There are three significant metrics from a typical *Uen(t)* profile, the basal

	Sample times									**N-samples**			**Relative sample cost**	**Real-time**	**P-S *Uen(t)***
	0	**10***	**15**	**20^**	**25**	**30**	**35**	**40**	**50**	**G**	**I**	**C**			
Fully-sampled	GIC	GIC	*G*IC	GIC	G*I*C	GIC	GIC	GIC	GIC	**9**	**9**	**9**	**$562**	N	**Y**
Short	-	GIC	*G*IC	GIC	-	GIC	-	GIC	-	**5**	**5**	**5**	**$312**	N	**Y**
DIST-E/SI	-	GIC	*G*IC	GI	G	GIC	GI	GI	-	**6**	**6**	**3**	**$270**	N	**Y**
Sparse	-	GIC	*G*IC	-	-	-	GIC	-	-	**3**	**3**	**3**	**$187**	N	**Y**
DIST-SI	-	GI	I	GI	G	GI	GI	GI	-	**6**	**6**	0	**$165**	N	N
DIST-SI-2	-	G	-	G	-	GI	-	GI	-	**4**	**2**	0	**$60**	N	N
DISTq-FS	G	G	*G*	G	G	G	G	G	G	**9**	0	0	**$22**	**Y**	N
DISTq-S	-	G	*G*	G	-	G	-	G	-	**5**	0	0	**$12**	**Y**	N
DISTq-30	-	G	-	-	-	-	-	G	-	**2**	0	0	**$5**	**Y**	N
HOMA	-	GI	-	-	-	-	-	-	-	**1**	1	0	**$27**	N	N

Table 1. DIST sampling schedules for the estimation of *SI*. G, I and C represent glucose, insulin and C-peptide assays. Italics show a sample ignored by the specific test's parameter identification method but which may allow identification methods from other DIST protocols. Sample costs are estimated in NZD$ (glucose-$2.50, insulin-$25 and C-peptide-$35). The final columns show which protocols allow real-time analysis and participant-specific *Uen(t)* profiles.

production rate (U_b), the peak first-phase secretion (U_M), and the second-phase production (U_S). The three C-peptide assays chosen are the minimum possible to uniquely identify these three metrics.

Sparse: The Sparse protocol significantly limits the number of assays, minimising clinical intensity. Only three samples are taken, which can be used to define the three major *Uen(t)* metrics and a *SI*. The second sample is taken 5 minutes after the glucose bolus, and the glucose concentration at this point is affected by mixing and is not used (Edsberg et al. 1987; Lotz 2007). Instead, the glucose bolus magnitude and population estimates of glucose distribution volume are used to define the concentration increase caused by the glucose bolus.

DIST-SI: The DIST-SI protocol, identifies only *SI*, without any participant-specific *Uen(t)* metrics. Thus, no C-peptide, measurements are taken. The DISTq population-based parameter estimations (Docherty et al. 2009) are used to define the *Uen(t)* profile instead.

DIST-SI-2: The DIST-SI-2 further reduces assays and clinical intensity by taking less samples and performing less assays than the DIST-SI protocol. The period of greatest importance to *SI* identification is the later part of the test protocol. Thus, only the two samples taken at the end of the test are assayed for insulin, while the full glucose response is identified with the four glucose assays.

DISTq-FS: The DISTq-FS utilises all of the available glucose samples to define *SI* in an iterative process. The method utilises only glucose samples and anatomical data to identify a *SI*. The DISTq-FS has been shown to replicate fully-sampled DIST *SI* values (Docherty et al. 2009; Docherty et al. 2010). That analysis is repeated here in brief to allow a complete comparison.

DISTq-S: This protocol mirrors the Short DIST and uses only four glucose samples to define a value for *SI*. The second glucose sample (at t=15) is not used by the identification method. However, taking this sample may allow for later analysis of the other species to obtain metrics for first-phase insulin production or to increase resolution of the result in a possible hierarchy of tests.

DISTq-30: The DISTq-30 aims to identify *SI* from very sparse data. Only two glucose samples are taken.

2.4 Test Hierarchy

The sampling schedule of the various protocols could allow more, or less, assays from the samples taken during one test to enable differing analyses. For example, the sampling protocol of the DIST-SI could be followed yielding 7 blood samples. Assaying only 2-4 of them would enable a DISTq-30 or DISTq-S analysis. However, if greater resolution were required to obtain an accurate diagnosis, stored samples could be re-assayed for insulin and/or C-peptide, as well as glucose where not done previously, to obtain a DIST-SI or Short DIST result (with *Uen(t)* metrics).

This approach increases storage, but minimises cost for participants who can be diagnosed with a lower resolution test. Additionally only one clinically invasive procedure is required for the participant. Table 2 shows all the potential outcomes for each sampling protocol defined.

	Analyses possible with samples								
Protocol completed	Fully-sampled	Short	DIST-E/SI	Sparse	DIST-SI	DIST-SI-2	DISTq-FS	DISTq-S	DISTq-30
Fully-sampled	-	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Short	N	-	N	N	N	**Y**	N	**Y**	**Y**
DIST-E/SI	N	**Y**	-	**Y**	N	**Y**	N	**Y**	**Y**
Sparse	N	N	N	-	N	N	N	N	N
DIST-SI	N	**Y**	**Y**	**Y**	-	**Y**	N	**Y**	**Y**
DIST-SI-2	N	N	N	N	N	-	N	**Y**	**Y**
DISTq-FS	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	-	**Y**	**Y**
DISTq-S	N	**Y**	N	N	N	**Y**	N	-	**Y**
DISTq-30	N	N	N	N	N	N	N	N	-

Table 2. Potential for different assay regimes to allow analyses with identification methods from other protocols.

2.5 Identification methods of the proposed protocols

The *Uen(t)* profiles for the various protocols are either defined using deconvolution (DC) or the population based estimates of the DISTq method (E_{DISTq}). The deconvolution method was developed by Eaton et al. (Eaton et al. 1980) and validated by Van Cauter et al. (Van Cauter et al. 1992). It has previously been used with the DIST (Lotz 2007; Lotz et al. 2008; McAuley et al. 2007). The DISTq methods and the population based estimates have been published previously (Docherty et al. 2009; Docherty et al. 2010). The final blood sample of the DIST-E/SI is not assayed for C-peptide, and the *Uen(t)* rate is assumed to be constant from the final point.

Insulin concentrations in the plasma and interstitium are either defined using the iterative integral method (IIM) (Docherty et al. 2009; Hann et al. 2005) or the DISTq methods. (The DIST-SI-2 uses the DISTq parameter estimation for basal insulin (I_b), and IMM to identify n_L with a fixed x_L)

Protocols which require DISTq parameter estimations for n_L, have x_L fixed at an average population value of 70% (Cobelli et al. 1998; Ferrannini & Cobelli 1987; Meier et al. 2005; Toffolo et al. 2006). Glucose related parameters are identified with the iterative integral method. The Sparse and DISTq-30 protocols do not have sufficient glucose data to identify the volume of glucose distribution (*Vg*). In these cases, it is estimated as a proportion (29%) of the lean body mass as calculated by (Hume 1966).

Table 3 summarises for which protocol each identification method is used.

	Uen(t)	Insulin	Glucose
Fully-sampled	DC	IIM	IIM
Short	DC	IIM	IIM
DIST-E/SI	DC*	IIM	IIM
Sparse	DC	IIM	IIM*
DIST-SI	E_{DISTq}	IIM	IIM
DIST-SI-2	E_{DISTq}	IIM-E_{DISTq}*	IIM
DISTq-FS	E_{DISTq}	E_{DISTq}	IIM
DISTq-S	E_{DISTq}	E_{DISTq}	IIM
DISTq-30	E_{DISTq}	E_{DISTq}	IIM*

Table 3. Identification methods for the various protocols. * indicates that the identification method must be adjusted to account for sparse sampling.

2.6 Analysis

The *SI*, U_b, U_M and U_S values from the protocols are compared to the same values obtained from the fully-sampled DIST protocol using Pearson's correlation coefficients and the gradients of the regression lines. The gradients allow a comparison of the proportional shift of identified metrics. The liver clearance of insulin parameters (n_L, x_L) have limited clinical diagnostic use and are not presented. Equation 1 is used to force the regression line through the origin to obtain a true proportional ratio between metric values (V) from the any given protocol:

$$G = \left\| V_{given} \right\|_2 \Big/ \left\| V_{fullysampled} \right\|_2 . \qquad (1)$$

The homeostasis assessment model (HOMA) is also compared to the fully sampled DIST as it is an established, sparsely sampled fasting metric.

3. RESULTS

Table 4 summarises the performance of all the proposed protocols with respect to their ability to replicate the *SI* and *Uen(t)* values identified using the fully-sampled DIST.

The sparser DIST-SI-2 method showed the greatest ability to replicate the *SI* metrics of the fully-sampled DIST by a small margin. It was closely followed by DIST-SI, the Short protocol, DIST-E/SI and the Sparse protocol. DISTq methods showed an expected, lesser ability to replicate *SI*. However, DISTq results were in line with previous findings (Docherty et al. 2009; Docherty et al. 2010) and represent a sound result.

DISTq-S and DISTq-30 correlated highly to DISTq-FS: R=0.938 and R=0.893 respectively. When considering the vast reduction in samples between the DISTq-FS and DISTq-30, R=0.893 indicates strong stability and robustness.

Protocols that sampled basal and first-phase C-peptide showed absolute equivalence U_b and U_M from the fully-sampled DIST. Reducing the number of C-peptide samples had a greater effect on U_S. The DISTq population estimates were strongest for U_S, weaker for U_b, and poor for U_M, although the gradient of 0.8 implies that the general magnitude of the U_M predictions were accurate.

HOMA showed a relative inability to replicate the insulin sensitivity metrics of the fully sampled DIST.

	SI **R**(G)	$\boldsymbol{U_b}$ **R**(G)	$\boldsymbol{U_M}$ **R**(G)	$\boldsymbol{U_S}$ **R**(G)
DIST	**1**(1)	**1**(1)	**1**(1)	**1**(1)
Short	**0.904** (1.17)	**1**(1)	**1**(1)	**0.885** (0.99)
DIST-E/SI	**0.901** (1.10)	**1**(1)	**1**(1)	**0.716** (1.12)
Sparse	**0.888** (1.03)	**1**(1)	**1**(1)	**0.881** (0.95)
DIST-SI	**0.908** (1.10)	**0.622** (0.94)	**0.073** (0.80)	**0.751** (0.90)
DIST-SI-2	**0.922** (1.07)	**0.683** (0.97)	**0.09** (0.81)	**0.736** (0.99)
DISTq-FS	**0.834** (1.10)	**0.563** (0.94)	**-0.074** (0.80)	**0.697** (0.90)
DISTq-S	**0.767** (1.27)	**0.526** (0.92)	**-0.137** (0.80)	**0.692** (0.89)
DISTq-30	**0.713** (1.24)	**0.527** (0.98)	**-0.143** (0.80)	**0.708** (1.04)
HOMA	**-0.351** (-)	-	-	-

Table 4. Ability of protocols to replicate *SI* and *Uen(t)* values from the fully-sampled DIST.

4. DISCUSSION

Relatively high correlations (R~0.9) between protocols that assayed insulin and the fully-sampled test show that the limited sampling protocols could be used as surrogates for the fully-sampled test without significantly diminishing test resolution. In particular, only three samples during a 25 minute protocol in the Sparse protocol correlated relatively well to the fully-sampled test (R=0.888) and captured all major dynamics of the *Uen(t)* profile.

DISTq results also showed a strong ability to replicate the *SI* value identified by more intense and costly fully-sampled methods. The DISTq-FS method performed in accordance with the previously published findings (Docherty et al. 2010). DISTq-S and DISTq-30 also correlated relatively well to the fully-sampled DIST, particularly compared to the well-accepted HOMA. These results suggest that they could also be used as surrogate *SI* tests when there is a reduced resolution requirement, such as in preliminary T2DM or metabolic risk screening. The DISTq was not designed to identify *Uen(t)* and, as such, the resolution of these metrics was not sufficient to enable any beta-cell diagnostic value from these three tests.

Most importantly, this spectrum of tests can be used in a hierarchy. In particular, the low-cost DISTq-S could be used in a metabolic risk screening programme. When a participant's result is close to a diagnostic threshold, stored blood samples could be assayed for insulin and/or C-peptide. These added assays would cost more, but would enable identification from either the Sparse or Short protocol to find new, higher resolution *SI* and *Uen(t)*, per Table 2.

For example, if approximately 20% of a screening programme's participants produce *SI* values close to a diagnostic threshold with the DISTq-S, the samples already obtained from the 20% of ambiguous tests could be re-analysed using more assays to enable the Short protocol identification methods. Thus, a higher resolution more accurate result is achieved. With a sample cost of $12 for a DISTq-S and $312 for the Short protocol, the average sample cost per test for a programme with diagnostic resolution equivalent to the Short protocol would be $72/participant.

This test evaluation was limited by the available data. Some such test evaluations are made with cross-over studies, which may include comparisons to gold standard tests. In this case, a self evaluation was used, wherein no inter-test repeatability was possible, and the results were thus potentially enhanced by the study design. In contrast, daily *SI* variations and assay errors did not reduce the correlations as they might in cross-over, inter-protocol studies.

Future investigations of alterations to the sampling regime could enhance the economy or information gained from the DISTq-styled protocols. If the glucose and insulin samples were combined, *SI* identification (with accuracy that is likely to be comparable to the DISTq-30) could be obtained from a single 20 minute test. Furthermore, if there were more time and samples between the glucose and insulin boluses, a patient-specific first-phase response to glucose could be estimated using only glucose data. However, these possibilities could not be investigated presently, as no such clinical data is yet available.

5. Conclusion

This article has presented a spectrum of tests from information rich, relatively frequently sampled, repeatable tests, to very sparsely sampled tests that produce limited information and moderate result accuracy. The accuracy and information available from dynamic tests is inherently linked to the cost in terms of protocol time, clinician intensity and assay cost. However, the results from the DIST-SI-2, Sparse and DISTq-30 protocols have shown that relatively high accuracy (R=0.92, R=0.89 and R=0.71 to the fully-sampled protocol respectively) is possible at the lower end of cost spectrum.

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