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ORIGINAL ARTICLES

Design and Clinical Pilot Testing of the Model-Based Dynamic Insulin Sensitivity and Secretion Test (DISST)

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Abstract

Background:
Insulin resistance is a significant risk factor in the pathogenesis of type 2 diabetes. This article presents pilot study results of the dynamic insulin sensitivity and secretion test (DISST), a high-resolution, low-intensity test to diagnose insulin sensitivity (IS) and characterize pancreatic insulin secretion in response to a (small) glucose challenge. This pilot study examines the effect of glucose and insulin dose on the DISST, and tests its repeatability.

Methods:
DISST tests were performed on 16 subjects randomly allocated to low (5 g glucose, 0.5 U insulin), medium (10 g glucose, 1 U insulin) and high dose (20 g glucose, 2 U insulin) protocols. Two or three tests were performed on each subject a few days apart.

Results:
Average variability in IS between low and medium dose was 10.3% ($p = .50$) and between medium and high dose 6.0% ($p = .87$). Geometric mean variability between tests was 6.0% (multiplicative standard deviation (MSD) 4.9%). Geometric mean variability in first phase endogenous insulin response was 6.8% (MSD 2.2%). Results were most consistent in subjects with low IS.

Conclusions:
These findings suggest that DISST may be an easily performed dynamic test to quantify IS with high resolution, especially among those with reduced IS.

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Abbreviations: (AUC) area under curve, (CV) coefficient of variation, (DISST) dynamic insulin sensitivity and secretion test, (EGP) endogenous glucose production, (EIC) euglycemic hyperinsulinemic clamp, (IFG) impaired fasting glucose, (IR) insulin resistance, (IS) insulin sensitivity, (ITT) insulin tolerance test, (IVGTT) intravenous glucose tolerance test, (MSD) multiplicative standard deviation, (NGT) normal glucose tolerance, (PK) pharmacokinetic, (SD) standard deviation, (T2DM) type 2 diabetes mellitus

Keywords: insulin resistance, insulin sensitivity, physiological modeling, pilot study, type 2 diabetes diagnosis

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Introduction

Insulin resistance (IR) is a key underlying abnormality in type 2 diabetes mellitus (T2DM) and a major risk factor for cardiovascular disease.[1,2] A longterm follow-up study by Martin and colleagues.[3] reported that 10 years ahead of a formal diagnosis of T2DM, those who developed the disease had a 60% higher mean IR than those who did not. McLaughlin and coworkers.[4] found that among obese individuals, IR is the strongest predictor of subsequent T2DM and cardiovascular disease risk.

Insulin sensitivity (IS = 1/IR) is not a discrete metric, but represents an attempt to quantify insulin-mediated glucose utilization. The relative contributions of the three major determinants of overall IS (peripheral sensitivity, hepatic sensitivity, and β-cell function) vary according to whether an individual is in the fasting or postprandial state and may change over time as the disease state progresses.[5] Methods of assessment vary in their ability to determine one, two, or three of the contributors, thus generating potentially discrepant results requiring careful interpretation.[6]

The euglycemic hyperinsulinemic clamp (EIC)[7] is the gold standard for assessing insulin sensitivity. It measures peripheral sensitivity by suppressing endogenous glucose production (EGP) and endogenous insulin secretion using high-dose infusions of insulin and glucose. Due to its complexity and duration,[6,8] simpler methods have arisen, including the insulin tolerance test (ITT)[9] and the intravenous glucose tolerance test (IVGTT) with minimal model assessment.[10] These tests have not achieved wide acceptance in a clinical environment given that they are too time consuming and complex and do not correlate particularly well with the EIC.[8,9] Other attempts at sample-reduced (12-sample),[11] or shorter (40-minute)[12] IVGTT protocols had the same model identification problems as the standard IVGTT,[13] as they too are based on minimal model assessment. Simple, fasting assessments such as the homeostasis model assessment (HOMA)[14] and the quantitative insulin sensitivity check index (QUICKI),[15] are appealing for large studies, however they assess combined hepatic and peripheral sensitivities in the fasting state, have poor reproducibility, and do not correlate well with the EIC. A sensitive, simple, repeatable measure of insulin sensitivity would have considerable value in clinical and research contexts, and in evaluating the impact of interventions.[16]

The dynamic insulin sensitivity and secretion test (DISST) is a dynamic test with mathematical model assessment, similar to the insulin-modified IVGTT. The integrated design of the clinical protocol, mathematical model, and data-fitting methods enable a shorter test duration, more physiological dosing, less frequent sampling, and higher robustness, compared with the EIC or IVGTT. In addition to a combined metric for hepatic and peripheral insulin sensitivity, detailed information about β-cell function can also be obtained.[17] During DISST development, a strong emphasis was put on practical aspects of the protocol and clinical applicability, which differentiates it from the IVGTT. A more detailed explanation of the test design considerations and differences to the IVGTT are given in **Appendix A**.

The DISST was designed and tested in Monte Carlo simulation studies[18] and has been shown to have good accuracy in repeatability with an intraindividual coefficient of variation (CV) of 4.5% (90% CI: 3.8–5.7%). As no simulation study can fully reproduce all metabolic effects in such a dynamic test, limited *in vivo* testing was required prior to the design of a full validation study. This pilot study was undertaken to qualitatively verify these simulation results *in vivo*, to assess the effect of glucose and insulin dosing on the outcome metrics, and to get an indication of the repeatability of the test in an outpatient setting. This pilot study was not intended to deliver a fully powered result on the DISST's performance, but rather deliver an indication of the feasibility of the test prior to a larger validation study against the EIC. A power calculation for a full validation study comparing the DISST to the EIC is proposed based on this study's results.

Methods

Subjects

A total of 16 adult volunteers were recruited by advertisements in the hospital and word of mouth. Subject 12 did not complete the full study protocol and was excluded from all further analysis. Insulin samples in two tests (two on subject 6 and two on subject 9) were exceptionally high, suggesting sampling errors and were therefore excluded. Subject 9 had to be excluded completely, as only a single remaining test was available. One subject was previously diagnosed with T2DM and on metformin

treatment. Medication was stopped a day prior to the testing. Written informed consent was obtained from all subjects, and height, weight, and family history of diabetes recorded. Subject characteristics are summarized in **Table 1**.

Study Design

All tests were performed at the Christchurch School of Medicine or the Department of Human Nutrition, University of Otago using exactly the same protocol. The clinical pilot study of the DISST aimed to investigate two aspects:

- Part 1: Effect of glucose and insulin dose on test outcome
- Part 2: Repeatability of the test at the same dose

In Part 1, the subjects had two tests on different days (3–8 days apart) using different glucose and insulin doses. Three dosing regimens were used: 5 g glucose and 0.5 U insulin (low), 10 g glucose and 1 U insulin (medium), or 20 g glucose and 2 U insulin (high). Each subject had a combination of either low/medium or medium/high dose tests.

In Part 2, the subjects had two tests (3–14 days apart) using the same glucose/insulin dose. Some subjects had three tests and were included in both parts of the study by repeating one of the dosing options. The order of the tests on each individual was picked randomly, and **Table 1** shows the doses given to each subject.

Experimental Protocol

The tests were performed in the morning after an overnight fast. A cannula was inserted in the antecubital fossa for venous blood sampling and administration of glucose and insulin. The catheter was flushed with saline after every sampling or injection step to reduce sample contamination. Two baseline blood samples were taken at t = -10 min and t = 0 min. Glucose (50% dextrose) was administered at t = 0 min, and insulin (Actrapid, Novo Nordisk, Copenhagen, Denmark) at t = 10 min. Blood samples were taken at t = 5, 10 15, 20, 25, 30, 35, and 45 min to assess the physiological response to the administered glucose and insulin. Blood samples were assayed for plasma glucose, insulin, and C-peptide concentrations. Glucose was analyzed by an enzymatic

Table 1.
Subject Characteristics and Tests Performed on Each Subject.

Subject	Gender	Age (year)	Weight (kg)	BMI (kg/m^2)	Fasting glucose (mg/dl)	Fasting insulin (pmol/liter)	T2DM or IFG[a]	Tests			Part	
								5 g 0.5 U	10 g 1 U	20 g 2 U	1	2
1	f	57	89	33.9	104.4	213.9	IFG		1	1	X	
2	f	59	67	25.5	106.2	9.7	IFG		1	1	X	
3	f	59	87	39.2	84.6	86.8			3			X
4	f	21	78	25.2	90.0	36.1		1	1		X	
5	m	41	76	21.7	72.0	3.5			2	1	X	X
6	f	45	76	25.4	73.8	11.8			2	1	X	
7	m	55	73	24.1	81.0	30.6		1	1		X	
8	f	51	67	27.2	77.4	9.7		1	1		X	
9	f	35	66	24	86.4	45.8			1	1	X	
10	f	30	50	19.5	75.6	22.2		2	1		X	X
11	f	55	85	30.1	122.4	63.9	T2DM	2	1		X	X
12	m	60	76	23.7	79.2	22.2			1			
13	f	48	91	33.4	93.6	66.0			3			X
14	f	41	111	41.3	81.0	27.1			2	1	X	X
15	m	29	84	25.9	91.0	17.4		2	1		X	X
16	m	49	105	35.1	113.4	115.3	IFG	2	1		X	X

[a] IFG denotes subjects who were not diagnosed with T2DM but had elevated fasting glucose levels >100 mg/dl, qualifying for a diagnosis of IFG on the day of the test (per American Diabetes Association guidelines[19]).

glucose hexokinase assay (C8000 Analyzer, Abbott Laboratories, Inc, Abbott Park, IL). Insulin and C-peptide were analyzed with an electrochemiluminescence (ECLIA) immunoassay (Roche Diagnostics Elecsys, Mannheim, Germany).

Modeling and Data Analysis

Sampled concentration profiles were analyzed by fitting metabolic models of glucose, insulin, and C-peptide to the data, as described in detail in **References 18**, **20** and **21**, and in **Appendix B**. The estimated model parameter value for IS, S_I, was used to describe the body's insulin sensitivity. In addition to IS, information about β-cell function (basal secretion, first phase response) and hepatic insulin clearance were obtained.

For added robustness, glucose samples taken within 10 minutes of glucose injection, and insulin samples taken within 10 minutes of insulin administration, were disregarded in the model fit to minimize errors introduced by effects of intravascular mixing.[22] This approach avoids overfitting of measurement errors, which can cause considerable parameter estimation problems.[13,21]

Statistical Analysis

The interdose repeatability of Part 1 of this study was calculated as the relative percentile difference in the insulin sensitivity parameter, S_I, of the higher dose test compared to the lower dose test, as shown in **Equation 1**. The mean result was taken if more than one test was done at a given dose.

$$\Delta S_I = \frac{\overline{S_{I\ higher}} - \overline{S_{I\ lower}}}{\overline{S_{I\ lower}}} \quad (1)$$

The variability in S_I at a given dose for Part 2 is defined as the maximum deviation from the mean S_I, divided by the mean S_I, as shown in **Equation 2**.

$$\Delta S_I = \max\left[\frac{\mathrm{abs}(S_{I1\ldots n} - \overline{S_I})}{\overline{S_I}}\right] \quad (2)$$

Where data distribution was normal, the mean and standard deviation (SD) were used to describe spread. Where the distribution was log-normal, the geometric mean and multiplicative standard deviation (MSD) were used. Statistical significance of the differences was assessed with the two sample Student's *t*-test.

Accuracy of the DISST was compared to the intraindividual CV in S_I, defined as the ratio of SD over the mean S_I (CV = SD/mean–S_I), simulated by Monte Carlo analysis on a virtual cohort generated from 146 euglycemic clamp tests.[18] The CV derived from the Monte Carlo analysis gives an indication of expected accuracy in a clinical testing environment. By comparing the simulated CV with the experimentally derived accuracy, an estimate is obtained of the variability attributable to other physiological factors not completely accounted for by the simulation method. In this pilot study, a meaningful intraindividual CV in S_I could not be calculated because only two or three tests were performed on each subject. Instead, the absolute deviations of the test results ΔS_I were compared to the range defined by ±2 SD (95% of subjects) obtained from the Monte Carlo results.[18] Despite this limitation, this comparison aimed to deliver an indication of the achievable accuracy in an *in vivo* environment, and the validity of the prior simulation study.

Ethical approval for the study was granted by the Upper South A Regional Ethics Committee.

Results

Part 1: Effect of Dosing

The estimated IS parameter, S_I, is shown in **Table 2** for Part 1 (by dose combination), along with basal insulin secretion rate u_B, first-phase insulin-secretion area under curve AUC_{10}, and peak secretion rate S_{max}. Differences in S_I, AUC_{10}, and S_{max} shown (denoted by Δ) are percentile difference of the higher dose result compared to the lower dose result.

Estimated S_I was lower in 8 of 12 subjects at the higher dose test, but the differences were not statistically significant (low/medium p = .50, medium/high p = .87). A noticeable reduction in the impact of dosing could

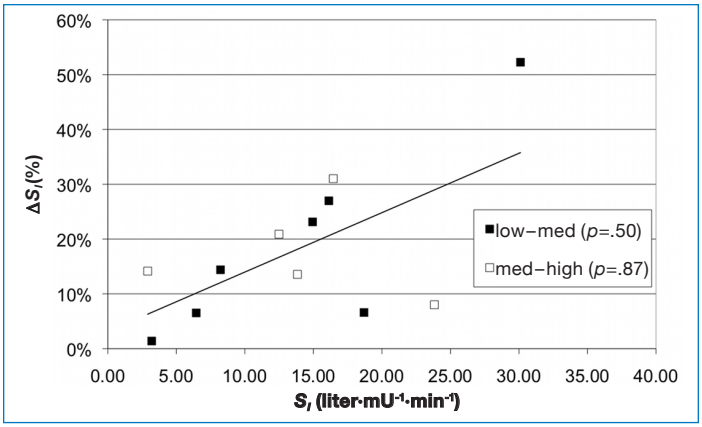

Figure 1. Part 1: Dose-dependent variability in insulin sensitivity S_I as a function of S_I. Black squares show relative percentile differences in estimated S_I values between the low and medium dose protocols, white squares between the medium and high dose protocols.

Table 2.
Results from Model Fit to Experimental Data from Part 1 of the Study.[a]

Subject	Dose	S_I (liter/mU/min)	ΔS_I (%)	u_B (pmol/min)	AUC_{10} (pmol)	ΔAUC_{10} (%)	S_{max} (pmol/min)	ΔS_{max} (%)
Low/medium (p = .50)								
4	5 g	13.39		136.1	1536		246	
	10 g	16.49	23.1	145.2	1764	14.8	292	18.8
7	5 g	19.33		172.9	2910		748	
	10 g	18.06	-6.6	171.5	5458	87.5	1061	41.9
8	5 g	18.64		79.2	2638		608	
	10 g	13.61	-27.0	88.9	5782	119.1	1327	118.0
10	5 g	43.73		95.1	3330		745	
	10 g	17.40		93.8	4364	25.8	1040	
	5 g	29.19	-52.3	108.3	3611		852	30.2
11	5 g	6.88		251.4	1400		189	
	10 g	6.73		235.4	1574	16.2	203	
	5 g	5.75	6.5	293.1	1308		220	-0.9
15	5 g	8.28		138.9	2776		795	
	10 g	7.39		144.5	4501	69.4	1007	
	5 g	8.99	-14.4	153.5	2538		728	32.3
16	5 g	3.27		435.5	1702		299	
	10 g	3.17		459.8	4011	213.8	569	
	5 g	3.16	-1.4	395.9	856		178	138.6
Mean			-10.3			78.1		54.1
SD			24.3			71.7		52.7
Medium/high (p = .87)								
1	10 g	3.13		492.4	2732		435	
	20 g	2.69	-14.1	478.5	7133	161.2	1222	180.9
2	10 g	19.47		61.8	1214		226	
	20 g	13.43	-31.0	83.3	1563	28.8	271	20.0
5	10 g	26.45		99.3	2851		529	
	20 g	25.07		75.7	3918	48.5	905	
	10 g	19.97	8.0	77.1	2425		481	79.3
6	10 g	14.84		118.1	2694		440	
	20 g	12.83	-13.6	119.5	3851	60.9	563	27.9
14	10 g	11.70		146.5	4837		1278	
	20 g	14.12		132.6	4630	-7.1	1003	
	10 g	11.65	20.9	179.2	5129		1252	-20.7
Mean			-6.0			58.5		57.5
SD			20.4			62.9		77.6

[a] S_I, insulin sensitivity; ΔS_I, change at higher dose; u_B, basal insulin secretion rate; AUC_{10}, total first phase insulin secretion; ΔAUC_{10}, change at higher dose; S_{max}, peak insulin secretion rate; ΔS_{max}, change at higher dose.

be seen in subjects with lower IS, as shown in the correlation plot in **Figure 1**. Basal insulin secretion u_B was consistently higher in subjects with lower S_I. Total first phase insulin secretion above basal, AUC_{10}, was increased at the higher dose in all but 1 subject, with a wide range in changes of -7.1 to 213.8%. The same was the case for the difference in peak secretion rate, S_{max}, which was in the range of -20.7 to 180.9%, and positive for all but 2 subjects.

Part 2: Repeatability

The study population for Part 2 consisted of 8 subjects; 4 completed two low-dose tests, and 4 completed two or three medium-dose tests. The estimated IS parameter, S_I, error in S_I and insulin secretion metrics are given in **Table 3**.

Variations in S_I were in the range of 0.2 to 24.7% with a geometric mean of 6% (MSD 4.9%). The repeat tests at each dose were not significantly different compared to the first tests (low dose p = .75, medium dose p =.56). Insulin secretion metrics were very consistent, with repeatability in basal secretion rate u_B in the range of 2.6 to 11.7%. Total first phase insulin AUC_{10} was estimated with high accuracy in repeatability, with a geometric mean value of 6.8% (MSD 2.2%) and a range of 2.9–33.1%, and repeatability in S_{max} resulted in a geometric mean of 7.4% (MSD 2.8%), with a range of 1.0–25.3%. The dependency of dosing on IS in Part 1 was evident in repeatability accuracy as well, but was less marked across the S_I range, as shown in **Figure 2**.

Diagnostic Relevance

Results from the full test protocol analysis on three subjects, including normal glucose tolerance (NGT), impaired fasting glucose (IFG), and T2DM is shown in **Appendix C** with a full discussion of the potential diagnostic relevance.

Table 3.
Results from Model Fit to Experimental Data from Part 2 of the Study.[a]

Subject	S_I (liter/mU/min)	ΔS_I (%)	u_B (pmol/min)	AUC_{10} (pmol)	ΔAUC_{10} (%)	S_{max} (pmol/min)	ΔS_{max} (%)
Low dose (5 g glucose, 0.5 U insulin) (p =.75)							
10	43.73		95.1	3330		745	
	29.19	19.9	108.3	3612	4.0	852	6.7
11	6.88		251.4	1400		189	
	5.75	8.9	293.1	1308	3.4	220	7.6
15	8.28		138.9	2776		795	
	8.99	4.1	153.5	2538	4.5	728	4.4
16	3.27		435.5	1702		299	
	3.16	1.7	395.9	856	33.1	178	25.3
Medium dose (10 g glucose, 1 U insulin) (p = .56)							
3	10.18		236.8	8390		1679	
	8.59		269.5	9892		1879	
	7.37	16.8	300.0	9140	8.2	2195	14.4
5	26.45		99.3	2851		529	
	19.97	14.0	77.1	2425	8.1	481	4.7
13	16.31		247.2	3155		506	
	13.51		251.4	3782		845	
	21.20	24.7	236.8	4125	11.9	706	23.2
14	11.70		146.5	4837		1278	
	11.65	0.2	178.5	5129	2.9	1252	1.0

[a] S_I, insulin sensitivity; ΔS_I, change at higher dose; u_B, basal insulin secretion rate; AUC_{10}, total first phase insulin secretion; ΔAUC_{10}, change at higher dose; S_{max}, peak insulin secretion rate; ΔS_{max}, change at higher dose.

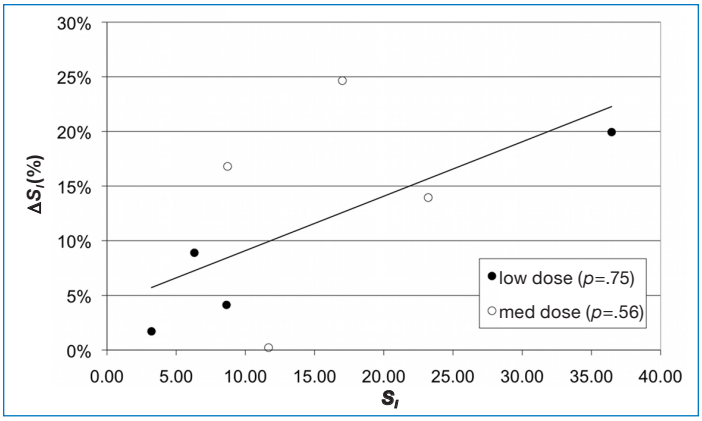

Figure 2. Part 2: Intradose variability in S_I. Accuracy in repeatability of estimated insulin sensitivity S_I as a function of S_I. Black circles show relative percentile differences around the mean of estimated S_I values during the low dose protocol, white circles during the medium dose protocol.

Discussion

The goal of this pilot study was to assess the feasibility and performance of the DISST in a clinical setting. The modeling and data fitting methods were customized to a clinical protocol to allow robust parameter identification and avoid the problems encountered with the IVGTT.[13,23,24] The study demonstrated a high level of acceptability of the test to participants, the only complaint being mild discomfort during the injection of 20 g glucose, probably due to the large volume injected within a short time frame. This did not occur at lower doses.

The protocol and fitting algorithm proved to be reliable and robust. In Part 1 of the study, estimated S_I was lower in 8 of 12 subjects in the higher dose test as compared with the lower dose test, but the difference was not statistically significant (p = .50, p = .87). This effect was also found by Prigeon and associates.[25] who reported lower IS values when an IVGTT was performed at different doses. In that study, injecting 4 U of insulin resulted in a 32% reduced IS value compared with injecting 2 U of insulin. A possible explanation could be saturation effects, which have been identified in other studies.[8,25,26] Saturation effects are less likely at lower doses, and this aspect could be improved by adding saturation dynamics to the model.[26,27]

This pilot study does not permit definitive conclusions with regard to optimum dose. A higher dose provides a stronger signal in the sampled concentration profiles but encounters stronger saturation effects and triggers stronger suppression of EGP, thus adding unknown variability. Suppression of EGP cannot be measured easily and thus is not accounted for in the model. On the other hand, a lower dose is less likely to be affected by saturation and counterregulatory responses but might be too small to provide an optimum signal. Lower doses are likely to be more physiological and involve less discomfort to the subject. In the clinical context, consistency is useful and a low to medium dose is probably the best choice.

The reason for the choice of a single dose across all subjects is practical, as it would allow a test kit to be compiled prior to knowing the subject's characteristics. This consistency is particularly useful in routine clinical testing environments. It is debatable whether a patient-specific dose calculation should be used in such a test. However, in this study, differences in estimated S_I at different dosing in the same subject had a stronger effect on lighter subjects with a body weight of less than 70 kg, in which estimated S_I was much lower at the higher dose. On all other subjects the effect was not systematic. It is unclear whether this effect is caused by the difference in weight or the fact that these lighter subjects were very insulin sensitive and thus more sensitive to assay error or measurement noise. A larger study is required to further analyze this aspect.

A further factor that could influence insulin sensitivity in a person is pain induced by the protocol, such as cannulation or administration of large volume 50% glucose solution. Pain has been shown to affect insulin sensitivity[28,29] and would add an unknown inaccuracy to the assessment. In this study, one person experienced discomfort during administration of 20 g of glucose, but not in the lower dose. By using a lower, more physiological dose, and more diluted glucose solutions, this effect could potentially be mitigated.

Part 2 assessed repeatability by performing the same low or medium dose test on each subject two or three times. Errors around the mean in each subject were in the range of 0–25%, and log-normally distributed, with a geometric mean of 6.0% (MSD 4.9%). The expected intraindividual accuracy assessed by the Monte Carlo simulation[18] resulted in a mean CV_{SI-MC} = 4.5% (90% CI: 3.8–5.7%) at the medium dose, CV_{SI-MC} = 6.9% (90% CI: 4.9–9.9%) at the low dose, and CV_{SI-MC} = 3.6% (90% CI: 3.0–4.5%) at the high dose. In other words, considering ±2 SD, an absolute deviation between 6.0–19.8% from the mean could be attributed to assay and protocol errors in ~95% of subjects. This outcome was also reflected in the hypothesis testing (p = 0.75, p = 0.56), indicating that repeatability of the DISST was good, even with the limited small sample size of this study.

Natural variability in IS, which was not included in the Monte Carlo simulation, could have been a source of additional variability in this pilot study.[30] Results of this study were thus in good accordance with the Monte Carlo simulation results, though possibly slightly more variable due to additional sources of variability, such as time of day,[30] state of health,[31,32] menstrual cycle,[33] or exercise.[34,35] Glucose samples were analyzed in the lab with an assay CV = 1–2%, similar to that simulated in the Monte Carlo study. If point of care glucose sensors were used with higher inaccuracies of CV = 2–8%, one could think that estimated IS could be slightly less repeatable. Due to the integrals involved in the model fitting method,[36] this impact is minimized if the variability is assumed to be normally distributed around the mean.

The data in **Figure 2** suggest more consistency in S_I at lower IS ranges. This effect is partly attributed to insulin and glucose assay variability, which carry over into the model. A dominant effect influencing the estimation of S_I in the modeling methodology is the decay rate of insulin concentrations immediately following the insulin injection. A smaller rate, as generally observed in insulin resistant subjects, is less affected by assay variability and results in a more consistent IS assessment. This increased accuracy in less sensitive subjects is a positive characteristic of the test, as these subjects represent the group among whom repeatability and accuracy are clinically the most relevant. In contrast to the DISST, the IVGTT can be much less sensitive in markedly insulin-resistant individuals and those with diabetes.[13,23,24]

In addition to IS, β-cell secretion metrics were estimated with the DISST from C-peptide concentrations (see **Appendix B**). Secretion metrics were estimated with good consistency, given assay errors. While basal secretion u_B and total first phase insulin above basal AUC_{10} were likely to be very accurate, peak secretion rate S_{max} may have been underestimated because of the lack of samples in the first 5 minutes after glucose injection. Additional modeling could improve this artefact. Since this error is systematic, comparison between tests remains valid. Considered alongside IS data, insulin secretion metrics help to provide a clear indication of the pathophysiology at any given state of the disease process. For example, an increased basal insulin secretion and blunted first phase response typically represents a fairly early stage in the progression of insulin resistance, as can be seen in Subject 16 (**Figure 4** in **Appendix C**). In addition to the quantitative metrics, these concentration profiles resulting from the DISST provide further valuable diagnostic data on an individual's metabolic status.

While the administration of insulin 10 minutes after glucose has clear benefits in identifiability of S_I, its limitations are in suppressing endogenous second phase insulin secretion.[37] A reliable estimation of second phase insulin secretion is thus not possible with the DISST in the current short protocol. A larger time gap between glucose and insulin administration would be required for second phase estimation.

Overall, this pilot study showed that the DISST is feasible to perform *in vivo* and the model and protocol assumptions discussed in detail in **Appendix A** are valid. The integrated approach combining a customized protocol, model, and identification method showed good performance in matching the results previously obtained in a Monte Carlo study.[18]

The full DISST protocol presented here can be completed in 50–60 minutes, which is appreciably shorter than the EIC (minimum 2–4 hours) and the IVGTT (3 hours). A single fasting blood test or oral glucose tolerance test is cheaper and simpler, but provides no dynamic information regarding the disease process. Instead, the benefit of the test is its accuracy and richness of information not obtainable with other simple tests. In addition to providing an indication of insulin secretion and sensitivity, the DISST has considerable potential for use as an accurate monitoring tool in metabolic studies and monitoring drug or lifestyle intervention programs. The ability to reduce the DISST's duration to ~30 minutes without any loss of performance will make it a more viable alternative to the EIC and IVGTT.

Optimal sample size power calculations for a clinical validation study of the DISST compared to the EIC were performed using the crossover study method described by Hauschke and colleagues[38] based on the expected accuracies in repeatability obtained from this pilot study. The method calculates the minimal sample size required to show clinical equivalence of two different tests. An optimal number of subjects required to show equivalence between both metrics within ±10% was determined to be between 24–49. A safe choice would thus be at least 50 subjects encompassing a wide range of individuals to ensure a broad spectrum of insulin sensitivities. The design of such a validation study should also ensure that both tests are performed only a few days apart to minimize errors introduced by natural

variability. This validation study has been designed based on these pilot trial results.

Conclusions

The clinical pilot study of a new DISST was presented. The DISST was previously designed and verified in Monte Carlo simulation and shown to be potentially repeatable and practicable in a clinical setting. This clinical pilot trial confirmed these simulated results and provided further insight on expected variability due to different dosing and unaccounted physiological variability.

Different insulin and glucose dosing can affect estimated outcomes, but these effects did not achieve statistical significance in this pilot study. Repeatability was within expected ranges of 6.0–19.8% (2 SD) identified in the previous Monte Carlo study[18] and showed good potential to correlate well to the EIC in clinical validation. Given the performance and practical aspects, a dose of 5–10 g glucose and 0.5–1 U insulin is recommended for further application of the protocol. This low level of dosing ensures a more physiological state and less effect on counterregulatory responses. In practical application, the protocol proved to be robust, and could be performed by a single person. Further reduction in the number of blood samples and test duration is possible.

Finally, this pilot study provided the results necessary to conservatively power a validation trial versus the EIC at n = 50+ subjects.

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Appendix A

Specific Differences between DISST and IVGTT

At first glance, the DISST looks very similar to an insulin-modified IVGTT. The general sequence of the test protocol is similar, followed by a physiological glucose model assessment. The IVGTT has been used in many studies and discussed widely, both benefits and problems, since the original landmark publication of the minimal model of glucose kinetics by Bergman and Cobelli.[39] In spite of its merits, many problems still exist with the IVGTT protocol, which constrain its use to a research-only environment. We analyzed these problems and attempted to design an insulin sensitivity test that was based on the IVGTT concept, but could be used in a clinical setting under physiological conditions and dosing. Such a test could enable more accurate insulin sensitivity testing in a wider group of people. The key differences in protocol, modeling, and identification are:

1. Clinical Protocol

A clinical protocol that is relatively simple to perform was a key objective for the development of the DISST. The three main aspects of improvement that were identified are duration, sampling frequency, and analytes.

1. To achieve a shorter duration than the IVGTT, i.e., less than 60 minutes, only the initial response after insulin administration is analyzed. This section of the glucose decay curve is mainly attributable to an insulin-dependent uptake, due to the relatively high concentration of plasma insulin. Furthermore, the counterregulatory glucagon response leading to an increase in EGP is not yet marked and does not strongly affect the sampled glucose concentrations. This time-reduced data set also better matches model assumptions, avoiding misidentification of certain parameters, such as insulin-independent glucose uptake p_G. In fact, the aspect mentioned here has also been recognized to clearly improve minimal model fitting of IVGTT data.[40]

2. The highly transient dynamics in the first 10 minutes after glucose or insulin administration are strongly affected by intravascular mixing, as can be seen in **Figure 3**, in which blood samples were taken from both arms during a DISST test. These effects have been observed to affect model fitting before, but were mainly attributed to a monocompartmental undermodeling approach.[13,41,42] High frequency of sampling as performed in the first 10–20 minutes of the IVGTT (1–2 minutes) adds to data resolution, but is difficult to perform, especially by a single person, due to the practical aspects involved in sampling and keeping track of timing. In our experience, a sample every 5 minutes is feasible, better every 10 minutes if less transient dynamics are observed. By disregarding the initial

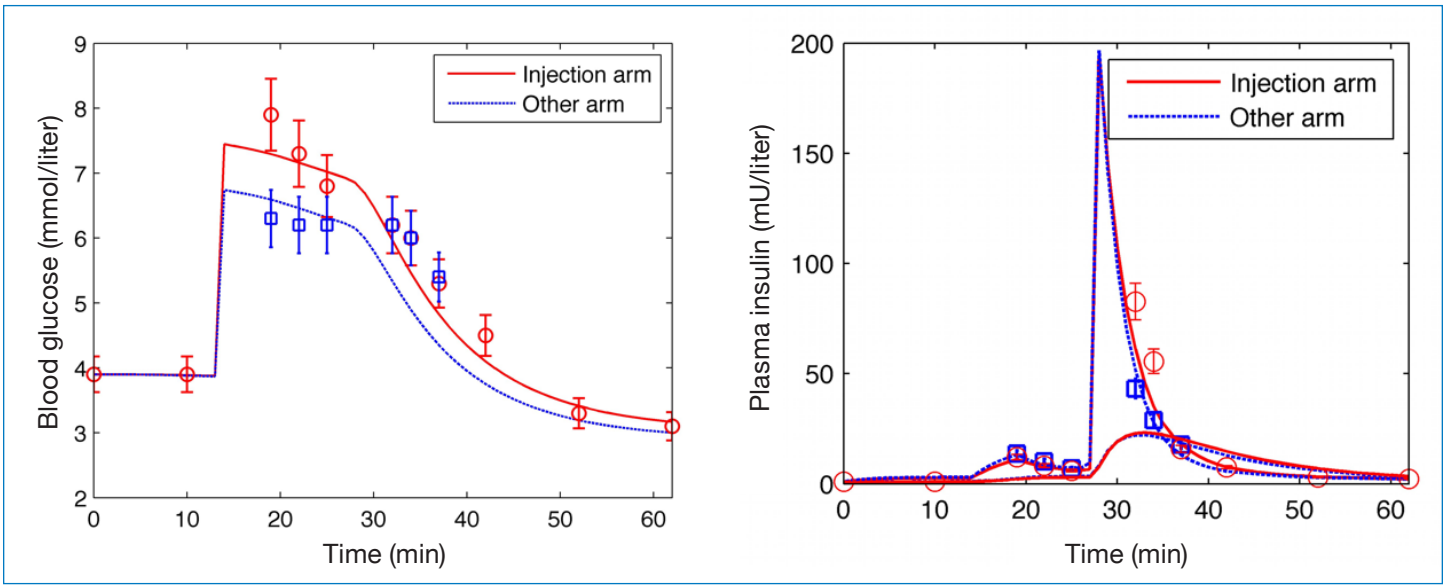

Figure 3. Effects of mixing. Shown are samples taken from both arms of the same subject after administration of glucose and insulin. Concentrations take about 10–15 minutes to equalize in both arms, a clear sign of intravascular mixing.

10 minutes after glucose or insulin administration, the DISST does not only avoid overfitting of unmodeled kinetics, but also concentrates on the latter part of the data, which better matches the model structure of a single glucose compartment,[41] avoiding parameter misidentifications.

3. Testing and modeling of analytes commonly tested by laboratories (glucose, insulin, and C-peptide) increase the practical use of the test. Use of glucose tracers, which can be used to estimate EGP[43,44] could improve the performance of the DISST, but add complexity and cost, and are thus purposefully avoided.

2. Modeling

Accuracy of identified model parameters can be improved by ensuring the model used matches the kinetics and dynamics observed and fitted in the data. Problems with minimal model fits of IVGTT data have commonly been attributed to undermodeling.[13,41] Whether the problem is undermodeling or overfitting of unmodeled effects remains to be debated. The model used in the DISST was adapted from the original minimal model to better match observed glucose and insulin behavior at the reduced sampling protocol, and to attempt to reduce misidentification issues observed in the past.[13,45] Furthermore, a modeling approach was followed that attempted to match assumptions made in the EIC to ensure good correlation with this gold standard test. These modeling aspects include:

Single Compartment Glucose Kinetics

By acknowledging intravascular mixing and disregarding the first 10 minutes of the glucose decay curve, the DISST approach concentrates on the latter part of the decay curve, which follows a monoexponential decay and can be identified well with a single compartment model. This approach avoids the use of glucose tracers and the requirement of more frequent sampling to identify the fast exponential.

Insulin Independent Clearance p_G Fixed

Robust identification of p_G requires a glucose decay signal in which insulin concentrations are low. As such, a state is not existent during the chosen protocol; the value of p_G is fixed at a value identified in other studies.[41,45-47] This is a well-recognized problem with the original minimal model, which identifies S_G from the final stages of the IVGTT in which insulin is low. As counterregulatory effects lead to increased EGP at this stage, S_G incorporates this effect and is clearly overestimated.[13,40,43] The DISST value of $p_G = 0.004$ min^{-1} is lower than commonly found minimal model values, because it only represents insulin-independent uptake, and does not lump suppression of EGP and basal glucose uptake into the same parameter.[47]

Constant EGP

Endogenous glucose production can be estimated only with the use of tracers,[43,48] and due to the lack of tracers, cannot be estimated in the DISST. To minimize intersubject variability by adding this dynamic, EGP is kept constant at a value estimated from the basal state. This assumption is likely a source of error, but the suppression effect at low insulin dosing is expected to be reduced at the low doses used in the DISST.[49] Monte Carlo simulations of this unmodeled effect showed only a small influence on the overall estimation of S_I.[18]

Physiologic Insulin Kinetics

By applying a physiologic insulin kinetics model, the estimated concentration of interstitial insulin, driving glucose uptake by the cells,[37] can be used directly to estimate insulin sensitivity S_I. A constant steady-state concentration ratio of $Q_{ss}/I_{ss} = 1/2$ is chosen[50,51] to *a priori* identify the diffusion rate n_I between both insulin compartments. This constraint removes another source of intersubject variability, and ensures a closer model match to the assumptions of the EIC.

Insulin Clearance n_L Constant

Hepatic insulin clearance n_L has been postulated to be variable, particularly at the early stage of an IVGTT in which first phase insulin secretion is very large.[52] This is likely due to a saturation of the receptor-based clearance pathway,[53,54] and is dependent on the magnitude of the first phase response. In the DISST, S_I estimation is mostly influenced by the insulin signal after insulin administration and it is very unlikely that a constant n_L will have a significant effect on it.

3. Model Identification

The model identification approach is a very important component of an integrated model-based diagnostic method. The goal is to ensure a robust overall parameter estimation that requires minimal human intervention and still delivers repeatable and reliable results. The DISST was designed to correlate well with the EIC, by attempting to assess similar physiologic effects, while requiring a shorter and more physiologic protocol. The key model identification aspects to achieve this are

Constrain Variability to S_I

Insulin independent clearance, p_G, is fixed at a population value, as explained before. This ensures that the glucose decay is purely attributed to insulin-mediated effects, represented by S_I, matching EIC assumptions.

Concentration on Strong Insulin Signal

By concentrating parameter estimation on data periods with high insulin concentrations, robustness of S_I estimation is improved. Due to the external administration of insulin, identification problems in low-sensitivity groups, commonly reported in the IVGTT[23,24,55] are eliminated.

Convex Fitting Method

The integral-based method used in the DISST is a convex parameter identification method that is not starting point dependent.[36] Due to the integration steps involved, the method further acts as a low-pass filter, reducing the effects of measurement noise.

Appendix B

The models and methods used to fit the experimentally sampled data are shown here. Further details on the development of the models and the fitting method employed can be found in **References 18**, **20**, **21**, **36** and **56**.

$$\frac{dG(t)}{dt} = -p_{GU}(G(t) - G_B) - S_I G(t) Q(t) + \frac{P(t)}{V_G} + EGP(t) \tag{3}$$

$$\frac{dI(t)}{dt} = -n_K I(t) - n_L \frac{I(t)}{1 + \alpha_I I(t)} - \frac{n_I}{V_P}(I(t) - Q(t)) + \frac{u_{ex}}{V_P} + (1 - x_L)\frac{u_{en}}{V_P} \tag{4}$$

$$\frac{dQ(t)}{dt} = -n_c Q(t) + \frac{n_I}{V_Q}(I(t) - Q(t)) \tag{5}$$

$$\frac{dC(t)}{dt} = k_2 Y(t) - (k_1 + k_3) C(t) + u_{en}(t) \tag{6}$$

$$\frac{dY(t)}{dt} = k_1 C(t) - k_2 Y(t) \tag{7}$$

where $G(t)$ represents plasma glucose concentration, G_B basal plasma glucose, V_G glucose distribution volume, $P(t)$ glucose input into plasma, S_I insulin sensitivity, p_{GU} noninsulin dependent glucose uptake, $EGP(t)$ endogenous glucose production, $I(t)$ plasma insulin, $Q(t)$ interstitial insulin, V_P plasma volume, V_Q interstitial volume, u_{ex} exogenous insulin input into plasma, u_{en} pancreatic insulin secretion, n_K renal insulin clearance rate, n_L hepatic insulin clearance rate, n_I diffusion constant for insulin transport between plasma and interstitium, x_L fractional first pass hepatic extraction of pancreatic insulin, α_I hepatic insulin clearance saturation, n_C insulin clearance at tissue cells, $C(t)$ plasma C-peptide, $Y(t)$ interstitial C-peptide, k_1-k_2 transport rates between C-peptide compartments, and k_3 renal clearance of C-peptide.

The pharmacokinetic (PK) and pharmacodynamic models shown in **Equations 3–7** are fitted to the sampled profiles of C-peptide, insulin, and glucose to obtain model-based information about the physiological response. The fitting process is performed in three steps:

Step 1: Insulin Secretion

Estimation of pancreatic insulin secretion $u_{en}(t)$ is performed with the model and methods presented by Eaton and coworkers.[56] and Van Cauter and associates.[57] Estimation of insulin secretion rate is performed with an integral-based identification method[36] resulting in a minute-wise step function of secretion rate. From this result, insulin secretory performance of the pancreas can be assessed in basal state, and during first phase secretion in response to an intravenous glucose loading. Values calculated in this study are basal secretion rate u_B (pmol/min), total insulin secreted over basal in the first 10 minutes after glucose injection AUC_{10} (pmol), and peak first phase secretion rate S_{max} (pmol/min).

Step 2: Insulin Kinetics

Insulin kinetics model parameters are estimated by fitting the model to the insulin profile data as described in Reference 20. Briefly, model parameters are estimated *a priori* where possible, using parallels to C-peptide kinetics, and remaining key parameters n_L and x_L estimated from the insulin profile. Estimated pancreatic secretion profile $u_{en}(t)$ from Step 1 is used as input to the insulin PK model. The fitting method employed is again the integral-based approach,[20,36] which has the advantage of being convex and less sensitive to assay variability.

Step 3: Glucose Pharmacodynamics

Insulin sensitivity S_I is estimated by fitting the glucose PK model to the glucose profile, using modeled interstitial insulin $Q(t)$ from Step 2 and known glucose administration $P(t)$. Noninsulin dependent glucose uptake p_{GU} cannot be identified reliably given the strong insulin signal in this experimental protocol. It was thus kept constant at a population value of $p_{GU} = 0.004$ min^{-1} [46,47] to avoid well known misidentification problems encountered by others.[13] Endogenous glucose production cannot be measured easily and is assumed to stay constant throughout the test at a steady-state value calculated from **Equation 3**, EGP = $S_I G_B Q_B$, with Q_B being basal interstitial insulin. A constant assumption for EGP ensures the bias of this unkown dynamic effect to be systematic, compared to a nonlinear assumption that would introduce additional intersubject variability to the outcome.

Appendix C

Diagnostic Relevance

Figure 4 shows example results of the full DISST analysis on three subjects, including normal glucose tolerance (NGT), impaired fasting glucose (IFG), and T2DM.

The progression of the disease can be visualized well on the examples shown in **Figure 4**. The NGT example, Subject 14, had an insulin sensitivity of $S_I = 11.7 \times 10^{-4}$ liter·mU^{-1}·min^{-1}, a fasting glucose level of 81.0 mg/dl and fasting insulin level of 20.8 pmol/liter. Basal insulin secretion rate was $u_B = 146.5$ pmol/min. The first phase β-cell response to a bolus injection of glucose was very distinct and large, peaking at $S_{max} = 1278$ pmol/min above the basal rate u_B and releasing a total amount of insulin above the basal rate of $AUC_{10} = 4841$ pmol. The first phase insulin secretion lasted about 5–10 minutes, after which the secretion rate immediately dropped back to nearly its basal rate.

The second example shows an IFG individual, Subject 16. IS was very low at $S_I = 3.2 \times 10^{-4}$ liter·mU^{-1}·min^{-1}, fasting glucose was elevated at 113.4 mg/dl and fasting insulin was also elevated at 115.3 pmol/liter. Basal insulin secretion rate was three times as high as in the NGT subject, at $u_B = 460$ pmol/min. In response to the glucose bolus, the pancreas increased its output, but a distinct first phase secretion peak was not pronounced. Insulin secretion peaked at $S_{max} = 569$ pmol/min above its basal secretion rate u_B and continued to produce at this rate until the end of the test. The β cells could only release additional $AUC_{10} = 4014$ pmol over the basal rate during the first phase. The pancreas was not able to fully compensate the low IS, and blood glucose levels drop only slowly. In addition to low IS, significant damage in β-cell function was evident in this subject.

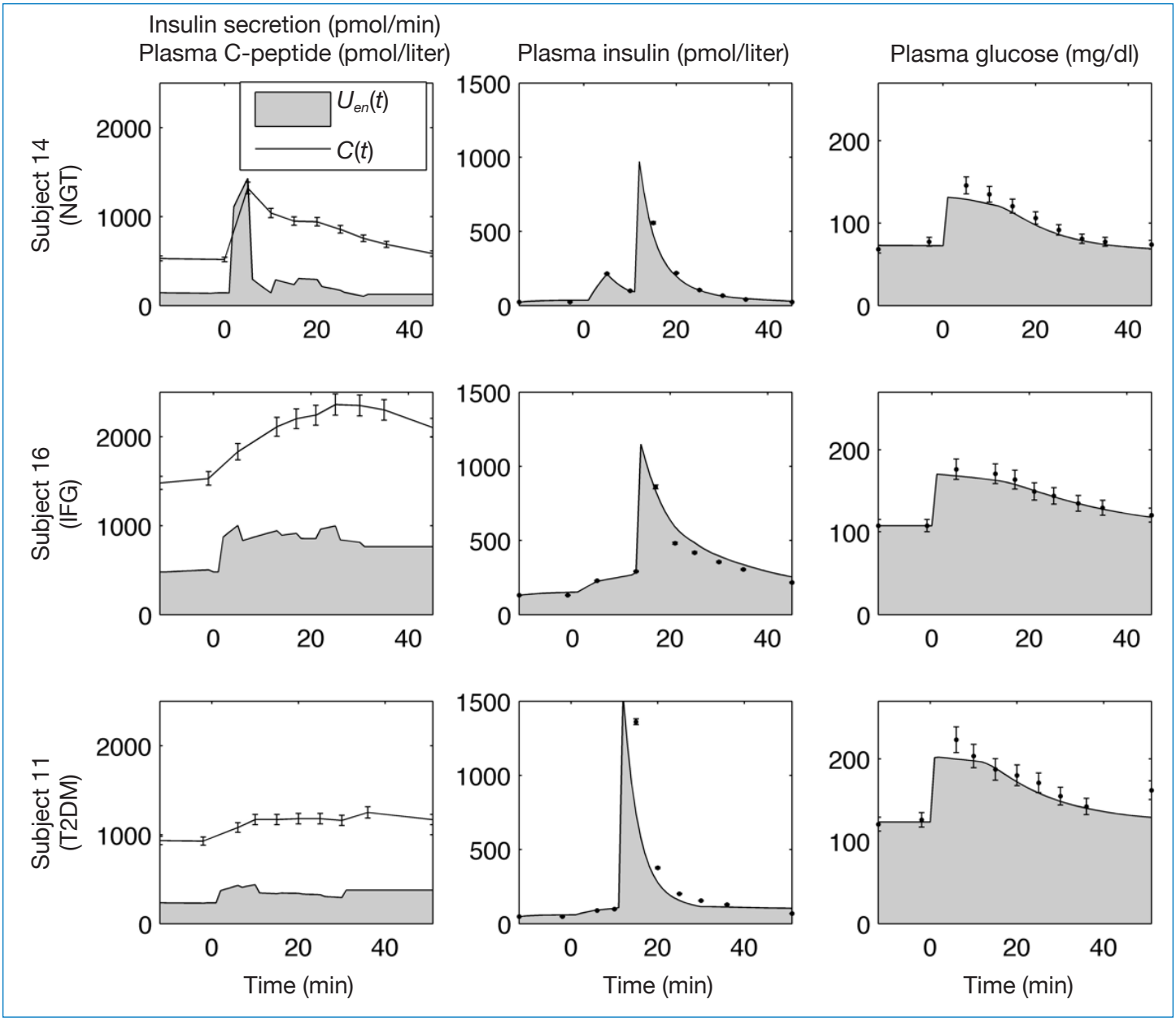

Figure 4. The exemplary test results using 10 g glucose and 1 U insulin on a NGT (**top**), IFG (**middle**), and T2DM subject (**bottom**). Shown are, from left to right, the estimated endogenous insulin secretion rate with overlaid plasma C-peptide concentration, the plasma insulin concentration, and the blood glucose concentration. Samples are shown with error bars, and areas show the model fits. The scale in the first column shows pmol/min for insulin secretion rate, and pmol/liter for plasma C-peptide concentration.

The third example shows Subject 11, who was diagnosed with T2DM. IS was higher than in the IFG example at S_I = 6.7×10−4 liter·mU^{-1}·min^{-1}, which could have been due to lasting effects of metformin, normally taken by this subject. The fasting glucose level was at 122.4 mg/dl, just below the T2DM diagnostic threshold of 126 mg/dl,[19] and fasting insulin was elevated at 9.2 mU/liter. Basal insulin secretion rate was u_B = 235.4 pmol/min, not nearly as high as in the IFG subject, a possible sign of β-cell exhaustion. Insulin secretion rate was slightly increased in response to the glucose bolus, but only AUC_{10} = 1577 pmol were produced above the basal rate u_B, with a secretion peak of only S_{max} = 201 pmol/min above the basal rate. The strongly diminished β-cell function could not compensate for the insulin resistance, resulting in fasting hyperglycemia.