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The Dynamic Insulin Sensitivity and Secretion Test (DISST) - a novel measure of insulin sensitivity.

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Abstract

Objective: To validate the methodology for the Dynamic Insulin Sensitivity and Secretion Test (DISST) and to demonstrate its potential in clinical and research settings.

Methods: 123 men and women had routine clinical and biochemical measurements, an oral glucose tolerance test and a DISST. For the DISST, participants were cannulated for blood sampling and bolus administration. Blood samples were drawn at *t*=0, 10, 15, 25 and 35 minutes for measurement of glucose, insulin and C-peptide. A 10g bolus of intravenous glucose at *t*=5 minutes and 1U of intravenous insulin immediately after the *t*=15 minute sample were given. Fifty participants also had a hyperinsulinaemic euglycaemic clamp. Relationships between DISST insulin sensitivity (*SI*) and the clamp, and both DISST *SI* and secretion and other metabolic variables were measured.

Results: A Bland-Altman plot showed little bias in the comparison of DISST with the clamp; with DISST underestimating the glucose clamp by $0.1 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$ (90%CI -0.2 to 0). The correlation between *SI* as measured by DISST and the clamp was 0.82, the c unit for the ROC analysis for the two tests was 0.96. Metabolic variables showed significant correlations with DISST IS, and the second phase of insulin release. DISST also appears able to distinguish different insulin secretion patterns in individuals with identical *SI* values.

Conclusions: DISST is a simple, dynamic test that compares favourably with the clamp in assessing *SI* and allows simultaneous assessment of insulin secretion. DISST has the potential to provide even more information about the pathophysiology of diabetes than more complicated tests.

Key Words: insulin sensitivity, beta cell function, insulin secretion, insulin resistance, type 2 diabetes mellitus.

Abbreviations: BMI: body mass index. DBP: Diastolic blood pressure. DISST: dynamic insulin sensitivity and secretion test. FPG: fasting plasma glucose. HOMA: homeostasis model assessment. IGT: impaired glucose tolerance. NGT: normo-glucose tolerance. IQR: interquartile range. IVGTT: intravenous glucose tolerance test. NGT: normal glucose tolerance. OGTT: oral glucose tolerance test. ROC: receiver operator curve. SBP: systolic blood pressure. SI: insulin sensitivity.

1. Introduction

Insulin resistance and β-cell dysfunction are prerequisites for the development of impaired fasting glucose, impaired glucose tolerance (IGT) and type 2 diabetes mellitus. However, the lack of a relatively simple test to reliably quantify both insulin sensitivity and secretion make it difficult to examine heterogeneity in epidemiological studies of prediabetes and diabetes and explore pathophysiology in studies of prevention and treatment. We have described a simple test – DISST [1, 2] which can provide quantitative measures of insulin sensitivity and insulin secretion.

The present paper has utilised a simple version of the DISST which involves five blood samples taken over a 35-minute protocol that uses low-dose, intravenous glucose (10g) and insulin (1U) boluses as stimulus. Thus, it is relatively short, and considerably less labour intensive than the gold-standard glucose clamp. The DISST model and identification method enable the sparse sampling protocol by fitting and refining physiological responses to the measured data [3, 4]. Unlike previous models, the DISST model of glucose and insulin kinetics accounts for patient-specific losses of insulin to the liver and the kidneys, saturation of insulin clearance at high concentrations and diffusion and mass conservation of insulin between the plasma and the interstitium [4]. In addition to assessing insulin sensitivity the test can be used to assess ß-cell function using established methods [5]. This aspect of the DISST is not novel.

The availability of such a test which can physiologically assess insulin sensitivity and simultaneously estimate insulin secretion provides the potential to explore heterogeneity in those who are currently labelled with the diagnosis of metabolic syndrome, prediabetes or type 2 diabetes mellitus, and to further understand responses to treatment with lifestyle measures and pharmacology.

This paper provides a validation of the DISST in the assessment of insulin sensitivity and illustrates its potential use.

2. Methods

Data from two separate studies undertaken by the same group of investigators have been combined. The first study cohort included 10 lean (BMI<25), 20 overweight (BMI>25, <30)

and 20 obese (BMI>30) participants, with even gender distribution in each category. The second study cohort included 73 women who were considered at-risk of metabolic diseases either by a BMI>25 or BMI>23 and a family history of diabetes. Participants were excluded if they suffered from any major medical or psychiatric illness or were known to have diabetes. Ethical approval for the first study was from the Upper South A Regional Ethics Committee. The second study was approved by the University of Otago Ethics Committee.

All 123 participants had weight, waist circumference (the midpoint of the lowest rib and highest part of the hip) and resting blood pressure measured. The 50 participants in the first study underwent a glucose clamp, the 4-sample OGTT and DISST protocols within 8 days, with at least one day between tests. The tests were given in random order such that each of the six possible combinations were equally represented. A pre-randomised test order was allocated to each participant based on order of recruitment. Participants of the second study underwent the DISST and the 2-sample OGTT in order to classify them as having a normal, or impaired glucose tolerance or type 2 diabetes [6]. All participants fasted from 10pm the night before each test and the tests were begun at 9am.

OGTT protocol

Fifty participants from the first study had an OGTT for assessment of insulin sensitivity using the Matsuda method [7]. Participants were given a standard 75g oral glucose load after a fasting blood sample. Further blood samples were collected at 30, 60 and 120 minutes. HOMA was also calculated for the first study participants using the basal assays of the OGTT and previously published methods [8, 9].

DISST protocol

Participants had a cannula inserted into the antecubital fossa for blood sampling and bolus administration. Blood samples were drawn at t=0, 10, 15, 25 and 35 minutes and glucose, insulin and C-peptide was measured on these samples. A 10g bolus of intravenous glucose was given at t=5 minutes and 1U of Actrapid insulin was given immediately after the t=15 minute sample. Participants were required to remain at the clinic for 30 minutes after the test and were provided with a small meal or snack.

The parameter identification methods of dynamic tests (such as the DISST) are sensitive to the timing of samples. Thus, the actual sample times were recorded. The integral method is

used to identify model-based insulin sensitivity (*SI*), glucose distribution volume (*Vg*), first-pass (x_L) and subsequent hepatic insulin clearance (n_L) [3, 10]. Metrics of β cell function are derived from insulin production profiles that are deconvolved from interpolated C-peptide data following the established method of Van Cauter et al. [3, 5]. The DISST model and identification method are briefly repeated in Appendix 1.

Three metrics were used to quantify β cell function. The basal rate (U_b) indicates the rate of insulin production the participant requires to maintain a constant fasting glucose measurement. AUC_{10} measures the first phase insulin production and is defined as the amount of insulin produced above the basal rate during the ten minutes after the glucose bolus. AUC_{2nd} quantifies the participant's second phase of insulin production as the total amount of insulin produced during the 20 minutes after the period measured by AUC_{10}.

The DISST method used in this study is a simpler version of the original DISST [3, 4] using 5 blood samples instead of 9. The impact of such sparse sampling on insulin sensitivity and insulin secretion metrics have been shown to be limited in previous studies [4, 11, 12]. Previous analysis by Docherty et al. found that insulin sensitivity and production values were barely affected by the omission of samples from the frequently sampled protocol used in the DISST pilot study [12]. The five-sample method was not significantly different from the original 9-sample method. The correlations between the outcomes of the pilot sampling protocol and the sampling protocol used here were r=0.90, 1.0, 1.0, and 0.89 for *SI*, U_b, AUC_{10}, and AUC_{2nd}, respectively.

Glucose Clamp protocol

The 50 participants in the first study underwent a glucose clamp. Participants had two cannulae inserted: one in the antecubital fossa; the other, a retrograde cannula, inserted in the dorsum of the hand. The hand was heated so that arterialised blood was obtained for sampling. Insulin was infused at 280pmol/min/m^2 and glucose was infused to achieve a target glucose concentration of 81mg/dl, or at the fasting level if this was between 72 and 90 mg/dl. The test lasted for 2 to 2.5 hours and data from the last 40 minutes was used to calculate insulin sensitivity index (*ISI*) in $mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$ [13]. Participants were required to remain at the clinic for 30 minutes after the test and were provided with a small meal or snack.

Unit correction

As the standard DISST and the clamp *SI* values have different units, a conversion needs to be made to compare the magnitude of values across the two different tests. The model-based *SI* identified by the DISST measures the glucose disposal as a function of the available glucose, glucose distribution volume and the modelled interstitial insulin. However, the clamp measures as a function of the absolute glucose disposal, steady-state plasma insulin concentration and the participant's bodyweight. Thus to achieve common units the DISST *SI* values must be converted:

$$ISI_{DISST} = SI_{modelled} \frac{1000 \cdot Gb \cdot Vg \cdot \gamma}{BW}$$

where: G_b is the basal glucose concentration [mmol·l^{-1}], Vg is the identified distribution volume of glucose [l], BW is bodyweight [kg], γ is the steady state ratio between plasma and interstitial insulin (0.5) [14], and the '1000' coefficient converts from dl to l and multiplies by 100 in accordance with the standard practice for reporting clamp metrics.

Laboratory analysis

Glucose values for the first study were analysed using YSI 2300 stat plus Glucose and L-Lactate analyser using whole blood. These were converted to plasma glucose with the equation recommended by the analyser manufacturer:

$$G_{plasma} = \frac{G_{wholebloodglucose}}{1 - \left(2.4 \cdot 10^{-3} \cdot Haematocrit(\%)\right)}$$

Plasma glucose levels taken in the second study were measured enzymatically with Roche kits and calibrators on a Cobas Mira Analyser. Samples for insulin and C-peptide were separated immediately and frozen. Measurements of insulin were undertaken by the Endolab, Canterbury Health Laboratories for the first study and by the University of Otago Nutrition Laboratory for the second study. Both laboratories used Roche Elecsys® after Peg precipitation of immunoglobulins (Roche Diagnostics, Mannheim, Germany). Consistency between laboratories was maintained. All C-peptide measurements were undertaken by Endolab, Canterbury Health Laboratories using the Roche Elecsys® method. Serum cholesterol and triglycerides were measured enzymatically with Roche kits and HDL was measured in the supernatant after precipitation of apolipoprotein B containing lipoproteins with phosphotungstate/magnesium chloride solution [15].

Statistical methods

The data are presented as means and standard deviations or median and upper and lower quartiles. Correlations were used to describe the associations between the insulin sensitivity values. A Bland Altman plot was used to compare the DISST with the glucose clamp. Analysis of variance was used to compare the three groups derived from the first insulin phase (AUC_{10}) and those derived from the second insulin phase (AUC_{2nd}). Comparisons between those with IGT and those with NGT are also presented.

3. Results

The range of DISST insulin sensitivity values for the 123 individuals was 0.2 to $3.4 \cdot 10^{-4}$ $\cdot l \cdot pmol^{-1} \cdot min^{-1}$ with a mean of 1.1 (SD 0.64), median 1.0 (IQR 0.7 to 1.4). The range for insulin sensitivity estimated by the glucose clamp (n=50) was 0.1 to $2.3 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$, mean 1.0 (SD 0.61), median 0.9 (IQR 0.6 to 1.4).

The Bland-Altman plot (Figure 1) shows the bias between the two tests, where the DISST underestimated the glucose clamp by $0.1 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$ (95% CI -0.2 to 0.0). The limits of agreement were -0.9 to $0.7 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$. Figure 2 shows the correlation between the DISST and the glucose clamp (r=0.82). Figure 3 presents a ROC curve for the DISST compared to the glucose clamp (c unit=0.96 using an insulin resistance cut off for the glucose clamp of $1.0 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$ [9]).

Correlations between the DISST and the variables known to be associated with insulin resistance are shown in Table 1 as well as the correlations between the DISST and the HOMA and the Matsuda index.

Characteristics of those separated into tertiles of first phase and second phase insulin secretion are shown in Tables 2 and 3. Of note, those with IGT were spread evenly across the tertiles of first phase insulin secretion. However, second phase insulin secretion was significantly associated with all of the features of the metabolic syndrome. Table 4 compares insulin secretion metrics across the NGT and IGT subgroups. In accordance with previous observations [16-18], the second phase insulin secretion was significantly higher in those with IGT.

Figure 4 shows the results of the DISST test for insulin sensitivity and insulin secretion metrics for four insulin resistant participants. All of the examples in this figure had the same

insulin sensitivity measured by the clamp ($0.8 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$), however the DISST profiles showed clear differences between these individuals. The range of insulin sensitivity estimated by the DISST was 0.95 to $1.36 \cdot 10^{-4} \cdot l \cdot pmol^{-1} \cdot min^{-1}$for these participants. However, of particular note were the distinct insulin production characteristics of these participants. Participants A and B showed contrasting profiles to participants C and D in terms of the magnitude of first phase release of insulin. Participant C had an increased second phase, and blunted first phase of insulin production, which coupled with an inability to return to the basal glucose concentration within 30 minutes, indicated insulin resistance and β cell dysfunction for this participant.

No serious adverse events were observed in participants and there were no episodes of symptomatic hypoglycaemia following the DISST.

4. Discussion

The DISST estimates both insulin sensitivity and β cell function including the first and second phases of insulin release. The protocol is well accepted by participants, and is straightforward to perform. The low-dose and low-intensity DISST protocol is unique in that it results in insulin concentrations that are comparable with daily excursions and are not affected by dose-dependent saturation effects [19] whereas established tests rely on non-physiological doses that exceed saturation level for insulin action [20, 21]. Thus, the model-based DISST can more directly account for dosing differences than the simpler M/I model of glucose clearance and uptake used in the clamp assessment, which varies directly with the insulin dosing due to insulin effect saturation [20, 21]. As a result, DISST *SI* values are more directly comparable across studies [3].

The DISST concurrently allows an assessment of insulin secretion with insulin sensitivity. The insulin secretion identification method was validated by Van Cauter et al. [5] and has been used by many leading insulin sensitivity research groups [22-25]. The second phase values of insulin secretion obtained from the low-intensity DISST correlated well with metabolic risk factors, and distinguished IGT and NGT subgroups. The DISST offers the possibility of relating the insulin secretion rate to their insulin sensitivity status, which is potentially useful in research and clinical practice. Insulin secretion typically increases with insulin resistance in the early stages of IGT and type 2 diabetes, but declines as β cell function is lost [13, 26, 27]. Thus, as illustrated in Figure 4, apparently healthy NGT individuals can

have insulin production rates similar to those of individuals that have considerable loss of β cell function. Current tests do not distinguish between these individuals with different insulin secretion responses [16].

The DISST insulin sensitivity values were converted to mimic the units of the gold-standard clamp, and thus could be compared to clamp values in terms of both correlation and bias. Furthermore, Table 1 compared the DISST insulin sensitivity values to co-factors related to insulin resistance and produced expected outcomes [28]. The moderate correlations presented in Table 1 must be considered with respect to the low resolution of the co-factors presented in terms of characterising insulin resistance.

In contrast to insulin sensitivity, there is no established gold standard for the evaluation of insulin secretion. Thus, this investigation has evaluated the insulin secretion values estimated by the DISST by comparison with established metabolic markers of insulin resistance. Furthermore, the low-intensity 5 sample protocol has been validated by Lotz et al. [11].

The DISST requires a significantly less intensive protocol than insulin sensitivity tests that produce similar correlations to the clamp [29-31]. The DISST can achieve this level of accuracy with improved parameter identification methods [10] and the adoption of a single model variable for glucose decay. The identification of two metrics that model glucose clearance has been an issue in previous studies using the Minimal Model approach [32, 33] and strategies used to ameliorate this problem require either Bayesian techniques [34, 35] or arduous, clinically intense, frequently sampled protocols. However, it has been shown that fixing the glucose dependent clearance term (that has limited clinical value) maximises identification stability and allows the considerably less intense protocol of the DISST to produce a stable and relevant metric of insulin sensitivity [4, 12]. The overall reduction in clinical intensity and improved parameter stability offered by the DISST comes at the cost of increased parameter identification complexity. However, this is a positive development, as it allows a lower per participant cost than the established, simple-model, intense-protocol tests for insulin sensitivity.

More intensive tests such as the glucose clamp [36] and the IVGTT [37] require specialist training for those performing the tests, involve a greater participant burden and are more costly, all of which generally limit their use to small research studies. They appear to be

comparable tests, although the IVGTT, with a coefficient of variation of 14 to 30%, is less reliable than the glucose clamp, coefficient of variation of 6 to 10%. The particularly high repeatability has earned the glucose clamp gold standard status [16]. However, the glucose clamp yields different results at different infusion rates which complicates comparisons between studies [20, 21]. The basic glucose clamp assumes all endogenous glucose and insulin secretion is fully suppressed, that all glucose uptake is mediated by insulin and that the uptake rate is proportional to the plasma insulin concentration [36]. In fact, insulin independent glucose uptake occurs and can be constant (to the brain and the central nervous system) or dependent on glucose concentration [38]. This is accounted for by the DISST [3].

An earlier study involving repeated tests demonstrated that the DISST was as reliable as the glucose clamp in measuring insulin sensitivity [2]. We report here a strong correlation between insulin sensitivity measured by the DISST and the glucose clamp (R=0.82). It is noteworthy that on average the DISST only under-estimated the clamp *ISI* by $0.1 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$, even though there were substantial differences between the two protocols. The ROC analysis, which is usually used to compare two very different tests, indicates that the DISST and the glucose clamp are reasonably comparable. Although both tests relate the rate of glucose uptake to an insulin concentration, the clamp involves a steady state, hyper-physiological protocol with suppression of insulin and glucose production and 2-3 hours of frequent sampling, whereas the DISST protocol involves only 35-minutes of less frequent sampling and does not significantly suppress endogenous insulin or glucose production. In contrast to the clamp, the DISST insulin sensitivity is a function of interstitial insulin and is measured with glucose and insulin concentrations that are typical of daily life. Furthermore, the DISST accounts for non-insulin mediated glucose uptake, which the clamp assumes is negligible. Thus, while the clamp was designed to maximise repeatability, the DISST was designed to be relevant to the participant's metabolic physiology.

In conclusion, we believe the DISST is a relatively low cost, practical test which yields substantially more information regarding glucose and insulin responses to stimuli than other available tests. DISST is safe and reliable and allows a reasonable estimation of insulin sensitivity. In addition estimates of insulin secretion can be obtained at the same time. It is a test which could be applied in clinical or research settings; either where a glucose clamp might be used or in larger trials where either an OGTT or the HOMA would be used. If the DISST were to be applied widely, it could greatly enhance our understanding of the

pathophysiology of type 2 diabetes mellitus and help to more clearly differentiate the very heterogeneous group who are at risk of type 2 diabetes mellitus.

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Author contributions.

KAM contributed to study concept and design, to acquisition of data, analysis and interpretation of data and the writing of the manuscript. JEB contributed to acquisition of data and reviewed manuscript. PDD contributed to acquisition of data, analysis and interpretation of data and contributed to writing of manuscript. TFL contributed to study concept and design, pilot work which forms the basis of this paper and revised the manuscript. LTM contributed to acquisition of data and contributed to writing of the manuscript. GMS contributed to study concept and design and acquisition of data and revised manuscript. SMW was the statistician and contributed to study design, analysis and interpretation of data and contributed to writing of the manuscript. JGC contributed to study concept and design, analysis and interpretation of data and contributed to the writing of the manuscript. JIM contributed to study concept and design, analysis and interpretation of data and contributed to the writing of the manuscript.

Appendix 1

The DISST defines the pharmaco-kinetics/dynamics of C-peptide, insulin and glucose with a physiological model. The model relates the rate of glucose decay to the concentration of insulin available in the interstitium to provide a metric of insulin sensitivity. The model equations are defined:

Plasma C-peptide
$$\dot{C} = k_2 Y - (k_1 + k_3)C + \frac{uen(t)}{Vp} \quad (1)$$

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

Plasma Insulin
$$\dot{I} = \frac{n_I}{Vp}(Q - I) - n_K I - n_L \frac{I}{1 + \alpha_I I} + \frac{uex(t)}{Vp} + (1 - x_L)\frac{uen(t)}{Vp} \quad (3)$$

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

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

where: $k_{1\text{-}3}$ are kinetic parameters (1/min); *C* and *Y* are plasma and interstitial C-peptide concentrations, respectively (pmol/l); *uen(t)* is the time variant rate of insulin production (pmol/min); *I* and *Q* are the plasma and interstitial insulin concentrations respectively (pmol/l); *Vp* and *Vq* are the distribution volumes of insulin in the plasma and interstitium respectively (l); n_K is the rate of insulin clearance by the kidney (1/min); n_I is the transition rate of insulin between the plasma and interstitium (l/min); n_L is the rate of hepatic insulin clearance (min^{-1}); α_I is the saturation of hepatic insulin clearance (l/pmol); n_C is the rate of insulin clearance to cells (1/min); *uex(t)* is the bolus input of insulin (pmol); x_L is the hepatic first pass extraction of insulin (1); p_{gu} is the glucose dependent (insulin-independent) rate of glucose disposal (1/min); *SI* is the modelled insulin sensitivity (l/pmol/min); *P* is the glucose bolus (mmol); *Vg* is the volume of distribution of glucose (l); G is the glucose concentration (mg/dL) and the '*b*' subscript denotes the basal concentration of the respective species.

The measured C-peptide, insulin and glucose data is used to identify participant-specific parameters with methods that have been exhaustively defined and justified in previous

publications [2-4, 10]. However, the methods will be summarised in brief: Initially, a false basal data point with concentrations equal to the measured basal sample was added immediately prior to the glucose bolus. This ensured that the influence of the basal period on the identified variables was equal across participants. The kinetic parameters of Equations 1 and 2 are quantified using functions of participant weight, height sex and age that were defined by Van Cauter et al. [5]. A piece-wise linear interpolation of the C-peptide data was used with these values in a deconvolution of Equations 1 and 2 to produce an endogenous insulin production profile (*uen(t)*). Finally, *SI*, *Vg*, n_L and x_L were identified using the deconvoluted endogenous insulin production profile, insulin and glucose data, Equations 3-5 and the integral method [3, 10]. Note that the t=10 minute glucose sample is assumed to be affected by mixing and is thus ignored in the identification of *SI* and *Vg* and is omitted from Figure 4.

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Table 1. Correlation between the DISST insulin sensitivity and variables known to be associated with insulin resistance as well as two simple surrogates for assessing insulin sensitivity, the HOMA and the Matsuda OGTT.

	Mean (n=123)	SD	Correlation with the DISST *SI*	P value
Age (years)	42	12.2	-0.16	0.09
Waist circumference (cm)	95.5	14.9	-0.51	<0.001
BMI (kg/m^2)	31.7	6.90	-0.45	<0.001
FPG (mg/dl)	86.4	8.64	-0.34	0.002
Fasting triglycerides (mmol/l)	1.30	0.94	-0.27	0.002
Fasting HDL cholesterol (mmol/l)	1.19	0.30	0.40	<0.001
Fasting insulin (pmol/l)	78.9	75.4	-0.63	<0.001
HOMA	2.4	2.27	-0.40	<0.001
Matsuda OGTT[a]	16.9	11.0	0.56	<0.001

[a] The Matsuda is on 50 participants only.

507 **Table 2**. Clinical and biochemical measures by tertiles of first phase of insulin release
508 (AUC_{10}, from 5 to 15 minutes) during the DISST (n=123).
509

	0-4250 pmol of insulin (n=41)	4251-7000 pmol of insulin (n=42)	7001- 22000 pmol of insulin (n= 40)	P value
Age (years)	44 (12)	42 (12)	38 (13)	0.09
Gender % Female	82	74	83	0.51
Weight (kg)	84.5 (18.3)	86.2 (18.0)	93.4 (24.6)	0.12
Waist (cm)	93.0 (15.0)	93.6 (12.7)	100.1 (16.1)	0.06
BMI (kg/m^2)	30.4 (6.6)	30.9 (6.0)	33.7 (7.7)	0.06
SBP (mmHg)	120 (14)	120 (14)	123 (19)	0.69
DBP (mmHg)	76 (10)	77 (11)	77 (8)	0.89
Fasting triglycerides (mmol/l)	1.30 (1.40)	1.21 (0.57)	1.39 (0.64)	0.68
Fasting HDL cholesterol (mmol/l)	1.24 (0.29)	1.22 (0.30)	1.11 (0.29)	0.09
FPG (mg/dl)	86.4 (9.0)	82.8 (7.2)	82.8 (7.2)	0.01
Fasting insulin (pmol/L)	61.6 (38.7)	66.9 (40.9)	109.1 (114.5)	0.007
IGT %	7	12	15	0.54
Insulin sensitivity (DISST)[a]	1.2 (0.69)	1.3 (0.69)	0.9 (0.48)	0.06

510 [a] Measured in $10^{-4} \cdot l \cdot pmol^{-1} \cdot min^{-1}$.
511

Table 3. Clinical and biochemical measures by tertiles of second phase of insulin release (AUC_{2nd}, from 15 to 35 minutes) during the DISST (n=123).

	0-5000 pmol of insulin (n=44)	5001-8000 pmol of insulin (n=38)	8001-16000 pmol of insulin (n=41)	P value
Age (years)	40 (12.8)	40 (11.4)	45 (12.0)	0.12
Gender % Female	80	79	80	0.98
Weight (kg)	76.6 (12.1)	86.0 (14.0)	102.2 (24.8)	<0.001
Waist (cm)	84.9 (8.5)	94.8 (11.5)	107.4 (14.3)	<0.001
BMI (kg/m^2)	27.2 (4.2)	31.3 (4.7)	36.8 (7.5)	<0.001
SBP (mmHg)	118 (14)	119 (14)	126 (18)	0.04
DBP (mmHg)	74 (9)	76 (8)	79 (12)	0.05
Fasting triglycerides (mmol/l)	0.86 (0.29)	1.57 (1.40)	1.51 (1.51)	0.005
Fasting HDL cholesterol (mmol/l)	1.29 (0.26)	1.29 (0.32)	0.99 (0.19)	<0.001
FPG (mg/dl)	79.2 (7.2)	82.8 (7.2)	88.2 (7.2)	<0.001
Insulin (pmol/l)	40.0 (19.2)	67.1 (24.3)	131.5 (108.5)	<0.001
IGT %	7	13	15	0.48
Insulin sensitivity (DISST)[a]	1.6 (0.69)	1.1 (0.39)	0.7 (0.25)	<0.001

[a]Measured in $10^{-4} \cdot l \cdot pmol^{-1} \cdot min^{-1}$.

Table 4. Measures indicating β cell function by glucose tolerance status (n=123).

	n=	Basal Insulin Production (U_b)[a] (pmol/min). Mean (SD)	First Phase Insulin Secretion (AUC_{10})[b] (pmol). Mean (SD)	Second Phase Insulin Secretion (AUC_{2nd})[c] (pmol). Mean (SD)
All data	123	235 (103)	6,060 (3,564)	6,889 (3,320)
NGT	109	230 (105)	5,973 (3,578)	6,660 (3,245)
IGT	14	276 (74)	6,739 (3,502)	8,668 (3,487)
P value		0.11	0.45	0.03

[a]U_b is the basal rate of insulin production; [b]AUC_{10} is the amount of insulin produced 10 minutes after the glucose bolus above the basal rate; [c]AUC_{2nd} is the total amount of insulin produced between t=15 and 35 minutes.

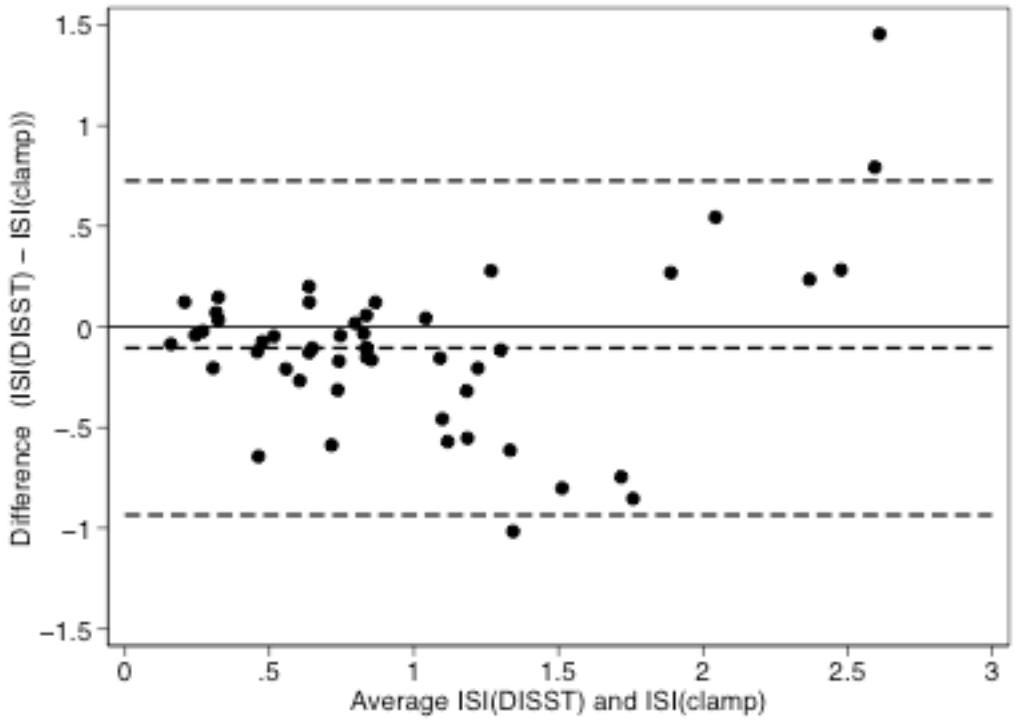

Difference (ISI(DISST) – ISI(clamp))
1.5
1
.5
0
−.5
−1
−1.5
0
.5
1
1.5
2
2.5
3
Average ISI(DISST) and ISI(clamp)

Figure 1.

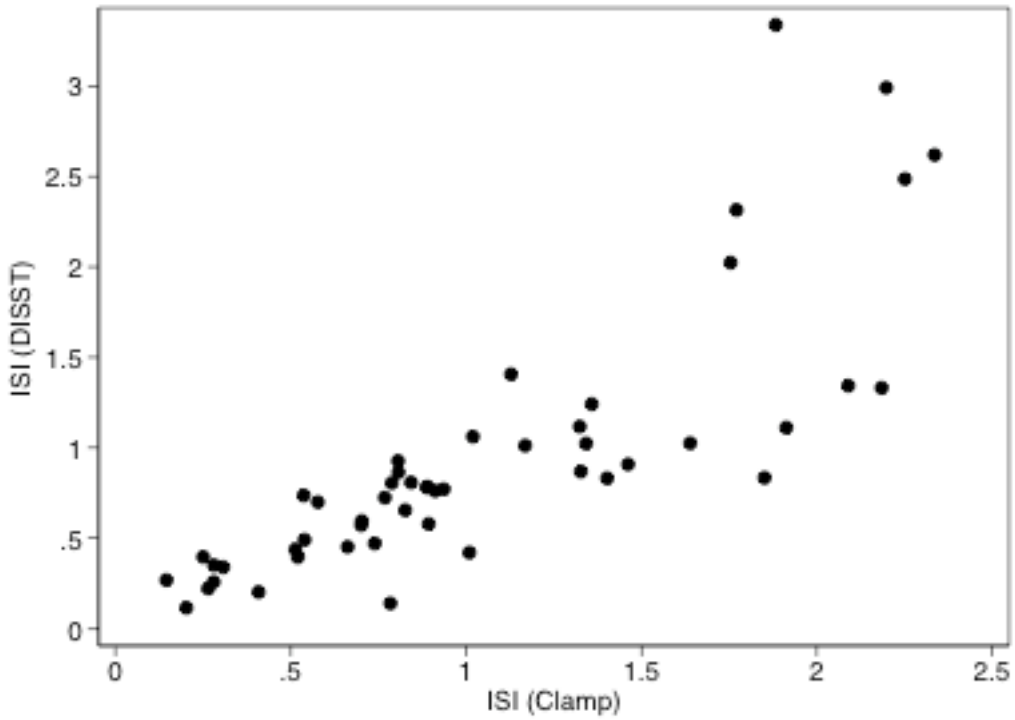
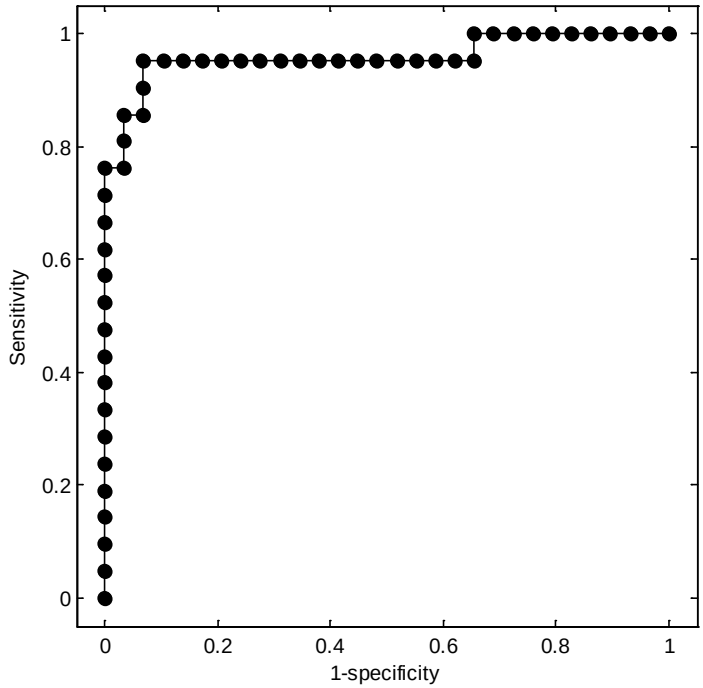


Figure 2.



528

529 **Figure 3.**

530

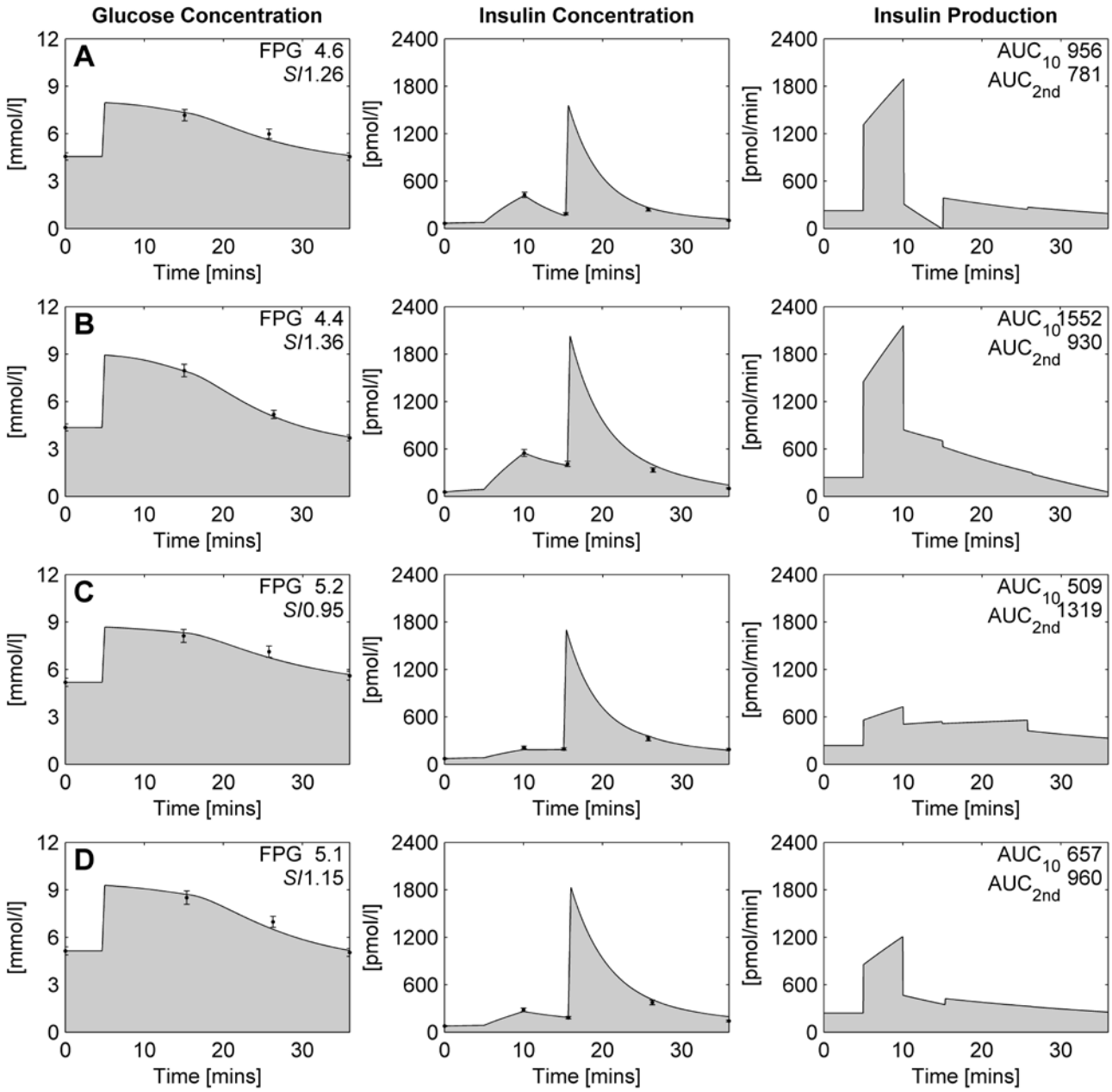

Glucose Concentration
Insulin Concentration
Insulin Production
A
FPG 4.6
S/1.26
AUC10 956
AUC2nd 781
B
FPG 4.4
S/1.36
AUC10 1552
AUC2nd 930
C
FPG 5.2
S/0.95
AUC10 509
AUC2nd 1319
D
FPG 5.1
S/1.15
AUC10 657
AUC2nd 960
[mmol/l]
[pmol/l]
[pmol/min]
Time [mins]

Figure 4.

Figure Legends

Figure 1. The Bland Altman plot of insulin sensitivity estimates derived from the DISST and the glucose clamp, showing the bias between the two tests, with the DISST overestimating the glucose clamp insulin sensitivity estimate by $0.1 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$ (95% CI -0.2 to 0.0). The limits of agreement are -0.9 to $0.7 \cdot 10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$.

Figure 2. The correlation of the DISST and the glucose clamp insulin sensitivity values (units are $10^{-2} \cdot mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$).

Figure 3. ROC curve of the DISST against the gold standard, the glucose clamp, c index =0.96.

Figure 4. Blood glucose, plasma insulin and insulin production responses of four individuals to the DISST stimulus. The second peak of the insulin concentration is due to the exogenous bolus of insulin used in the DISST protocol.