Evaluation and Development of the Dynamic Insulin Sensitivity and Secretion Test for Numerous Clinical Applications

by Paul D. Docherty

Submitted for the degree of Doctor of Philosophy

in Mechanical Engineering

at the University of Canterbury,

Christchurch, New Zealand

7th March 2011

Foreword

Type 2 diabetes mellitus is an irreversible disease state that is characterised by the sufferer's inability to produce sufficient insulin to maintain safe blood glucose concentrations. Although the precise mechanism is ambiguous, it is generally agreed that insulin resistance is the predominant driver of the pathogenesis of type 2 diabetes mellitus. Thus, identification of the insulin resistant state could prevent or delay the onset of diabetes, and potentially ameliorate the worst symptoms of the disease.

Many test protocols have been proposed to quantify a participant's insulin sensitivity. However, these tests are generally either too intensive, or inaccurate to be used in widespread screening programmes and thus limit etiological investigations of type 2 diabetes. This thesis presents a validation investigation of the dynamic insulin sensitivity and secretion test (DISST) and a series of mathematical and clinical developments. In particular:

Chapter 1 describes the physiology that is relevant to insulin sensitivity testing and the pathogenesis of type 2 diabetes.

Chapter 2 describes and compares the established insulin sensitivity tests.

Chapter 3 shows various model parameter identification methods.

Chapter 4 defines the DISST protocol, model and identification method. It also presents the outcomes of the DISST pilot investigation.

Chapter 5 introduces a novel *a-priori* model identifiability analysis that can uniquely define parameter trade-off with respect to assay error.

Chapter 6 presents the validation study outcomes of the DISST. The study measured the descriptive capability and gold-standard equivalence of the DISST

Chapter 7 compared the efficacy of the DISST model against the much-touted Minimal Model for the identification of insulin sensitivity from sparse data

Chapter 8 presents the quick DIST (DISTq) which is a novel real-time capable very low-cost insulin sensitivity test.

Chapter 9 shows how DISST based tests can form a spectrum of tests of differing compromises of accuracy, economy and information. A hierarchal system was also defined to enable low cost yet very high resolution diagnosis.

Chapter 10 presents two DISST protocol variations. While the former reduces clinical intensity, the latter increases information yield.

Chapter 11 describes how the DISST test can aid the tracking of insulin sensitizer and secretagogue drugs *in-silico*.

Chapter 12 summarises and concludes the outcomes of this thesis.

Chapter 13 summarises the future work and possibilities that may arise from the outcomes of this thesis.

Acknowledgments

I owe the greatest depth of gratitude to my beautiful wife, Tracey, who selflessly agreed to allow me to undertake this course of study and supported me throughout. I also owe thanks to my only sunshines, Charlotte and Iona, who effortlessly cheered me up on many occasions.

Many great thanks also go to my supervisor, Prof. Geoff Chase, who is unfatigable in providing with a great deal of help well above what could be reasonably expected from a supervisor. His work ethic and expertise is at once, inspiring, and daunting.

I would also like to thank my co-supervisors, Dr Thomas Lotz and Dr Chris Hann who introduced me to this project and have been extremely helpful in providing me with their significant expertise in physiology, modelling and mathematics.
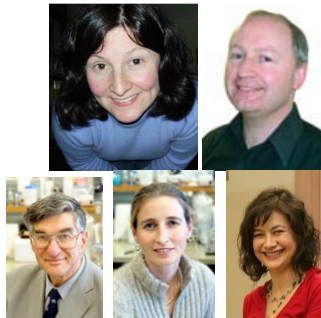
Huge apologies as well as thanks must go the clinicians involved in our studies, Juliet, Geoff, Jim, Kirsten and Lisa. Apologies for my initial ignorance of many clinical procedures and methods, and thanks for patiently explaining some of that ignorance away!

Many thanks also go the members of the glycaemic team at the University of Canterbury (Aaron, Ash, Chris, Fatanah, Jess, Jackie, Matt, Normy, and Ummu)

Thanks must also go to my parents. Their encouragement of independent thought and general tolerance of my insistence on doing things in unorthodox ways have been foundational to my development as an innovative researcher.

A massive debt of thanks must be paid to all of the clinical trial volunteers whose selflessness enabled all of the developments presented in this thesis.

And finally God, who got my life back on track.

Given the high and increasing social, health and economic costs of type 2 diabetes, early diagnosis and prevention are critical. Insulin sensitivity and insulin secretion are important etiological factors of type 2 diabetes and are used to define an individual's risk or progression to the disease state. The dynamic insulin sensitivity and secretion test (DISST) concurrently measures insulin sensitivity and insulin secretion. The protocol uses glucose and insulin boluses as stimulus, and the participant response is observed during a relatively short protocol via glucose, insulin and C-peptide assays.

In this research, the DISST insulin sensitivity value was successfully validated against the gold standard euglycaemic clamp with a high correlation (R=0.82), a high insulin resistance diagnostic equivalence (ROC c-unit=0.96), and low bias (-10.6%). Endogenous insulin secretion metrics obtained via the DISST were able to describe clinically important distinctions in participant physiology that were not observed with euglycaemic clamp, and are not available via most established insulin sensitivity tests.

The quick dynamic insulin sensitivity test (DISTq) is a major extension of the DISST that uses the same protocol but uses only glucose assays. As glucose assays are usually available immediately, the DISTq is capable of providing insulin sensitivity results immediately after the final blood sample, creating a real-time clinical diagnostic. The DISTq correlated well with the euglycaemic clamp (R=0.76), had a high insulin resistance diagnostic equivalence (ROC c-unit=0.89), and limited bias (0.7%). These DISTq results meet or exceed the outcomes of most validation studies from established insulin sensitivity tests such as the IVGTT, HOMA and OGTT metrics. Furthermore, none of the established insulin sensitivity tests are capable of providing immediate or real-time results. Finally, and most of the established tests require considerably more intense clinical protocols than the DISTq.

A range of DISST-based tests that used the DISST protocol and varying assay regimens were generated to provide optimum compromises for any given clinical or screening application. Eight DISST-based variants were postulated and assessed via their ability to replicate the fully sampled DISST results. The variants that utilised insulin assays correlated well to the fully sampled DISST insulin sensitivity values R~0.90 and the variants that assayed C-peptide produced endogenous insulin secretion metrics that correlated well to the fully-sampled DISST values (R~0.90 to 1). By taking advantage of the common clinical protocol, tests in the spectrum could be used in a hierarchical system. For example, if a DISTq result is close to a diagnostic threshold, stored samples could be re-assayed for insulin, and the insulin sensitivity value could be 'upgraded' without an additional protocol. Equally, adding C-peptide assays would provide additional insulin secretion information. Importantly, one clinical procedure thus yields potentially several test results.

In-silico investigations were undertaken to evaluate the efficacy of two additional, specific DISTq protocol variations and to observe the pharmacokinetics of anti-diabetic drugs. The first variation combined the boluses used in the DISTq and reduced the overall test time to 20 minutes with only two glucose assays. The results of this investigation implied no significant degradation of insulin sensitivity values is caused by the change in protocol and suggested that clinical trials of this protocol are warranted. The second protocol variant added glucose content to the insulin bolus to enable observation of first phase insulin secretion concurrently with insulin sensitivity from glucose data alone. Although concurrent observation was possible without simulated assay noise, when clinically realistic noise was added, model identifiability was lost. Hence, this protocol is not recommended for clinical investigation.

Similar analyses are used to apply the overall dynamic, model-based clinical test approach to other therapeutics. *In-silico* analysis showed that although the pharmacokinetics of insulin sensitizers drugs were described well by the dynamic protocol. However, the pharmacokinetics of insulin secretion enhancement drugs were less observable.

The overall thesis is supported by a common model parameter identification method. The iterative integral parameter identification method is a development of a single, simple integral method. The iterative method was compared to the established non-linear Levenberg-Marquardt parameter identification method. Although the iterative integral method is limited in the type of models it can be used with, it is more robust, accurate and less computationally intense than the Levenberg-Marquardt method.

Finally, a novel, integral-based method for the evaluation of *a-priori* structural model identifiability is also presented. This method differs significantly from established, derivative based approaches as it accounts for sample placement, measurement error, and probable system responses. Hence, it is capable of defining the true nature of identifiability, which is analogous, not binary as assumed by the established methods.

The investigations described in this thesis were centred on model-based insulin sensitivity and secretion identification from dynamic insulin sensitivity tests with a strong focus on maximising clinical efficacy. The low intensity and informative DISST was successfully validated against the euglycaemic clamp. DISTq further reduces the clinical cost and burden, and was also validated against the euglycaemic clamp. DISTq represents a new paradigm in the field of low-cost insulin sensitivity testing as it does not require insulin assays. A number of *in-silico* investigations were undertaken and provided insight regarding the suitability of the methods for clinical trials. Finally, two novel mathematical methods were developed to identify model parameters and asses their identifiability, respectively.

Table of chapters

Table of contents

List of Figures

Chapter 11. Observing insulin sensitizer and secretagogue drug kinetics *in-silico* 201

List of Tables

Abbreviations

Physiological conditions

T1DM or IDDM	Type 1 diabetes mellitus (insulin dependent)
T2DM or NIDDM	Type 2 diabetes mellitus (non-insulin dependent)
IGT	Impaired glucose tolerance
IFG	Impaired fasting glucose
NGT	Normal glucose tolerance
IR	Insulin resistant

Insulin sensitivity tests

EIC	Euglycaemic hyperinsulinaemic EIC
IVGTT	Intra-venous glucose tolerance test
IM-IVGTT	Insulin-modified IVGTT
FS-IVGTT	Frequently sampled IVGTT
ITT	Insulin tolerance test
OGTT	Oral glucose tolerance test
MTT	Meal tolerance test
HOMA	Homeostasis model assessment
DISST	Dynamic insulin sensitivity and secretion test
DISTq	Quick dynamic insulin sensitivity test

Statistical nomenclature

SD	Standard deviation
CV	Coefficient of variation
ROC	Reciever operator curve
c-ROC	area under the ROC curve
Q_1 Q_2 Q_3	Quartiles

Institutions

ADA	American Diabetes Association
NZMoH	New Zealand Ministry of Health

Blood assays

TAG	Triglyceride
FPG or G_B	Fasting plasma glucose
IPG or I_B	Fasting plasma insulin
C_B	Fasting plasma C-peptide

Chol	Cholesterol	
HDL-C	High-density lipoprotein cholesterol	
IV	Intra-venous	

Modelling terms

PK	Pharmaco-kinetic	
PD	Pharmaco-dynamic	

Common model parameters

SI	Insulin sensitivity	L/mU/min
U_N	Endogenous insulin production rate	mU/min
U_B, U_I and U_2	Basal, first and second phase insulin production rates	mU/min
G	Glucose concentration	mmol/L
I	Plasma insulin concentration	mU/L
Q	Interstitial insulin concentration	mU/L
C	Plasma C-peptide concentration	pmol/L
Y	Intersitital C-peptide concentration	pmol/L
p_G	Glucose dependant glucose clearance	1/min
V_G	Glucose distribution volume	L
V_P and V_Q	Plasma and interstitial insulin distribution volumes	L
BW	bodyweight	kg
n_L	Hepatic plasma insulin clearance	1/min
x_L	Hepatic portal insulin clearance	1
n_K	Renal insulin clearance	1/min
n_I	transition of insulin between plasma and intersitital	L/min
k_I, k_2 and k_3	C-peptide transport rates	1/min
P_X	Exogenous glucose bolus	mmol
U_X	Exogenous insulin bolus	mU
EGP	Endogenous glucose production	mmol/L

PART ONE: Background

Chapter 1. Relevant physiology

The contents of this chapter summarise the physiology that is associated with insulin resistance and the pathogenesis of type 2 diabetes mellitus.

1.1 Glucose

Glucose is a simple sugar that is used by the body as a primary energy source. It is absorbed by the body as a significant constituent of dietary carbohydrate intake. Glucose is absorbed into the blood stream from the small intestine after being broken into the simple form from longer chain carbohydrate molecules found in food. Figure 1.01 shows the molecule structure.
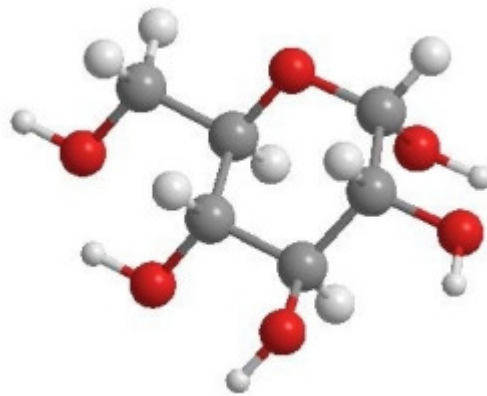
Figure 1.01. A glucose molecule (grey-carbon, white-hydrogen, red-oxygen)

Glucose is transported around the body passively in the blood stream. It can be directly taken up by the brain and some specific types of intestinal cells. However, most of the body's cells require the hormone insulin to mediate uptake. In this form of uptake, insulin, acts as a biochemical signal to unlock the cell so glucose can pass inside.

In the cells, glucose can either be stored or consumed to release energy. Storage (glycogenesis) involves conversion of glucose to glycogen, which, in turn, is later broken down for release of the stored energy (glycogenolysis). Glucose can also be metabolised to release energy (glycolysis). Glycolysis involves a series of transformations, some of which release significant energy, and results in the pyruvate molecule ($C_6H_{12}O_6$ to $C_3H_3O_3$). Amino acids or other dietary monosaccharides can also undertake the glycolysis process, or be converted to glycogen to store energy.

Blood glucose concentration in the body is highly regulated in healthy individuals. A healthy glucose concentration is approximately 4 to 5 mmol/L with brief, regulated excursions after meals. Prolonged malnutrition or exposure to insulin can result in hypoglycaemia (low blood glucose). Severe hypoglycaemia (<2.2 mmol/L) can limit the availability of energy to the brain and nervous system and result in un-consciousness or death. Hyperglycaemia occurs when blood glucose is elevated above safe levels. Incidents of hyperglycaemia can result in coma (<25-30 mmol/L), stupor, polyphagia (excessive hunger), polydipsia (excessive thirst), and polyuria (excessive urinary output) amongst other symptoms (>10 mmol/L). Prolonged hyperglycaemia is also highly toxic to a wide range of tissues and can result in diabetic retinopathy, leading to partial blindness, and decay of peripheral capillaries, which may finally require hands or feet be amputated. Thus, continual tight glucose control is very important for positive ongoing health outcomes.

1.2 Insulin

Insulin is a hormone that is produced in the β-cells of the pancreas with the primary function of reducing glucose concentration in the blood stream. Insulin's principle function is to stimulate cellular uptake of glucose. Insulin reacts with specific insulin-receptors on the cell wall and causes the cell to open channels of transport that allow glucose to transition into the cell where it is used as the primary energy source. Figure 1.02 shows six insulin molecules arranged in a hexamer configuration.
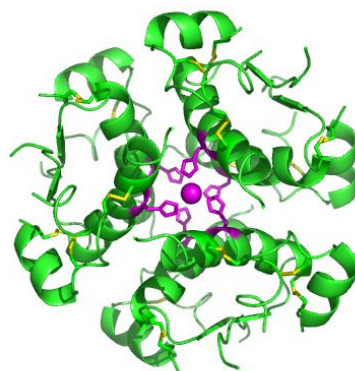
Figure 1.02. Ribbon diagram of the insulin hexamer

Insulin is generally released by the pancreas in response to an increase in blood glucose concentration. Insulin release generally consists of two distinct phases. The first phase is a

release of stored insulin and is in reaction to sudden, larger changes in glucose concentration. Cherrington found that the magnitude of the first phase of insulin release was proportional to the rate of change in glucose and the glucose gradient between the periphery and the portal vein (Cherrington 1999). In daily life, this behaviour only occurs as a result of food ingestion. The second phase of insulin production is in response to the glucose concentration. The rate of production is a function of glucose concentration over a basal level of 4 to 5 mmol/L and is designed to slowly reduce elevated glucose levels.

Insulin is produced by β-cells, which are located in islets of Langerhans in the pancreas. These specialised β-cells first produce long peptide chains called proinsulin. This long peptide is then cleaved yielding a single insulin molecule and a single C-peptide molecule. Insulin is then secreted along with C-peptide in equimolar amounts into the portal vein, which drains directly to the liver and then the bloodstream via the hepatic vein, vena cava, then finally the arterial system. Transport to the interstitium, where cellular glucose uptake occurs in the periphery, is achieved via passive diffusion across membranes.

It should be noted that insulin production can be suppressed by high insulin concentrations (Argoud *et al*. 1987), acting as a form of necessary counter-regulation. Equally, low glucose levels shut off insulin production and lead the pancreas to secrete counter-regulatory hormones (e.g. glucagon) to raise blood glucose. Together the system regulates overall glucose levels.

Endogenous insulin is predominantly cleared by the liver but also by the kidney in smaller quantities and broken down at the cell after utilisation. Initially, endogenous insulin travels through the portal vein to the liver, where much of it is extracted and used in the creation and storage of glycogen. Specifically, 60-80% of the portal vein insulin content is extracted by the liver during the first pass after secretion (Cobelli & Pacini 1988; Ferrannini & Cobelli 1987; Meier *et al*. 2005; Toffolo *et al*. 2006). Insulin is then subsequently further extracted from the blood stream by the liver or kidney, or passes into the interstitial regions of the body where it is broken down at the cellular level after mediating glucose uptake.

In the interstitium, insulin binds to receptors of muscle and adipose cell outer membranes and activates an intracellular reaction that results in glucose uptake. Specifically, insulin prompts the insulin receptor to initiate a series of intracellular reactions that result in the translocation of glucose transporter proteins (GLUT-4) from the interior of the cell to the cell membrane. Once GLUT-4 is situated in the cell membrane, it opens a channel through which glucose can diffuse. The glucose concentration gradient across the cell membrane drives diffusion. Once glucose enters the cell, it undergoes a transformation to glucose-6-phosphate. Glucose-6-phosphate then

either undergoes energy-releasing glycolysis, or is converted to glycogen, a rich source of stored energy for the cell.

Insulin also performs a number of functions other than mediation of cellular glucose uptake that have an effect on glycaemia. Importantly, it suppresses the hepatic release of stored glucose. Hepatic glucose release or endogenous glucose concentration (EGP) is primarily suppressed by a postprandial (post-meal) increase in glucose concentration. However, increased insulin concentrations also occur postprandial and serve to amplify the suppression of glucose release. Insulin causes storage of glucose in the liver through promotion of glycogen synthesis and insulin reduces the production of glucose from non-carbohydrates sources through the inhibition of the gluconeogeneisis process. In summary, every glycaemic-related function of insulin serves to reduce the blood glucose concentration.

Insulin also mediates a number of non-glycaemic-related functions as well. It increases the uptake of lipids and fatty acids in the blood stream for conversion to triglycerides. Insulin also increases the uptake of amino acids and potassium to the cell. Finally, it can cause the arterial muscle to relax increasing the blood flow rate acting as a dilatory agent (Barrett *et al*. 2009). Insulin can also improve cognitive ability (Benedict *et al*. 2004; Craft *et al*. 1999) and mediates the hunger impulse (Hallschmid & Schultes 2008).

1.3 Type 2 diabetes mellitus

The first recorded use of the term 'diabetes' was first used in 1st century Greece wherein the Greek word for 'siphon' was used to characterise the sufferers excessive urinary output. The suffix 'mellitus' meaning 'honey' was added in the 17th century. This latter term describes the sweetness of the urine that occurs as a result of the high glucose content in the urine caused by renal glucose clearance. Renal clearance of glucose only occurs when (unregulated) glucose levels are very high (Arleth *et al*. 2000) and thus, does not occur in healthy individuals who regulate glucose via insulin.

There are three main types of diabetes: Type 1, Type 2 and gestational diabetes. Type 1 diabetes occurs when the body's immune system attacks β-cells causing a total, or near total, loss of insulin production. Thus, type 1 sufferers must use exogenous insulin to maintain safe glucose concentrations. Gestational diabetes occurs during pregnancy and is caused by some of the hormonal changes associated with pregnancy. Gestational diabetes is typically alleviated post-partum, but can occasionally trigger type 2 diabetes. Type 2 diabetes occurs when the

participant's ability to produce insulin becomes insufficient to maintain healthy glucose concentrations. Over time, excessive insulin resistance and increased insulin production requirements lead to an eventual loss of β-cell function. It is generally agreed that the development of type 2 diabetes is predominantly driven by increased resistance to insulin. Insulin resistance is typically caused by obesity or a genetic disposition. Thus, etiological studies of the disease frequently measure participant's insulin resistance with purpose designed clinical tests.

Type 2 diabetes is characterised by the sufferer's inability to regulate their blood glucose concentrations with their own endogenous insulin production response. Untreated or uncontrolled diabetic individuals often experience hyperglycaemia, wherein glucose concentrations are above healthy levels. Healthy individuals have a basal glucose concentration of approximately 4.2 and 4.7 mmol/L, whereas diabetic individuals can have basal blood sugar concentrations between 7 and 25 mmol/L.

Blood glucose concentrations over 20 mmol/L can trigger seizures or coma, while concentrations of over 11 mmol/L cause oxidative processes to take place that have a damaging effect on a wide range of tissues. This damage is the driver of the worst long-term diabetic symptoms and complications. Blood vessels are most susceptible to this oxidative process and, as such, the most prevalent symptom of uncontrolled diabetes is cardiovascular disease. Other significant negative outcomes include renal failure, loss of feeling or amputation of hands and feet, and damage to the optical nerve or retinopathy. In particular, damage to the optical nerve results in diabetic retinopathy wherein blood vessels close to the eye rupture and release small amounts of blood onto the retina, damaging vision. Equally, renal failure requires these patients to undergo kidney dialysis up to 3 times per week, a time consuming, costly and debilitating procedure. The overall outcome is decreased function and quality of life, and often earlier mortality.

The first irregularity sufferers of type 2 diabetes often notice is excessive urinary output. This symptom is a result of the sufferers thirst response to elevated glucose concentrations. Drinking a lot of water has a diluting effect on blood glucose. However, the body responds to the increase in body fluid and the kidney generates more urine.

Diagnosis of diabetes can be made with a fasting glucose measurement of over 7.0 mmol/L, a glucose concentration of over 11.1 mmol/L 2 hours after a 75g oral glucose load, and, more recently, a glycosylated haemoglobin (HbA1c) of over 6.5% has also been an accepted diagnostic (ADA 2010). Confirmation of the disease state must be made with a second test undertaken on a separate day. However, these diagnostic thresholds are set at levels that maximise positive predictive value and thus for many patients some damage and complication may already have set in.

Type 2 diabetes can be treated a number of ways. Typically, subjects attempt to maintain safe glucose concentrations though diet and exercise control, or with the aid of insulin sensitizers or insulin production enhancement drugs. However, the worst sufferers are not sufficiently controlled with such measures and require exogenous boluses of insulin to maintain safe glucose levels. These individuals use skin pricks to determine blood glucose concentrations and must use varying doses of insulin to maintain safe levels of glycaemia, essentially like type 1 diabetic individuals. In effect, the resulting control can be stable, but in the absence of adequate control systems, or as a result of rapidly changing physiological requirements, this control can become instable, potentially causing dangerous hyperglycaemia or hypoglycaemia. With adequate glucose control, diabetic subjects can live a relatively normal life. However, long-term β-cell damage is currently irreversible and ongoing treatment will be required.

The New Zealand Ministry of Health has predicted that 180,000 individuals (~5% of NZ population) will have diagnosed type 2 diabetes in 2011 (NZMoH 2007). This represents an increase of 45% from 2001. It is claimed that two thirds of this increase is associated with increased life expectancy and population growth. However, the remaining third can be attributed to the growing obesity epidemic (NZMoH 2007). Current health spending associated with diabetes is approximately $800 M/year in New Zealand with a projected cost increase to $1.4 to 1.8 B/year in 2021 (PriceWaterhouseCoopers 2007). Thus, although the health cost associated with type 2 diabetes currently places a significant burden on national health resources approaching 1% of GDP, it is likely to increase significantly in the coming decades.

Equally, this is not just a New Zealand problem. There are currently an estimated 220M sufferers of type 2 diabetics worldwide, with significant increases expected in developing countries in the coming decades (Gakidou *et al*. 2010). Perhaps more importantly, the number of un-diagnosed type 2 diabetic individuals is estimated at 30-50% of all such individuals, implying an unknown increase of 60-100% over those already diagnosed (Gakidou *et al*. 2010).

1.4 Loss of insulin production and the pathogenesis of diabetes

Type 1 diabetes occurs when the pancreas ceases to produce insulin as a result of an immune response irregularity (Bluestone *et al*. 2010). The individual's natural immune system begins to attack the insulin producing β-cells of the pancreas. This generally occurs in juvenile individuals, but can occur at any stage of life.

In contrast, type 2 diabetes is generally brought on by excessive insulin demand on the pancreas resulting in a loss of β-cell mass, and insulin production capability. Although the precise mechanism for the loss of insulin production during the pathogenesis of type 2 diabetes is unknown, it is generally agreed that it is often an artefact of obesity due to excessive dietary intake over an extended period, a genetic predisposition or as an effect of another disease state (Ahrén & Pacini 2005; Kahn *et al*. 2006). The initial stage of the pathogenesis of type 2 diabetes is always marked by an increase in insulin resistance. Thus, the body requires a greater amount of insulin to maintain safe glucose concentrations.

In particular, the pancreas is generally capable of producing much more insulin than is required by relatively healthy, insulin sensitive individuals. Although individuals with increased insulin demand can usually maintain safe, normal appearing glucose concentrations, their decreased insulin sensitivity implies that they have impaired glucose tolerance (IGT) (ADA 2005). However, if insulin resistance is significantly exasperated, the maximal possible rate of insulin production can become incapable of maintaining safe, normal basal glucose concentrations. At this stage, type 2 diabetes can first be diagnosed with typical tests.

Further exacerbation of insulin resistance or prolonged exposure to this state often results in β-cell damage. Due to over-working of the pancreas, or damage relating to elevated glucose, the β-cell mass is diminished, reducing the amount of insulin that the participant can produce. Thus, long-term and recently-diagnosed type 2 diabetic individuals can be readily distinguished by their distinctive insulin production rates. While the recently-diagnosed diabetic individual's endogenous insulin production is elevated, the long-term diabetic individual's endogenous production is significantly reduced and may be near zero.

Figure 1.03 shows the pathogenesis of type 2 diabetes by tracking the typical daily glucose concentration, typical daily insulin production demand and insulin sensitivity. The increase and subsequent decline of insulin production is known as Starlings curve of the pancreas (Clark *et al*. 2001; Gastaldelli *et al*. 2004). A similar curve was also shown by Ferrannini *et al*. (Ferrannini 1997). Figure 1.03 clearly shows that glucose concentration does not rise to allow diagnosis until well after the loss of insulin sensitivity.
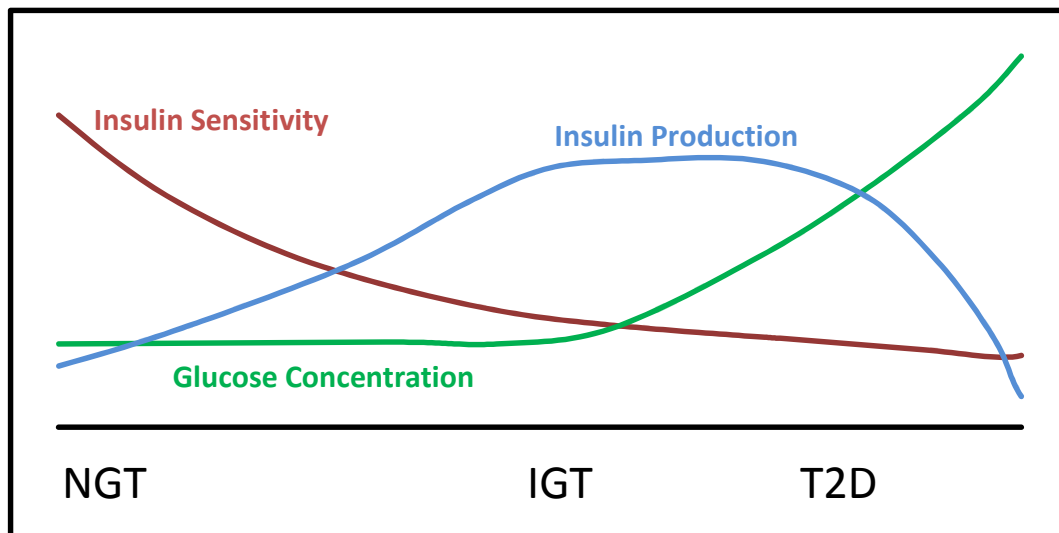
Figure 1.03. A generalisation of the inter-relationships between insulin production, sensitivity and glucose concentration during the pathogenesis of type 2 diabetes.

Therefore, unfortunately, the IGT state often goes unnoticed, and diagnosis often only occurs when insulin production becomes impaired. Thus, some irreversible damage is usually done prior to the initiation of measures that could potentially mitigate the development or symptoms of diabetes. Hence, the disease can be well established before diagnosis and many individuals may go undiagnosed. Early diagnosis based on insulin resistance (reduced insulin sensitivity) could allow earlier treatment to better reduce costly damaging long-term complications. Hence, there is a need for accurate insulin sensitivity based diagnostic tests. Ideally, the test would provide a picture of participant status relative factors shown on Figure 1.03 and the aetiology of diabetes.

Chapter 2. Insulin sensitivity tests

In order to appraise the tests that are presented later in this thesis properly, they should be placed in context of the insulin sensitivity tests that are currently available. Thus, this chapter describes the established, commonly used insulin sensitivity tests. This chapter reviews these tests in terms of parameter resolution and diagnostic accuracy, as well as clinical burden and cost.

2.1 Introduction

Most insulin sensitivity tests aim to measure the efficiency that insulin has on reducing blood glucose concentrations. The meaning of insulin sensitivity, or even the role of insulin sensitivity tests, is controversial as insulin predominantly reduces blood glucose concentrations in two ways. Primarily, it facilitates the transport of glucose into cells, removing it from the bloodstream, thus reducing concentration (peripheral sensitivity). Secondly, insulin suppresses endogenous glucose production (hepatic sensitivity). Either effect, or a combination of effects will be measured depending on a particular test's design. Thus, although the general aim of insulin sensitivity tests is agreed upon, the subtleties of the effect measured are different, and the correct use or definition is somewhat ambiguous (Pacini & Mari 2003). Notably, virtually none of the established tests consider the other factors in Figure 1.03 relating to insulin production.

There is considerable evidence that a loss of peripheral insulin sensitivity is a critical factor on the pathogenesis of type 2 diabetes (DeFronzo & Ferrannini 1991; Ferrannini 1997; Harris *et al*. 2003; Lorenzo *et al*. 2010; Martin *et al*. 1992; Zethelius *et al*. 2004). Whereas some isolated studies have shown that the disease state is sometimes also characterised by the loss of the insulin suppression of hepatic glucose release effect that is observed in healthy individuals (Consoli 1992; Martin *et al*. 1992). While there is a clear and consistently observed physiological contribution of the former to the pathogenesis of type 2 diabetes, the latter is also an important symptom of the disease state. Thus, both conditions have relevant clinical implications for the risk assessment, diagnosis and treatment of diabetes.

Unfortunately, these two distinct effects are very difficult to observe concurrently in a clinical trial. To do so requires labelled tracer elements, and the outcomes require complex modelling approaches that are often very sensitive to noise, limiting clinical utility (Avogaro *et al*. 1989; Cobelli *et al*. 1986; Mari 1998). Ultimately, the noise-sensitivity of such tracer studies has

limited their use in recent years and ongoing studies seem to have been abandoned in the literature.

Hence, most insulin sensitivity test protocols seek to reduce the suppression effect that insulin has on hepatic glucose release, while observing peripheral insulin sensitivity. In contrast, some protocols measure a combined effect of insulin to reduce plasma glucose concentration and hepatic glucose release. While the former measures peripheral sensitivity, the latter measures what is named 'whole body' sensitivity. However, many tests do not clearly state specifically which effect they are measuring, which can lead to confusion in comparing results across studies.

2.2 Uses for insulin sensitivity tests

Insulin sensitivity is a relatively strong predictor of the development of type 2 diabetes and could potentially be used as a risk assessment tool (Ader & Bergman 1987; Groop *et al*. 1993; Martin *et al*. 1992; Vozarova *et al*. 2002). Thus, instead of identifying the diabetic state on the right of Figure 1.03, the initial decline of insulin sensitivity could be identified and the subject could be given a diagnosis of increased risk. Mitigative measures could then be prompted that may alleviate the loss of β-cell function.

In a general practice setting, the current techniques with regard to assessing and mitigating the risk of diabetes are limited to recommending weight loss where appropriate and perhaps monitoring basal blood glucose when obesity is sustained. If the blood glucose is elevated for a prolonged period, the practitioner may recommend an oral glucose tolerance test (OGTT, described in Section 2.3.3) for an official diagnosis of type 2 diabetes mellitus. Thus, significant interventional measures are only actively made once the patient's glucose control is significantly inhibited, and poor lifestyle choices have usually become ingrained. Unfortunately, this stage usually happens only after significant and often irreversible β-cell damage has occurred. In particular, many obese individuals with significantly reduced insulin sensitivity can be normo-glycaemic and produce false healthy OGTT results (McAuley *et al*. 2001).

However, if the risk of diabetes were considered a possibility and an insulin sensitivity test were administered, the interventional measures could potentially be applied to prevent, offset or reduce the intensity of type 2 diabetes. Thus, the significant health costs associated with long-term type 2 diabetes could be ameliorated, and the individual could maintain a better quality of life for longer. Hence, early and accurate diagnosis is the key to reducing the human and economic costs of (rising) type 2 diabetes costs.

However, improved lifestyle outcomes and reduced incidence of type 2 diabetes is dependent on the high-risk patients' compliance to, and the effect of intervention measures. Such interventions have observed positive impact on participant weight and insulin sensitivity over the study or intervention duration (Clark *et al.* 2004; McAuley *et al.* 2002). However, it remains to be established whether such interventions have a significant positive long-term effect. These important considerations are outside the scope of the investigations presented in this thesis, as they first rely upon having effective early diagnosis, which is the critical element considered here.

2.3   Various existing insulin sensitivity tests

Insulin sensitivity tests can be classified as steady state, dynamic or fasting. Steady state tests generally apply continuous stimulus and measure the resultant steady state condition of the participant. Dynamic tests typically use bolus stimulus and observe the species kinetics that result. Fasting protocols do not use stimulus and merely measure some key molecular concentrations in the fasted participant. This section will detail the protocols of some well-known insulin sensitivity tests, and focus on their diagnostic use, clinical ease or burden, and overall capability.

2.3.1   The hyper-insulinaemic euglycaemic clamp test (EIC)

The EIC test was first proposed by Defronzo *et al* in 1979 (DeFronzo *et al.* 1979) and has since achieved gold standard status in the field of assessment of insulin sensitivity (Ferrannini & Mari 1998; Pacini & Mari 2003). Sensitivity metrics of the test have been shown to be accurate in terms of repeatability (DeFronzo *et al.* 1979; Mari *et al.* 2001; Monzillo & Hamdy 2003) and relevant in terms of its use for risk assessment of a series of metabolic conditions. These conditions include: type 2 diabetes (DeFronzo & Ferrannini 1991; Ferrannini 1997; Hanley *et al.* 2003; Zethelius *et al.* 2004), cardiovascular disease (McLaughlin *et al.* 2007) including congestive heart failure (Ingelsson *et al.* 2005), the metabolic syndrome (Hanley *et al.* 2005; Zimmet *et al.* 1999), and, in some cases, liver disease (Hanley *et al.* 2005). These conditions are all associated with poor diet and exercise lifestyles and, with the exception of type 2 diabetes, no immediate etiologic pathway exists between insulin resistance and any of these diseases.

The EIC test protocol requires a cannula placed in the antecubital fossa to enable insulin and glucose infusions. A second cannula is placed retrograde in the dorsum of the hand to enable

blood sampling. Most study designs include the heating of the hand surface to mobilize blood in the capillaries and enable samples that are more representative of overall, whole body interstitial concentrations. The surface of the hand is usually warmed using a purpose-built heated-hand-box, which heats and circulates air around the participant's hand. The temperature of the heated air is generally controlled between 40 and 70°C.

The test proper begins when insulin is first infused and is generally infused at a rate proportional to the participant's size. The rate is usually defined to achieve a certain plasma insulin concentration (100 mU/L is a typical target). This concentration is relatively hyper-physiological as a typical insulin concentration of a healthy fasting person is 2-10 mU/L and 50-70 mU/L at the postprandial peak.

A glucose infusion begins soon after the insulin infusion to maintain euglycaemia between 4 to 5 mmol/L. Blood samples are taken with relatively high frequency (2-15 minute resolution) and assayed for glucose at the bedside. This frequent sampling allows manual alterations of the glucose infusion for feedback control of the participant's blood glucose concentration. The insulin and glucose infusions are generally continued for 2.5 to 4 hours, but can continue for up to 24 hours in some studies (Swinnen *et al*. 2008). Some samples are spun and frozen to be assayed for plasma insulin typically during steady state. Basal insulin is usually measured, as well as at t=15 to 30-minute intervals after an hour of infusion.

The first hour of the test generally involves transient rates of glucose infusion as the hyperinsulinaemic conditions begin to suppress the endogenous productions of insulin and glucose (Argoud *et al*. 1987; DeFronzo *et al*. 1979; Ferrannini & Mari 1998) and the clinician begins to understand the particular metabolic dynamics of the participant. After approximately 60-90 minutes, a steady rate of glucose infusion can be achieved as the participant reaches steady state. However, if the participant's glycaemia is poorly controlled in the early stages of the test, the participants may exhibit counter-regulatory effects that can destabilise the remainder of the test and disable insulin sensitivity identification.

The EIC insulin sensitivity value is calculated as the rate that glucose is infused to the participant in the later steady-state stages of the test. Most studies normalise this infusion rate by the participant's weight or fat-free mass, while others normalise further by the particular insulin concentration achieved during the identification period. A "space correction" can also be used to account for changes in the participant's glucose concentration during the identification period of the test.

The EIC protocol suppresses most of the daily variations that can partially obscure the resolution of other insulin sensitivity tests. The strong insulin and glucose stimulus of the EIC protocol suppresses endogenous glucose and insulin production and also minimises the influence of variation in these effects on the identified sensitivity value. Thus, the EIC is highly repeatable. However, the protocol is arduous for both the participant and the clinician, and a steady-state infusion rate is not always assured at the end of the test. Thus, a result is not always guaranteed.

The insulin sensitivity value identified by the protocol is dependent on the concentration of insulin achieved, which, in turn, is a function of the specific dose and protocol used. In addition, the effect of insulin is saturable (Natali *et al*. 2000; Prigeon *et al*. 1996). For example, if the insulin concentration achieved were doubled, glucose clearance may only increase by 80%. Some studies have investigated this effect and its impact on EIC results. While some studies repeated the protocol on different days with varying insulin infusion rates (Kolterman *et al*. 1980), others used arduous stepped EIC protocols, wherein once steady state was established, the insulin infusion rate was changed (Laakso *et al*. 1990; Natali *et al*. 2000; Rizza *et al*. 1981). The average plasma insulin concentrations at half-maximal effect found by these studies was 160 mU/L. This value is close to the 100 mU/L insulin concentration target of most typical EIC protocols. As such, EIC metrics, although highly repeatable at consistent doses, lose relevance either in different tests or in EIC tests with a different dosing. Thus, insulin sensitivity values from the EIC are not directly comparable across studies.

The EIC has achieved gold standard status in the field of insulin sensitivity testing. However, this outcome does not necessarily imply that it is the optimal test for the screening for type 2 diabetes risk. In particular, the test cannot provide an evaluation of the participant's insulin production capability, nor an indication of participant's expected daily glucose concentration. Figure 2.01 summarises the ability of the EIC to track the pathogenesis of type 2 diabetes with reference to Figure 1.03, where the dotted lines indicate aspects not measured by the EIC.
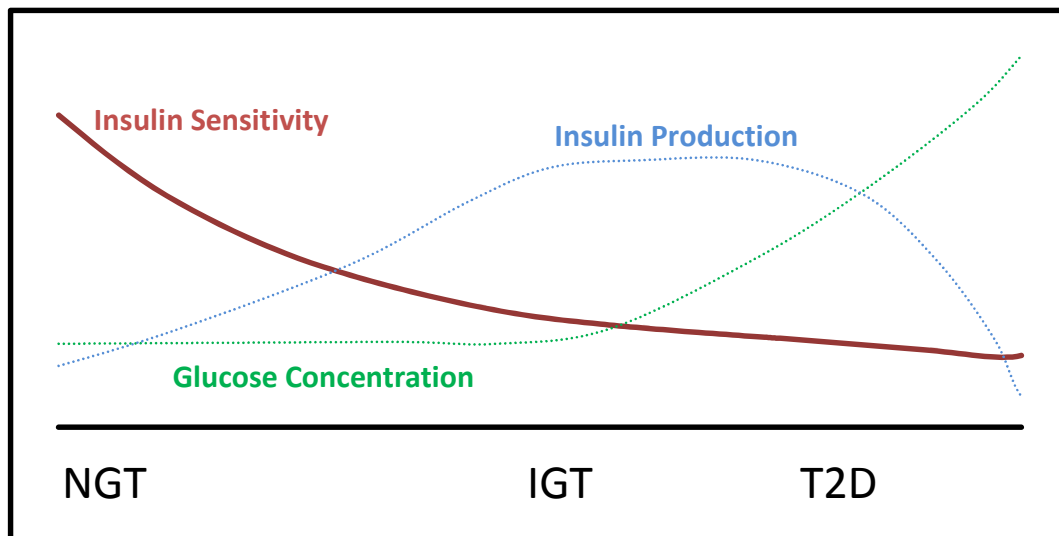
Figure 2.01. Characteristics of the pathogenesis of type 2 diabetes observable by the typical EIC protocol. The hyperinsulinaemic EIC is designed to solely quantify the participant's insulin sensitivity, insulin production is not measured.

A second EIC test protocol is available for the quantification of a participant's β-cell function. Instead of a sustained hyper-physiological insulin concentration, a sustained hyper-physiological glucose concentration is used. Thus, in the steady state the pancreas will respond to the raised glucose concentration and produce insulin. This protocol is not extensively utilised and cannot be used in conjunction with the hyper-insulinaemic EIC. Insulin production and insulin sensitivity cannot both be identified concurrently with the EIC approach.

2.3.2 Dynamic intra-venous tests

Typical dynamic insulin sensitivity test protocols use bolus glucose and take a series of assays to measure the participant's glucose decay and insulin concentration. The insulin concentration is often increased by the participant's endogenous physiological response to the test stimulus. However, some protocols strengthen the insulin signal with the addition of an exogenous bolus, this is particularly useful for type 1 and some type 2 diabetic subjects. The sensitivity metrics of such tests are often derived using models of the insulin/glucose pharmaco-dynamics and relatively complex mathematical processes. Many dynamic protocols have been developed to produce data that can enable insulin sensitivity identification using various specific or generic models and mathematical methods or algorithms.

The most popular dynamic protocol is the intravenous glucose tolerance test (IVGTT). There are a number of distinct protocols that are regarded as IVGTT tests. However, a typical protocol

includes cannula in the dorsum of the hand, and/or the antecubital fossa for blood samples and the application of a bolus glucose input. A series of fasting blood samples are taken to establish the basal condition of the participant. This sampling is followed by a glucose bolus (at t=0). The magnitude of the glucose bolus is usually a function of the participant's size. A typical dose may be 25g of glucose for a 75 kg participant. Further samples are taken after the bolus. The timing of the samples often reduces in frequency from about 2 to 3 minutes immediately after the bolus to 15 to 30 minutes 2 to 3 hours into the test protocol. The duration of these tests varies significantly between 1.5 to 4 hours. All samples are assayed for glucose and either a significant proportion or all of the samples are assayed for insulin. Some IVGTT protocols are suffixed with 'FS-' to denote the frequent sampling (FS-IVGTT).

The data can be used to quantify insulin sensitivity in a variety of ways. However, insulin sensitivity is usually estimated using the non-linear least-squares parameter identification method with the Minimal-Model of insulin glucose dynamics proposed by Bergman *et al.* (Bergman *et al.* 1979; Bergman *et al.* 1981; Bergman *et al.* 1987; Boston *et al.* 2003; Caumo *et al.* 1999). Although this method has been extensively validated since it was devised (Bergman *et al.* 1987; Donner *et al.* 1985; Finegood *et al.* 1984; Saad *et al.* 1994), numerous issues have been identified.

These issues include the over-parameterisation of the Minimal-Model that causes the inability to distinguish between insulin and non-insulin mediated glucose disposal, and the convergence to local, not global, error minima during the non-linear least-square identification process (Pillonetto *et al.* 2002; Quon *et al.* 1994b). A novel investigation of this particular issue is presented later in Section 5.4.2. The issue of over parameterisation can be mitigated somewhat by the application of complex Bayesian identification methods (Cobelli *et al.* 1999; Denti *et al.* 2009; Erichsen *et al.* 2004; Pillonetto *et al.* 2002). However, there are no reported, complete solutions for all these issues, which affect the diagnostic resolution and repeatability of these tests.

The Minimal Model defines the rate of glucose disposal as a function of glucose concentration (G) over the basal concentration (G_B), and the 'action' of insulin (X) multiplied by the available glucose. The model is defined:

$$\dot{G} = -S_G(G - G_B) - XG + \frac{P_X}{V_G} \qquad\qquad 2.01$$

$$\dot{X} = -p_2 X + p_3(I - I_B) \qquad\qquad 2.02$$

where: S_G represents the decay of glucose as a function of glucose concentration over basal [1/min]; p_2 lumps the rate of insulin transport into the in-accessible compartment (interstitium) and efficiency of insulin into a single parameter [1/min]; p_3 lumps the clearance of insulin from the in-accessible compartment [L/mU/min^2]; V_G is the volume of distribution of glucose [L]; and I-I_B is the plasma insulin concentration above basal [mU/L].

Insulin sensitivity (SI) is estimated as a function of p_2 and p_3:

$$SI = \frac{p_3}{p_2}$$
2.03

Both SI and S_G metrics are functions of glucose concentration and quantify glucose decay. Thus, parameter value tradeoffs can occur during the identification process and intra-participant repeatability between tests can be poor (Gelding *et al*. 1994; Monzillo & Hamdy 2003; Pillonetto *et al*. 2003; Quon *et al*. 1994a). This error is propagated by the susceptibility of clinical data to measurement noise from assay error, physiological mixing, ineffective cannula flushing or species decay prior to assay.

Metrics of insulin sensitivity from the IVGTT correlate relatively well to the gold standard EIC in most studies, but some studies have shown significant differences (R=0.44-0.85) (Donner *et al*. 1985; Foley *et al*. 1985; Galvin *et al*. 1992). This uncertainty, coupled with the over-parameterisation issue and the limited accessibility of the complex computer identification algorithms has limited the widespread acceptance of the IVGTT for use outside of research focused clinical trials.

IM-IVGTT and TM-IVGTT

Two variations of the IVGTT are also used. The first incorporates an insulin bolus or occasionally infusion (Ward *et al*. 2001) 10 to 20 minutes after the glucose bolus: the insulin modified IVGTT (IM-IVGTT). The second variation incorporates a tolbutamide bolus 10 to 20 minutes after the glucose bolus (TM-IVGTT). Tolbutamide stimulates a large, almost immediate, release of endogenous insulin. These protocols produce a greater consistency of insulin concentration across participants and a stronger signal for the identification methods. As such, the results are generally more stable and correlate somewhat more consistently with the EIC (R=0.70 to 0.89) (Bergman *et al*. 1987; Saad *et al*. 1997).

Figure 2.02 illustrates the aspects of the pathogenesis of type 2 diabetes that the various IM-IVGTT protocols can typically quantify accurately. Note that insulin sensitivity becomes poorly identified in the IGT/T2D regions and that insulin production is not measured at all.
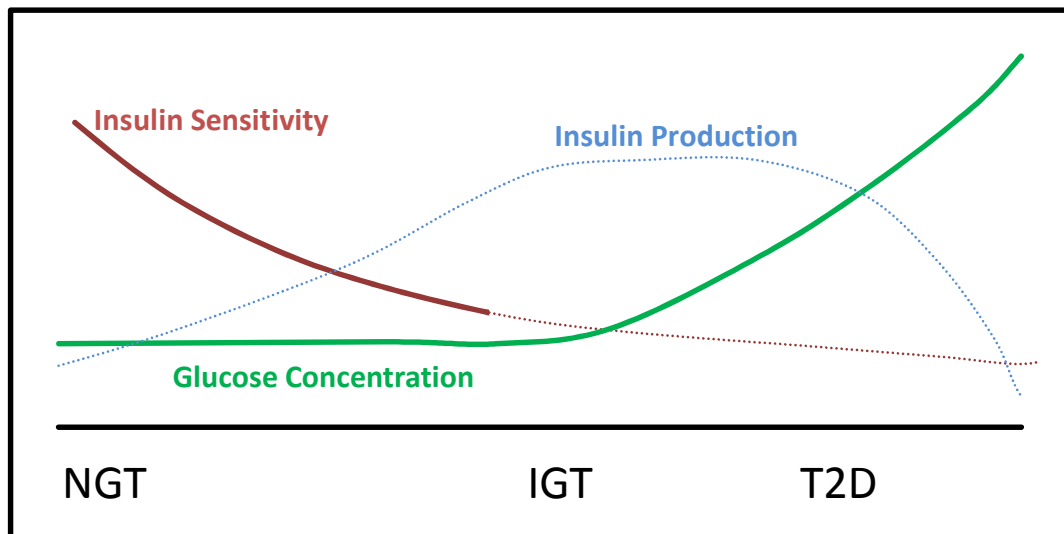
Figure 2.02. Characteristics of the pathogenesis of type 2 diabetes observable by a typical IVGTT protocol. Typical metric identification methods of the IVGTT cannot differentiate between insulin sensitivity of participants in the latter stages of the pathogenesis of type 2 diabetes.

ITT

The insulin tolerance test (ITT) is a dynamic test that involves the administration of a relatively large bolus of insulin to a fasted participant. Once a threshold of hypoglycaemia is reached, usually <2.2 mmol/L, the clinician quickly administers glucose to aid a return to euglycaemia. The rate that the participant's glucose concentration declines is measured and a metric of insulin sensitivity is derived. The test protocol is very dangerous, as severe hypoglycaemia is consistently and purposefully induced. As such, it is has been abandoned for insulin sensitivity testing and is only used very infrequently to detect very unusual conditions, such as pituitary or adrenal dysfunction, amongst others. However, the ITT has correlated particularly well to the EIC (R=0.83 to 0.9) (Bonora *et al*. 1989; Grulet *et al*. 1993), and it is relatively low in required clinical effort.

2.3.3 Oral glucose tolerance test (OGTT)

The oral glucose tolerance test involves the oral consumption of a drink with pre-defined glucose content. Various doses and sampling protocols are used. However, the most common are the 75g 2-hour trial and the 50g 1-hour trial. The 75g protocol can be frequently sampled with assays for insulin and glucose, or sparsely sampled with only one blood test at the end of the test. The 50g protocol usually only involves a single blood sample which is taken at the end of the trial. Diagnosis is typically made using the last glucose sample to determine whether the participant has the ability to reduce glucose loads adequately. Thus, diagnostic use of the OGTT does not typically calculate insulin sensitivity directly.

When samples from the frequently sampled 75g protocol are assayed for glucose and insulin a surrogate metric of insulin sensitivity can be obtained. These surrogate metrics tend to involve simple mathematical equations of the assay values and have been extensively published (Cederholm & Wibell 1990; Gutt *et al*. 2000; Matsuda & DeFronzo 1999; Piché *et al*. 2007; Stumvoll *et al*. 2000). Equations 2.04-2.07 show the equations of some popular OGTT metrics.

$$ISI_{Matsuda} = \frac{10000}{\sqrt{G_b I_b \bar{G} \bar{I}}} \tag{2.04}$$

$$ISI_{Cederholm} = \frac{75000 + 39.3BW(G_b - G_{2h})}{120 \ln(\bar{I})\,\bar{G}} \tag{2.05}$$

$$ISI_{Gutt} = \frac{75000 + 0.19BW(G_b - G_{2h})}{120 \ln \dfrac{(I_b + \bar{I})(G_b + \bar{G})}{4}} \tag{2.06}$$

$$ISI_{Stumvoll} = 0.226 - 3.2\,e^{-3}BMI - 6.45e^{-6}I_{120} - 3.75e^{-3}G_{90} \tag{2.07}$$

where: $\bar{G}$ and $\bar{I}$ represent the average of the *t*=0, 30 , 60 and 120 glucose and insulin assays, respectively.

Some model-based methods for parameter estimation have also been proposed (Dalla Man *et al*. 2005b; Mari *et al*. 2005; Mari *et al*. 2001). However, the variable rate of absorption of glucose from the gut to the bloodstream is difficult to measure and causes significant variability in results and computational parameter identification issues. The identified insulin sensitivity values in particular are affected by the variable rate of absorption. Thus, variations in absorption or appearance rates play a major complicating role.

One such model modifies Bergman's Minimal Model to include a term for the absorption of glucose (Breda *et al*. 2002; Dalla Man *et al*. 2005a). Bayesian techniques coupled with tracer elements enable high correlations to the EIC. However, the limited availability of tracer glucose and the complexity of the modelling and identification required when using this approach has

limited it's broader use. Table 2.01 shows the correlations reported between these metrics and the gold standard EIC.

OGTT Metric	EIC *SI* correlation	References
Matsuda	0.57-78	(Lorenzo *et al*. 2010; Malita *et al*. 2010; Piché *et al*. 2007; Pigeon *et al*. 2009; Soonthornpun *et al*. 2003)
Cederholm	0.53-79	(Piché *et al*. 2007; Pigeon *et al*. 2009; Soonthornpun *et al*. 2003)
Gutt	0.58-0.63	(Gutt *et al*. 2000; Soonthornpun *et al*. 2003)
Stumvoll	0.51-0.75	(Malita *et al*. 2010; Piché *et al*. 2007; Soonthornpun *et al*. 2003)
Oral Minimal Model	0.71-0.81	(Breda *et al*. 2002; Dalla Man *et al*. 2005a)

Table 2.01. Reported performance evaluation values (when available) for the presented OGTT surrogate insulin sensitivity metrics.

The OGTT is not often used in clinical investigations that require accurate insulin sensitivity values, nor is it used for the assessment of diabetes risk. However, the OGTT is currently an accepted method for the diagnosis of type 2 diabetes. Thus, the single blood test 2 hour 75g form of the OGTT will continue to be a commonly used glycaemic test. Figure 2.03 shows that the participant's insulin sensitivity and glucose concentration can be measured through the pathogenesis of type 2 diabetes. However, insulin production characteristics are not frequently reported.

An alternative version of the OGTT includes the administration of a pre-defined meal instead of the sugary drink (MTT). The meal is usually a standard size with a reported energy composition by carbohydrate, protein and fats. The MTT has been used to both predict insulin sensitivity (Caumo *et al*. 2000; Steil *et al*. 2004) and insulin production (Hovorka *et al*. 1998; Mari *et al*. 2002). MTT studies have shown a moderate *SI* identification equivalence with the EIC (R=0.76) (Steil *et al*. 2004), and an improved correlation with the FSIVGTT 0.89 (Caumo *et al*. 2000).
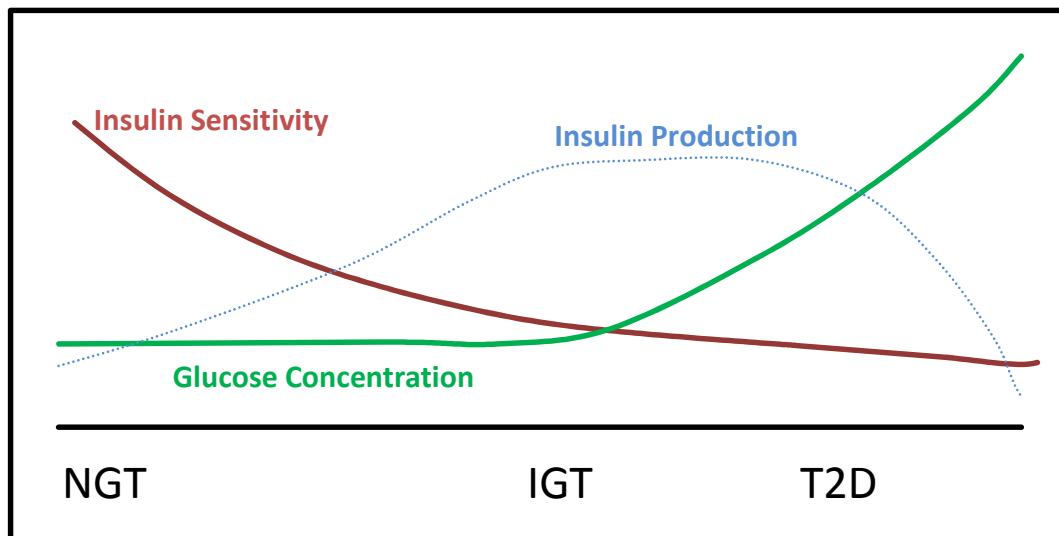
Figure 2.03. Characteristics of the pathogenesis of type 2 diabetes observable by the OGTT.

2.3.4 Fasting metrics

Numerous fasting measures can be used to asses an individual's metabolic health. However, only those which are relevant to the pathogenesis of diabetes are discussed here. Garcia-Estevez *et al.* and Ruige *et al.* contain more complete reviews of such tests (Garcia-Estevez *et al.* 2003; Ruige *et al.* 2006).

Fasting glucose

Fasting plasma glucose (G_B) is the most frequently used test to assess a subject's diabetic status. It is the least expensive and lowest intensity test available other than using anatomical characteristics like BMI or waist measurements. Samples can be assayed in real-time in a general practice setting. However, fasting glucose measurements can only become informative once an insulin production defect has already occurred (between IGT and T2DM in Figure 1.03). Thus, it is a very poor predictor of insulin sensitivity or the risk of developing type 2 diabetes.

Studies have correlated fasting plasma glucose to the EIC with results in the range R= -0.31 to -0.56 (Gutt *et al.* 2000; Ruige *et al.* 2006)

HOMA

The homeostasis model assessment (HOMA) measures blood glucose and insulin in the fasting state. The most common use of HOMA assays a single fasting sample for insulin and glucose. The concentrations of these species are multiplied and scaled to produce a measure of insulin

resistance (Equation 2.08). This value can thus be inverted for an insulin sensitivity value as in Equation 2.09.

$$HOMA_{IR} = \frac{G_b I_b}{22.5} \qquad\qquad 2.08$$

$$HOMA_{SI} = \frac{22.5}{G_b I_b} \qquad\qquad 2.09$$

However, this method was not what was intended by the creators of the metric. The initial process requires a series of samples to be taken on subsequent days (Wallace *et al*. 2004). Thus, the clinical intensity of the initially proposed method is significantly increased. Almost all studies that use HOMA use the single sample method.

HOMA has been validated against the EIC a number of times yielding a wide spread of correlations values between R=0.22 and R=0.93 (Bonora *et al*. 2000; Lorenzo *et al*. 2010; Mari *et al*. 2001; Mather *et al*. 2001; Matthews *et al*. 1985; Pacini & Mari 2003; Piché *et al*. 2007). Relatively high CV values have also been reported (10 to 20%) (Bonora *et al*. 2000; Matthews *et al*. 1985; Wallace *et al*. 2004).

Figure 1.03 indicates that the pathogenesis of type 2 diabetes is intrinsically linked to insulin sensitivity and can be reasonably observed in changes in the subject's insulin and glucose concentrations. The HOMA metric thus assumes that insulin sensitivity will be inversely proportional to both insulin and glucose concentrations. This assumption is generally correct until β-cell damage occurs and insulin production is impaired. However, the lack of test stimulus means that the effect of the pulsatile release of insulin (Del Prato *et al*. 2002; Meier *et al*. 2005) and assay error have a notable and adverse confounding effect on the identified insulin sensitivity.

HOMA is currently used by some general practitioners, primarily for a diagnostic of insulin resistance. However, the relatively high coefficient of variation and the lack of real-time available results limit the tests value. Thus, in many cases a simple fasting glucose is used, and is considered more practical in a general clinical setting.

Figure 2.04 shows that the HOMA basal glucose measurement could approximate the location of the participant along the latter stages of the pathogenesis of type 2 diabetes. However, during this stage of pathogenesis when the insulin production becomes inhibited, insulin sensitivity estimation becomes erroneous. For example, a mildly resistant individual with normal fasting glucose and corresponding increased basal insulin might have an equal HOMA value to a diabetic individual with elevated glucose and depleted insulin production capability. Thus, the resolution of the HOMA is poorest in the region of maximum clinical utility. The insulin assay may infer

the participant's insulin production capability to an extent. However, it is not a direct or relied upon measurement.
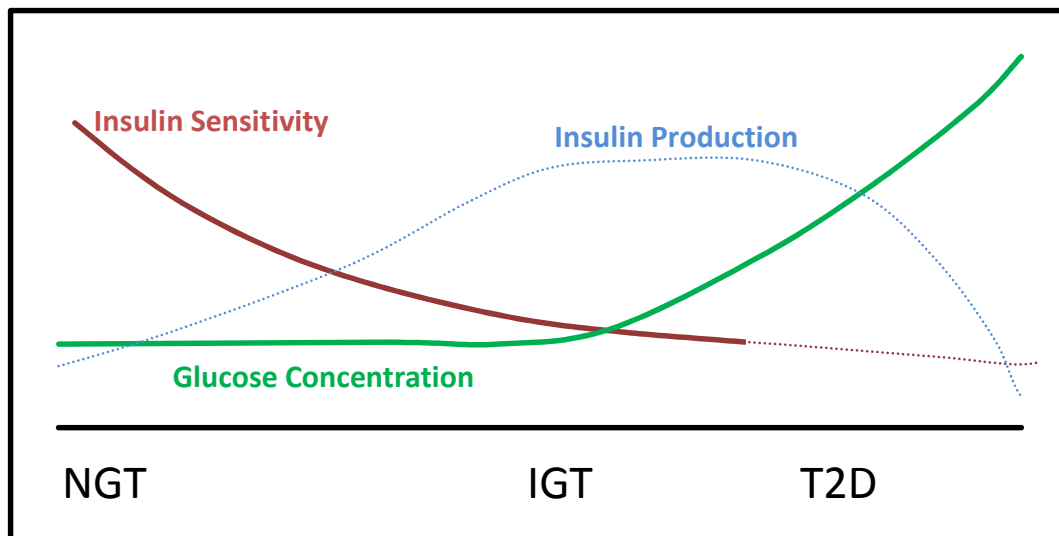
Figure 2.04. Characteristics of the pathogenesis of type 2 diabetes observable with the HOMA metric (and contributory assays).

QUICKI

QUICKI is a simple inversion and log transform of the HOMA-IR value and as such has effectively performed the same as HOMA in validation studies (Chen *et al*. 2005; Hrebicek *et al*. 2002; Katz *et al*. 2000). There is thus no difference between them from the prospective of this research.

HbA1C

HbA1c (glycated haemoglobin) effectively allows a measurement of the subject's recent average plasma glucose concentration. The indicative timeframe of HbA1c values has not been conclusively confirmed. However most estimates fall between 2 weeks and 3 months. This range is based on the lifetime of a red blood cell of 13 weeks. However, with a 5 week half-life, the values are likely to be weighted towards a shorter-term period. Hence, HbA1c is not an insulin sensitivity test. Rather, it is an appraisal of the participant's glucose control capability, which is a function of the subject's insulin sensitivity and insulin production. HbA1c assays are being used with increasing frequency to measure and quantify individuals' glycaemic health, and have recently become an accepted test for diabetes diagnosis (ADA 2010).

Specifically, haemoglobin makes up approximately 97% of the red blood cell and facilitates the transport of oxygen around the body. However, glucose molecules in the plasma can become

attached to haemoglobin and remain attached for the duration of the cell's three-month life cycle. The rate of attachment is proportional to the blood glucose concentration and thus, the proportion of glycated haemoglobin to total haemoglobin can be used to predict or illustrate the average blood glucose concentration of the subject during the life cycle of the haemoglobin.

Nathan *et al*. (Nathan *et al*. 2008) presented a linear regression between 2700 glucose assays from 661 subjects and their associated HbA1c levels. The cohort was weighted towards individuals with type 1 and type 2 diabetes, but had a significant number of NGT participants. Equation 2.10 presents the regression line proposed by Nathan *et al*. that links HbA1c with average glucose concentration ($\bar{G}$).

$$\bar{G} = 1.59 HbA1c - 2.59 \hspace{3cm} 2.10$$

Equation 2.10 produces a particularly strong adherence to the measured data (R^2=0.89).

Barr *et al*. present a relatively low intra-participant CV of 2-4% and promotes the use of HbA1c assays for the diagnosis of diabetes (Barr *et al*. 2002). Recently, HbA1c has increasingly become accepted and used as a method of diabetes diagnosis. Until very recently the American Diabetes Association (ADA) has only recommended HbA1c as a tool to define optimal treatment of diabetes (ADA 2007). However, now the organisation accepts the use of HbA1c for diabetes diagnosis (ADA 2010). A threshold of 6.5% is used for diagnosis, which corresponds to recent average plasma glucose concentrations of approximately 7.8 mmol/L.

Testing for HbA1c has some unique benefits over the sensitivity tests presented. It does not require that the subject is fasted, and it only requires a single blood test. Thus, the clinical intensity of the test is very low. However, it must be re-iterated that it is not an insulin sensitivity test, it is a test for average glucose. Thus, it is not capable of measuring changes in sensitivity during clinical trials or after interventions. Furthermore, in reference to Figure 1.03, it is only capable of capturing the period around IGT when glucose is beginning to creep up. It cannot observe when declining insulin sensitivity is counter-regulated by an increase in insulin production. Thus, HbA1c can only provide a risk assessment for type 2 diabetes in the very late stages of pathogenesis when diabetes is imminent. Figure 2.05 shows this outcome graphically. Nonetheless, the test remains an important tool for the diagnosis, treatment and investigation of the disease state and etiology of type 2 diabetes.
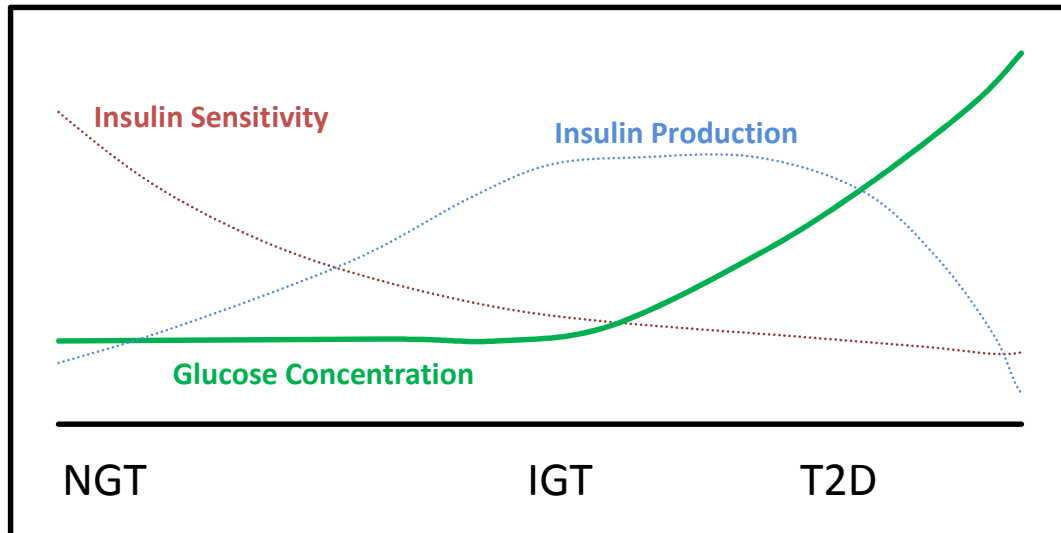
Figure 2.05. Characteristics of the pathogenesis of type 2 diabetes observable using the HbA1c assay. Note that HbA1c cannot discern insulin sensitivity or insulin production

McAuley index

The McAuley index is a surrogate insulin sensitivity value for that uses fasting assays of insulin and triglyceride (*TAG*) from a single blood test. Triglycerides store the energy of dietary fat intake for later use. Insulin promotes the generation of triglycerides that are thus elevated in insulin resistant individuals. Equation 2.11 shows the McAuley index that was derived using a multivariable regressive analysis of data from a series of clinical studies (McAuley *et al*. 2002).

$$ISI_{McAuley} = e^{2.63 - 0.28\ln(I_b) - 0.31\ln(TAG)} \qquad 2.11$$

The McAuley index correlates relatively well to the EIC considering the low intensity of the test (R=0.48-0.61) (McAuley *et al*. 2002; Oterdoom *et al*. 2005; Ruige *et al*. 2006).

Other studies have used functions of fasting assays, including fasting triglyceride levels, as surrogates for the EIC similar to this index. They have found correlations in the range of R=0.40-0.72 (Amato *et al*. 2010; Guerrero-Romero *et al*. 2010; Schwartz *et al*. 2008). Hence, none provides a significant advance on the established methods, although they do incorporate more relevant physiological markers associated with type 2 diabetes and its etiology.

2.4 Relative uses, accuracies and costs

The accuracy in terms of intra-participant repeatability and correlation to the gold standard EIC of a series of insulin sensitivity tests is summarised in Table 2.02.

Insulin sensitivity test	*SI* CV	EIC-*SI* corr'n	Clinical Intensity	Assays Required	References
EIC	6-10%	-	very high	12-36 Glucose 3-10 Insulin	(DeFronzo *et al.* 1979; Mari *et al.* 2001; Monzillo & Hamdy 2003)
IVGTT (incl FSIVGTT)	14-82%	0.44-0.85	high	12-36 Glucose 12-36 Insulin	(Donner *et al.* 1985; Ferrannini & Mari 1998; Ferrari *et al.* 1991; Foley *et al.* 1985; Galvin *et al.* 1992; Mari & Valerio 1997; Scheen *et al.* 1994)
IM-IVGTT TM-IVGTT	6-88%	0.70-0.89	high	12-36 Glucose 12-36 Insulin	(Bergman *et al.* 1987; Erichsen *et al.* 2004; Rostami-Hodjegan *et al.* 1998; Saad *et al.* 1997)
ITT	7-31%	0.81-0.9	extreme inhibitive	10-15 Glucose	(Bonora *et al.* 1989; Gelding *et al.* 1994; Grulet *et al.* 1993; Monzillo & Hamdy 2003)
2hr-OGTT for T2 Diagnosis	15-40%	0.43-0.74	low	1 Glucose	(Ferrannini *et al.* 2005; Levy *et al.* 1999; Stumvoll *et al.* 2000)
OGTT surrogate metrics	7-15%	0.51-0.79	moderately low	4-8 Glucose 4-8 Insulin	(Gutt *et al.* 2000; Lorenzo *et al.* 2010; Malita *et al.* 2010; Piché *et al.* 2007; Pigeon *et al.* 2009; Soonthornpun *et al.* 2003)
OGTT Minimal Model	12-15%	0.70-0.81	moderately low	4-8 Glucose 4-8 Insulin	(Breda *et al.* 2002; Dalla Man *et al.* 2005a)
MTT	15%	0.76	moderately low	4-8 Glucose 4-8 Insulin	(Hovorka *et al.* 1998; Steil *et al.* 2004)
HOMA	10-20%	0.22-0.93	very low	1 Glucose 1 Insulin	(Bonora *et al.* 2000; Katsuki *et al.* 2001; Katsuki *et al.* 2002; Lotz *et al.* 2008; Mari *et al.* 2001; Mather *et al.* 2001; Matthews *et al.* 1985; Pacini & Mari 2003; Piché *et al.* 2007; Wallace *et al.* 2004)
Fasting plasma glucose	~3%	-0.46	very low	1 Glucose	Validation study data
TAG metrics (incl McAuley index)	21%+	0.42-0.72	very low	1 TAG 1 Insulin (or similar)	(Amato *et al.* 2010; Antuna-Puente *et al.* 2009; Guerrero-Romero *et al.* 2010; McAuley *et al.* 2002; Oterdoom *et al.* 2005; Ruige *et al.* 2006; Schwartz *et al.* 2008; Widjaja *et al.* 1999)
HbA1c	2-4%*	-	extremely low	1 HbA1c	(Barr *et al.* 2002)

Table 2.02. Comparative statistics of the various tests (* the CV of the HbA1c assay is not indicative of the coefficient of variation of the mean glucose).

In summary, the hyperinsulinaemic EIC (EIC) is the gold standard of insulin sensitivity testing by merit of its consistent, irrefutably low intra-participant variation. However, the test evaluates the efficiency of a supra-physiological insulin concentration to clear glucose to the periphery (skeletal muscle). This information is an imprecise representation of the participant's daily metabolic physiology. In addition, the results are dose dependent and not readily comparable between protocols. Furthermore, the procedure is arduous, for the clinician and participant and a result is not necessarily guaranteed. Hence, the significant clinical burden and cost of this protocol has limited it's use to either small scale or very expensive clinical research programmes.

Dynamic tests that utilise intravenous stimulus (IVGTT, FS-IVGTT, IM-IVGTT and TM-IVGTT) have shown ambiguous outcomes in terms of both coefficients of variation and correlation to the EIC. However, it is likely that the poorer outcomes may have occurred as a result of poor clinical data collection practice or model application. Equally, they may represent flaws in models or identification methods or a lack of repeatability for other reasons. Furthermore, the stronger outcomes are generally from studies that utilise Bayesian statistical methods to maximise performance outcomes, but do not provide a transparent value of insulin sensitivity at an individual level.

The poor identifiability of the Minimal Model of glucose disposal has also obscured the outcomes of many studies that use dynamic IV test protocols. Attempts to mitigate this poor identifiability have caused protocols to incorporate increasing sample frequency. Thus, the clinical intensity of some FS-IVGTT dynamic test protocols has crept past that of the EIC. Hence, like the EIC, such dynamic IV tests are typically only used in clinical research settings.

The oral tests such as the OGTT, 2hr-OGTT and MTT generally produce less accurate insulin sensitivity values (if they produce one at all) compared to the EIC or dynamic IV protocols. However, the clinical intensity of the protocol is significantly reduced. The tests are used to quantify insulin sensitivity in some studies, particularly when insulin sensitivity is the secondary consideration of a study. The 2hr-OGTT is primarily used to diagnose diabetes status, both in research and for outpatients, but without an insulin sensitivity value. Hence, it is not used to provide an early diagnosis or risk.

Fasting tests have shown varied surrogate performance for the euglycaemic clamp and some ambiguity exists to the utility of such metrics. HOMA and fasting insulin are frequently reported in studies that include insulin sensitivity as a secondary consideration. Fasting glucose concentrations are occasionally used to characterise subgroups. Triglyceride based metrics are relatively new and have not yet been incorporated into many insulin sensitivity studies.

Although HbA1c provides virtually no indication of insulin sensitivity, it is likely that it will become the norm of type 2 diabetes diagnosis. It is a very low intensity test that needs only a single blood test from an un-fasted participant. The assay allows a relatively accurate inference of the participant's recent mean glucose concentration, which is a very good indicator of the test recipients recent glycaemic health. However, the symptoms of diabetes occur as a result of excessive glucose concentration, and generally only after a long period of increased insulin resistance. As such, quantification of mean daily glucose concentration is a very relevant diabetes diagnostic, but cannot provide an early diagnosis of risk.

Figure 2.06 schematically summarises the cost and accuracy of the various tests presented. Cost incorporates assay cost, clinical time and intensity, and participant time and discomfort. Accuracy measures the intra-participant repeatability and correlation to gold standard test. The weighting of the contributing attributes cannot be fairly established. Thus, the figure is dimensionless and only shows indicative costs and accuracies of the various tests.
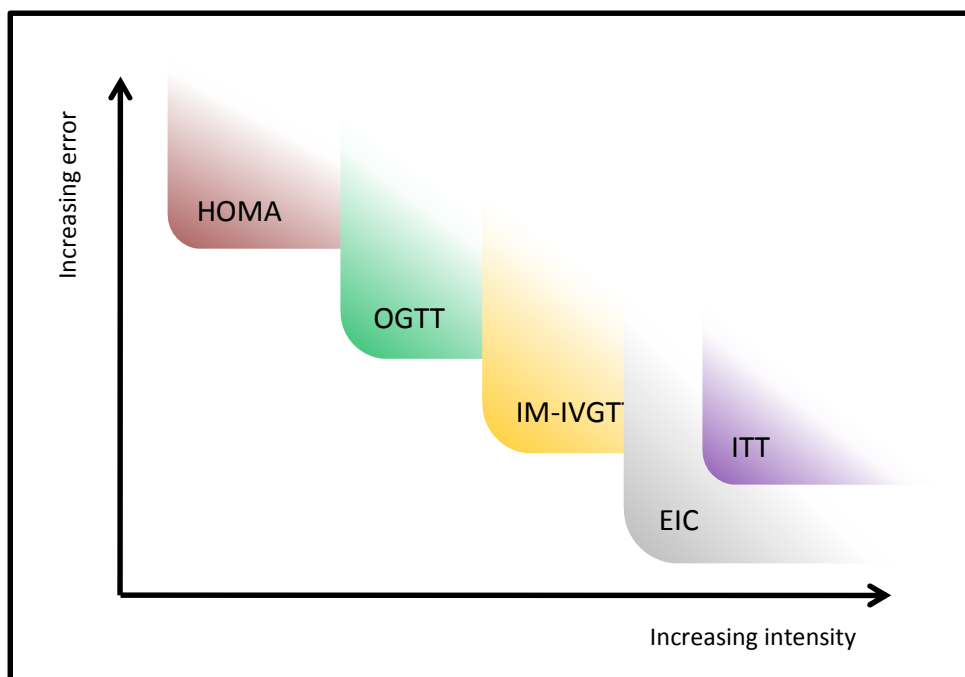
Figure 2.06. The relative costs and accuracies of the established insulin sensitivity tests.

The most notable feature of Figure 2.06 is the region toward the origin that is un-occupied by established tests. This region presents an opportunity for the development of new approaches for the quantification of insulin sensitivity that incorporate low cost and high accuracy attributes.

PART TWO: DISST model and parameter identification methods

Chapter 3. Parameter identification

This chapter describes some of the model parameter identification methods used in the analyses contained in subsequent chapters. The integral method (Hann *et al*. 2005b) was used in the pilot and validation investigations of the DISST test (Lotz *et al*. 2010). The iterative integral method is a development of the integral method that achieves greater accuracy. The nonlinear least-squares method is included as it is typically used to identify insulin sensitivity parameters from dynamic tests and thus serves as a good comparator.

3.1 Introduction and motivation

Mathematical models have been developed for numerous phenomenons, and in the case of insulin sensitivity tests, many models have been postulated (Carson & Cobelli 2001; Caumo & Cobelli 1993; Chase *et al*. 2010; Sherwin *et al*. 1974). Most such models are systems of first order linear or nonlinear ordinary differential equations that describe the kinetics of insulin, or the dynamics of insulin dependent glucose decay. A good model should be able to quantify the variation in the kinetics and dynamics between test subjects with distinct physiological responses by varying the values of key specific model parameters. Typically, the parameter values that produce the closest adherence between the model simulations and observed reactions are used to quantify and distinguish the test participant's responses.

There are a number of methods to identify the most accurate parameter values relative to clinical data observations. Furthermore, there are a number of ways to define precisely what 'accurate parameter values' means in the context of parameter identification. Typically, parameter values that define the best fit between the measured data and the model-based simulation are considered the most accurate. However, emerging Bayesian techniques can be used to shift identified values toward known population trends and away from the most accurate fit to data (Cobelli *et al*. 1999; Denti *et al*. 2009; Erichsen *et al*. 2004). Thus, accuracy is considered in context of population trends by Bayesian methods. Furthermore, some observations can be considered more representative than others, within any method, and thus the error mitigation is biased to what may be considered more relevant data points by the identification method applicator.

3.2 Integral method

The integral method was introduced by Hann *et al.* (Hann *et al.* 2005a) and functions by obtaining the coefficients of the discretised integral formulation of the governing model equations. The integral method was also used to define the parameter values of the pilot and validation investigations of the DISST test (Lotz *et al.* 2010).

3.2.1 Generalised integral method process

The integral method processes is demonstrated with a generalised system. Consider a coupled first order differential equation system defined by Equations 3.01 and 3.02, and sampled in *X*.

$$\dot{X} = f(X, Y, U, a, b, c_i) \tag{3.01}$$
$$\dot{Y} = f(X, Y, U, c_i, d_i) \tag{3.02}$$

where: *X* and *Y* are time variant mutually dependent species concentrations. *U* is a known time variant, *a* and *b* are variable parameters and *c* and *d* are known *a-priori* parameters. Assume that data is obtained in terms of *X*.

The integral method uses the following steps to identify model parameters.

1. The model is separated into linear coefficients of the parameters

$$\dot{X} = af_1(X, Y, U, c_i) + bf_2(X, Y, U, c_i) + f_3(X, Y, U, c_i) \tag{3.01a}$$

$$\int \dot{X} = a \int f_1(X, Y, U, c_i) + b \int f_2(X, Y, U, c_i) + \int f_3(X, Y, U, c_i) \tag{3.01b}$$

2. The functions are evaluated using the best possible representation of the required parameters and time variant profiles. In some cases, the best possible representation will be obtained by interpolation between measured points, and in other cases, physiological behaviour is simulated to approximate the system behaviour with reference to measured data. Function 3.01b is integrated from *i* to *j*:

$$X_j - X_i = a \int_i^j f_1(X, Y, U, c_i) + b \int_i^j f_2(X, Y, U, c_i) + \int_i^j f_3(X, Y, U, c_i) \tag{3.03}$$

where: i and j represent different discrete times. The time between $t=i$ and $t=j$ represents a period over which the integral method is applied to identify the parameters. For example, i could equal 0, while j = 0, 2, 4, ..., n-2, n indicating a series of periods that begin at $t=0$ and end on 2 minute intervals. In contrast, i could equal 0, 2, 4, …, n-4, n-2. While j=2, 4, 6, …, n-2, n representing a series of two minute intervals, the first being $t=0$ to 2 minutes then $t=2$ to 4 minutes. These intervals are defined differently depending on the data resolution, model and potential parameter variations over time (Hann *et al*. 2006; Hann *et al*. 2005b).

3. The selected periods are evaluated using the estimated parameters and profiles and then entered into a matrix equation defined:

$$
\begin{bmatrix}
\int_{i1}^{j1} f_1(X,Y,U,c) & \int_{i1}^{j1} f_2(X,Y,U,c) \\
\int_{i2}^{j2} f_1(X,Y,U,c) & \int_{i2}^{j2} f_2(X,Y,U,c) \\
\vdots & \vdots \\
\int_{in}^{jn} f_1(X,Y,U,c) & \int_{in}^{jn} f_2(X,Y,U,c)
\end{bmatrix}
\begin{bmatrix} a \\ b \end{bmatrix}
=
\begin{bmatrix}
X_{j1} - X_{i1} - \int_{i1}^{j1} f_3(X,Y,U,c) \\
X_{j2} - X_{i2} - \int_{i2}^{j2} f_3(X,Y,U,c) \\
\vdots \\
X_{jn} - X_{in} - \int_{in}^{jn} f_3(X,Y,U,c)
\end{bmatrix}
\quad 3.03a
$$

Assuming sufficient periods are evaluated, the parameters a and b are over-defined and can be uniquely identified using linear least squares approaches. Constraints can be placed on the values of a and b to meet physiological assumptions (e.g. non-negative clearance rates).

3.2.2 Summary of the integral method

The advantages of the integral method include:

- Very fast identification time as it does not require numerous simulations to complete identification, and numerical integration of data is computationally efficient.
- Convex, and thus very stable in computation.
- Does not require accurate initial value estimation of parameters prior to identification, in contrast to non-linear least squares approaches.
- Results are dependent on period selection, thus careful positioning can limit the effect of incorrect assays, or increase the influence of particular areas of the test

Disadvantages of the method include

- Results are dependent on estimation of contributing species concentration. Parameter identification accuracy is linked to the overall accuracy of the initial simulations.
- The integral method is not iterative, and thus convergence of parameters and minimisation of residuals does not occur.
- The model must be linearisable in terms of the variable parameter coefficients. The governing equation must be separated into linear functions of the parameters. All parameters must be included in the governing equation of the assayed concentration (although the model may be re-arranged to achieve this in some cases). This point is not a disadvantage for the models in this thesis, but does limit its use in some applications.

3.3 Non-linear least squares

Non-linear least squares is a parameter identification method frequently used with physiological models. It is the dominant method used in a wide range of forms for physiological and metabolic system modelling. The principle of the method is essentially similar to that of Newton's derivative root finding method applied to error. Parameter estimation values shift between iterations and are driven by derivative of a multidimensional error surface with respect to infinitesimal changes in the model parameters.

3.3.1 Generalised non-linear least square method process

Although there are numerous ways to apply non-linear least squares approaches, the general underlying method is presented using the generalised model of Equation 3.01 and 3.02.

$$\dot{X} = f(X, Y, U, a, b, c_i) \qquad\qquad 3.01$$

$$\dot{Y} = f(X, Y, U, c_i, d_i) \qquad\qquad 3.02$$

1. The first step requires the initial value estimation of a and b (a_k and b_k). X and Y are then simulated (X_{Simk} and Y_{Simk}) which adds significant computational cost for complex models. The error between the assayed species measurements (X_{meas}) and the simulated value

(X_{Simk}) at the measurement times (t_S) are squared then summed to produce the residual value (R).

$$R = \sum_{1}^{n} \left(X_{Simk}(t_S) - X_{Meas}(t_S) \right)^2$$

3.04

2. A Jacobian is then generated in which there are differential gradient values measuring the effect of parameter changes on residual error is generated.

$$J = \begin{bmatrix} \dfrac{dR_{t_S}}{da_1} \\ \dfrac{dR_{t_S}}{db_1} \end{bmatrix}$$

3.05

3. The values of a and b are then updated using, a function of the current parameter values and the Jacobian in Equation 3.05. A local, or perhaps global, residual minima will occur when the Jacobian equals zero. As such, the parameter values are driven in the negative direction of the residual slope. The typical Gauss-Newton method uses Equation 3.05 to derive a_{k+1} and b_{k+1}.

$$\{a_{k+1}, b_{k+1}\} = \delta + \{a_k, b_k\}$$

3.06

$$\text{where} \quad \delta = \frac{J^T(X_{Simk}(t_S) - X_{Meas}(t_S))}{J^T J}$$

3.06a

A great number of alternatives exist to stabilise or accelerate the overall convergence of the method. Most notable is the Levenberg-Marquardt algorithm which introduces a damping factor (λ) to Equation 3.06a (Equation 3.06b) (Levenberg 1944; Marquardt 1963). The damping factor limits the rate of model parameter change between iterations, thus preventing potential overshoot and instability. Therefore, although the Levenberg-Marquardt method is more stable than the basic method, it requires more iterations for convergence.

$$\delta = \frac{J^T W(X_{Simk}(t_S) - X_{Meas}(t_S))}{J^T W J + \lambda I}$$

3.06b

where: I is the identity matrix; W is a weighting factor that allows parameter levels of influence for different data points (not exclusive to the Levenberg Marquardt algorithm); and λ is the damping factor.

4. Steps 1 to 3 are iterated until the Jacobian value is below a certain threshold and either convergence is declared, or the maximum number of iterations is reached and failure to converge is declared.

3.3.2 Evaluation of non-linear least squares

Non-linear least squares is the preferred method for parameter identification in the field of insulin sensitivity testing and in a number of other model-based applications. Positive aspects of the method include:

- Capable of functioning with almost all model configurations
- When convergence occurs, the method finds a local or global residual minima
- Experienced users can tune the method to optimise stability and speed trade-off

The method also has some intrinsic faults that can produce very inaccurate results. In particular:

- Initial parameter estimates are required, and solutions are starting point dependent.
- There is no way of confirming whether the result is a global or local minima.
- Stability is not assured and is dependent on the initial parameter estimation and convergence rate.
- Each iterative step requires numerous full simulations of each relevant species. This requirement can require extreme and, at times, inhibitive computation power or time. In particular, if there is no analytical solution to the model computationally expensive Runga-Kutta algorithms (or similar) are required numerous times for each step.

The primary strength of the non-linear least squares method is in the unlimited number of models to which it can be applied. This key aspect is the reason the method is widely used by investigators. However, the method is very slow, particularly for models that do not have analytical solutions. The method can also converge to values that are representative of local and not global minima. Overall, the utility of the method is sufficient to convince most researchers to overlook the problems with the method, and its regular use has made it the effective gold-standard method of parameter identification.

3.4 Iterative integral method

The iterative integral method is a significant development and extension of the integral method. The method does not require an accurate initial estimation of the relevant species time variant profiles as the single integral method does, which can afford significant advantages in terms of test sampling intervals. The iterative method uses updating time-variant profiles to converge to accurate model parameter values and thus, sampling does not have to capture all specific dynamics comprehensively.

3.4.1 Generalised iterative integral method process

The iterative integral method uses a similar process to the single iterative method. However, there are some notable and important changes. The model of Equations 3.01 and 3.02 is used for this example also.

$$\dot{X} = f(X, Y, U, a, b, c_i) \qquad\qquad 3.01$$

$$\dot{Y} = f(X, Y, U, c_i, d_i) \qquad\qquad 3.02$$

The method uses the following steps to identify model parameters a and b.

1. The single and iterative integral methods both linearise the equation of the assayed species into the coefficients of the parameters. The governing equation is integrated:

$$\dot{X} = af_1(X, Y, U, c) + bf_2(X, Y, U, c) + f_3(X, Y, U, c) \qquad 3.01a$$

$$\int \dot{X} = a \int f_1(X, Y, U, c) + b \int f_2(X, Y, U, c) + \int f_3(X, Y, U, c) \qquad 3.01b$$

2. In contrast to the single integral method, the periods over which identification is evaluated are defined by the sample timing. Periods are defined between the first sample of the test and the times of each subsequent sample time. Thus, if there are N available data points, there will be n-1 available identification periods. In this case $j = t(s_2), t(s_3), \ldots t(s_{end})$.

$$X_j - X_0 = a \int_0^j f_1(X,Y,U,c) + b \int_0^j f_2(X,Y,U,c) + \int_0^j f_3(X,Y,U,c) \quad 3.07$$

3. These periods are evaluated using the estimated profiles. In contrast to the single iterative method, the initial profile estimation can be quite incorrect without parameter dependence, subsequent profiles estimations use more accurate profiles estimations. The outcomes of the period evaluations are entered into the matrix equation:

$$\begin{bmatrix} \int_0^{ts_1} f_1(X,Y,U,c) & \int_0^{ts_1} f_2(X,Y,U,c) \\ \int_0^{ts_2} f_1(X,Y,U,c) & \int_0^{ts_2} f_2(X,Y,U,c) \\ \vdots & \vdots \\ \int_0^{ts_n} f_1(X,Y,U,c) & \int_0^{ts_n} f_2(X,Y,U,c) \end{bmatrix} \begin{bmatrix} a \\ b \end{bmatrix} = \begin{bmatrix} X_{ts_1} - X_0 - \int_0^{ts_1} f_3(X,Y,U,c) \\ X_{ts_2} - X_0 - \int_0^{ts_2} f_3(X,Y,U,c) \\ \vdots \\ X_{ts_n} - X_0 - \int_0^{ts_n} f_3(X,Y,U,c) \end{bmatrix} \quad 3.07a$$

Parameters are identified using equation 3.07a and linear least squares methods with all the relevant advantages.

4. In contrast to the single iterative method, the identified parameters are used to re-simulate the relevant profiles. Two possible methods are possible, the first is stable, while the second much faster.

Method 1. The relevant profiles are identified using the analytical solution, a Picard iteration or stepwise methods such as Runga-Kutta or similar. Analytical solutions to model equations do not always exist. Thus, although they are the quickest method, they are only possible for simple linear models. Stepwise methods are capable of simulating most models. However, there is a trade-off between accuracy and computational time. These methods tend to require a lot of computational time to achieve simulations with the required level of accuracy. Picard iterations are capable of simulating nonlinear systems particularly quickly and have been under-utilised in physiological model simulations (Kim & Kim 2007; Youssef & El-Arabawy 2007). To simulate Equations 3.01 and 3.02 using Picard iterations the model equations are discretised and rearranged:

$$X_t = X_0 + a \int_0^t f_1(X,Y,U,c) + b \int_0^t f_2(X,Y,U,c) + \int_0^t f_3(X,Y,U,c) \quad 3.01c$$

$$Y_t = Y_0 + \int_0^t f(X, Y, U, c_i, d_i) \qquad\qquad \text{3.02c}$$

where: t=0, 1, 2,…,end.

Equations 3.01c and 3.02c must be evaluated a number of times in an iterative process to enable convergence. However, due to the nature of computational process architecture, this process is quite fast. Numerous methods can also speed up the process, such as the bounding of the simulation, or the damping of convergence (contrary to assumed behaviour damping actually increases the convergence rate).

Method 2. The profiles used in the evaluation of Equation 3.07 can be used to directly solve for X_t.

$$X_t = X_0 + a\int_0^t f_1(X, Y, U, c) + b\int_0^t f_2(X, Y, U, c) + \int_0^t f_3(X, Y, U, c) \quad \text{3.01d}$$

where: t=0, 1, 2,… end

The remote species can then be simulated using the analytical solution or Picard iterations.

Thus, no iterative process is required and a simulation can be achieved with very little computational cost. However, given sufficiently inaccurate initial estimates of the relevant profiles this method can become unstable, which negates a significant advantage of this overall approach. Thus, this method must be used with particular care.

5. The updated profiles are used to re-evaluate Equation 3.07 in Step 2. Steps 2-4 are iterated until convergence of the parameters is declared.

3.4.2 Acceleration of the iterative integral method

The iterative integral method can converge slowly depending on the level of coefficient similarity between the model parameters. However, the rate of convergence can be amplified using positive feedback control of the derivative term to increase the convergence rate. The conventional engineering strategy for dynamic control includes proportional and integral terms, as well as

derivative (damping) feedback. However, concerning model parameter identification, the set point is the residual minima final solution, which is presumably unknown during identification. Thus, only derivative control, which measures the rate of change between iterations, can be used effectively.

In particular, a gain is placed on the rate of change of the parameters between iterative steps. Equation 3.07a is thus altered:

$$\begin{bmatrix} a_k \\ b_k \end{bmatrix} = \theta N^{-1}M - (\theta - 1)\begin{bmatrix} a_{k-1} \\ b_{k-1} \end{bmatrix} \qquad\qquad 3.08$$

where: θ is the gain used to increase the rate of change between iterations and M/N is evaluated via the Moore-Penrose pseudo division for non-square matrix systems and M and N are defined:

$$M = \begin{bmatrix} X_{ts_1} - X_0 - \int_0^{ts_1} f_3(X,Y,U,c) \\ X_{ts_2} - X_0 - \int_0^{ts_2} f_3(X,Y,U,c) \\ \vdots \\ X_{ts_n} - X_0 - \int_0^{ts_n} f_3(X,Y,U,c) \end{bmatrix}; \text{ and } N = \begin{bmatrix} \int_0^{ts_1} f_1(X,Y,U,c) & \int_0^{ts_1} f_2(X,Y,U,c) \\ \int_0^{ts_2} f_1(X,Y,U,c) & \int_0^{ts_2} f_2(X,Y,U,c) \\ \vdots & \vdots \\ \int_0^{ts_n} f_1(X,Y,U,c) & \int_0^{ts_n} f_2(X,Y,U,c) \end{bmatrix}$$

3.08a

Increasing the value of θ increases the rate of convergence. However, it also increases the instability of the method. Thus, care must be taken when employing this method in practice. When applied correctly the accelerating method can speed up the rate of convergence by up to 400%. In small studies using clinical data, the added complexity may not be justified. However, in large *in-silico* analyses, when thousands of identification processes are required, an 80% reduction in computational time is particularly valuable to the investigator.

3.4.3 Summary of the attributes of the iterative integral method

The iterative integral method overcomes many of the issues presented by the single iterative method. The particular advantages of the method include:

- The method is convex and converges to a global minima
- The simple method is universally stable. Instability only occurs when acceleration of the process is attempted without appropriate care.

- The method does not necessarily require simulations between iterations. Thus, it has greatly reduced computational intensity for non-linear models, particularly in comparison to non-linear least squares and similar gradient decent based methods.

- Results are not dependent on initial estimates.

Disadvantages of the method include:

- Only models that have linearisable parameters in the equation of the assayed species can be identified, although the model may be re-arranged to achieve this in some cases.

- More complex than the single iterative method and thus more difficult to apply, but not as complex as most non-linear least-squares process.

- In contrast to non-linear least squares, no proprietary 'toolboxes' or other 'freeware' are available for the iterative integral method.

Chapter 4. DISST model, protocol and pilot investigation

Chapter 4 presents the dynamic insulin sensitivity and secretion test (DISST) that is the foundation of this research. The DISST was developed and piloted during the doctoral investigation of Dr Thomas Lotz (Lotz 2007). Much of the contents of this chapter are exhaustively described in publications by Lotz *et al*. (Lotz *et al*. 2008; Lotz *et al*. 2010).

4.1 Introduction

The DISST test was developed as a low cost, accurate alternative to the EIC during the doctoral investigation of Dr Thomas Lotz (Lotz 2007). To provide any benefit and become an accepted insulin sensitivity test, the proposed test must occupy a region of Figure 2.06 that is toward the origin from the existing tests. Thus, the DISST test was developed to improve upon the IM-IVGTT type tests by maximising the identifiability of the model and thus allowing a reduction in the protocol intensity while also improving accuracy. Improvements to identifiability enable reduced sampling frequency and time, and reduce the overall cost of the test, better enabling low-cost, wide-scale applications like insulin-resistance screening.

A significant contributor to the burden of insulin sensitivity tests is the clinician and participant hours. The established high accuracy tests all involve long, arduous protocols. Thus, reducing the protocol time has a profound effect on the overall burden of a test, more so than reducing the cost of assays. The DISST therefore incorporates C-peptide assays that provide information into the participant's endogenous insulin production. Although these assays are relatively expensive at an individual level, their contribution to the observation of the test participant's condition allows a significant reduction in sample frequency, effectively lowering the overall cost of the test. Fewer samples are required to simulate the participant's insulin concentration as their time-variant insulin production rate can be quantified.

Furthermore, Figure 1.03 shows the importance of insulin production as well as insulin sensitivity for the risk assessment of type 2 diabetes. Thus, the DISST is unique amongst the established tests as it provides both insulin sensitivity and insulin production metrics in a single, relatively short-duration and low-intensity test.

The DISST clinical protocol is effectively a low-dose, infrequently sampled insulin modified intravenous glucose tolerance test (IM-IVGTT). IM-IVGTT tests often involve participant-specific doses of glucose and insulin that are approximately 3 times the size of that administered in the DISST. Thus, saturation effects are triggered during most IM-IVGTT tests, but are avoided by the DISST. These effects vary across test participants and are difficult to identify (Natali *et al*. 2000; Prigeon *et al*. 1996). The DISST can be administered with as little as five samples in a 30-minute protocol. In comparison, the IM-IVGTT often requires 20 to 40 samples during a 2 to 4 hour protocol with intense sampling rates of 1 to 3 minutes close to the boluses. Hence, the DISST was designed to maximise accuracy and resolution, to minimise the impact of variation, and to minimise clinical effort.

The modelling strategy of the DISST provides the greatest distinction to the IM-IVGTT. In contrast to IM-IVGTT tests, the DISST samples C-peptide, which can provide a direct estimate of the participants insulin production response to the test stimulus (Van Cauter *et al*. 1992). This information is used to supplement a concentration-based simulation of the insulin pharmaco-kinetics. Thus, relatively sparse sampling is possible.

In contrast, the IM-IVGTT forgoes C-peptide data and in the absence of an insulin production profile defines plasma insulin as a linear interpolation of frequently sampled data (Bergman *et al*. 1979). Thus, accurate IM-IVGTT plasma insulin profiles are dependent of the resolution of insulin assays, and thus the protocols designed for Minimal Model analysis have become particularly intense. The intense clinical protocol is not necessarily intrinsic to the nature of IVGTT test; rather it is a requirement of the frequently adopted modelling strategy.

The lower intensity of the DISST clinical procedure comes at the expense of some increased modelling and mathematical complexity. However, this represents a very positive development in the field of insulin sensitivity testing. Once the model parameter identification processes have been established, numerous simple tests can be undertaken that can enable high accuracy values for a lower overall cost-per-test. In contrast, arduous tests that only need simple identification methods often limit the quantity of tests that are possible within a time or economic budget.

4.2 DISST protocol

The DISST protocol uses a series of blood samples to trace the participant's response to glucose and insulin bolus stimuli. Participants attend the test in the morning having fasted overnight

(water allowed). The participants' height, weight, sex and age are recorded prior to each test. Participants sit in a supine position for the duration of the test.

In the pilot investigation of the DISST, blood samples were scheduled at t=0, 5, 10, 15, 20, 25, 30, 35, 40 and 50 minutes. However, some DISST protocols can use a much sparser sample frequency or shortened test duration. Such protocols will be discussed in later chapters. Due to cannulation issues and other clinical complications some samples are not necessarily taken at the desired times. The actual sample times were recorded during testing and can be readily accounted for in the model identification methods. Glucose and insulin boluses were administered immediately after the t=5 and t=15 minute samples, respectively. During the pilot investigation of the DISST test, either high, medium or low doses were used. The boluses used for the high dose contained 20 grams of dextrose in a 50% dilution and 2 units of insulin. The medium dose used 10g of dextrose and 1U of insulin, while the low dose used 5g of dextrose and 0.5U of insulin. These alternative doses were used to investigate the DISST model's dose dependency (presented in Section 4.4.3).

The DISST cost savings are achieved by the sparse nature of the DISST data from the low intensity clinical protocol. However, a validated, high-resolution physiological model of the participant's pharmaco-dynamics (PD) and pharmacokinetics (PK) must be used to enable identification of insulin sensitivity and insulin production.

4.3 DISST model

The DISST model incorporates successful elements of existing models with improvements of other models to produce a comprehensive model that can accurately capture a participant's glucose and insulin concentration as well as insulin production profiles, in response to the test stimuli.

The DISST model can be separated into the representations of the three assayed species: glucose, insulin and C-peptide. The DISST model uses C-peptide assays to identify the endogenous insulin production response to the test stimulus. This profile is used to supplement insulin assays and a physiological simulation of plasma and interstitial insulin. Insulin sensitivity is defined as the ratio of proportional glucose clearance and interstitial insulin concentration.

4.3.1 Endogenous insulin production

The DISST uses the endogenous insulin production estimation model defined by Eaton *et al*. (Eaton *et al*. 1980) and the deconvolution approach validated by Van Cauter *et al*. (Van Cauter *et al*. 1992). C-peptide kinetics are modelled using a two compartment model that incorporates an accessible plasma compartment and a passive, un-measurable interstitial compartment. Blood samples are representative of the plasma compartment. C-peptide is only cleared in significant amounts by the kidney and clearance does not occur in the interstitium. Hence, C-peptide assays can provide a much better indication of insulin secretion than insulin-based analysis.

Equations 4.01 and 4.02 represent the plasma and interstitial C-peptide concentrations, respectively:

$$\dot{C} = k_2 Y - (k_1 + k_3)C + \frac{U_N}{V_P}$$ (4.01)

$$\dot{Y} = k_1 C - k_2 Y$$ (4.02)

where: C and Y are the plasma and interstitial C-peptide concentrations respectively [pmol/L]; k_1, k_2 and k_3 are rate constants [1/min]; U_N is the rate of insulin production (which is assumed to be equimolar with insulin) [mU/min]; V_P is the volume of plasma distribution [L] and φ is a conversion factor between pmol and mU [6.94 pmol/mU].

The rate constants are a function of the participants anatomical characteristics (weight, height, age, sex) as defined by Van Cauter *et al*. (Van Cauter *et al*. 1992). Equations 4.03 to 4.11 describe the method by which these parameters are obtained. Initially, the participant's body mass index (BMI) is identified.

$$BMI = \frac{BW(kg)}{height(m)^2}$$ (4.03)

Passive clearance in a two-compartment model is best defined with a double exponential representation. A regressive analysis by VanCauter *et al*. (Van Cauter *et al*. 1992) observed distinctly different rates between healthy and obese individuals for the short half-life decay, while the long half-life was most dependant on age.

if BMI>30 $halflife_1 = 4.55$ (4.04)

$$F = 0.78$$

if BMI<30 $$halflife_1 = 4.95 \qquad 4.05$$

$$F = 0.76$$

$$halflife_2 = 0.14 * age + 29.2 \qquad 4.06$$

These clearance and transport half-lives must be converted to enable applicability to the first-order linear physiological model of Equations 4.01 and 4.02.

$$a = \frac{ln_e(2)}{halflife_1} \qquad 4.07$$

$$b = \frac{ln_e(2)}{halflife_2} \qquad 4.08$$

$$k_2 = F * (b - a) + a \qquad 4.09$$

$$k_3 = \frac{ab}{2k_2} \qquad 4.10$$

$$k_1 = a + b - k_2 - k_3 \qquad 4.11$$

The volume of distribution of plasma and interstitium insulin and C-peptide is defined using a function of body surface area (BSA).

$$BSA = \frac{1}{6} \sqrt[2]{BW(kg) * height(m)} \qquad 4.12$$

if male $$V_P = 1.92 * BSA + 0.64 \qquad 4.13$$

if female $$V_P = 1.11 * BSA + 2.04 \qquad 4.14$$

4.3.2 Insulin pharmaco-kinetics

The insulin pharmaco-kinetics are described by a two compartment model that incorporates plasma and interstitial compartments. Insulin assays are drawn from the plasma compartment, which incorporates insulin clearance to the kidney and liver, and exogenous and endogenous insulin input. The interstitial compartment incorporates clearance of insulin to cells, and is representative of the amount of insulin available for the clearance of glucose to cells. It is thus the most critical to the identification of insulin sensitivity.

The model equations are defined:

$$\dot{I} = -n_K I - n_L \frac{I}{1 + \alpha_I I} + \frac{n_I}{V_P}(Q - I) + \frac{U_X}{V_P} + (1 - x_L)\frac{U_N}{V_P} \qquad 4.15$$

$$\dot{Q} = \frac{n_I}{V_Q}(I - Q) - n_C Q \qquad 4.16$$

where I and Q represent the insulin concentrations in the plasma and interstitium respectively [mU/L]; n_K is the kidney clearance rate [1/min]; n_L is the hepatic clearance [1/min]; α_I is the saturation of hepatic insulin clearance [L/mU]; n_I is the transition rate between the plasma and interstitial compartments [L/min]; U_X is the exogenous insulin bolus [mU/min]; x_L is the proportional first pass hepatic extraction of endogenously produced insulin [1]; V_Q is the distribution volume of insulin in the interstitium [L] and n_C is the rate of insulin clearance to cells in the interstitium [1/min].

The DISST identification method typically identifies n_L and x_L as variable parameters, while the other parameters are defined *a-priori* based on anatomical functions. The values of V_Q, n_K and n_I are defined:

$$V_Q = \frac{k_1}{k_2}V_P \qquad 4.17$$

$$n_K = k_3 \qquad 4.18$$

$$n_I = k_2 V_Q \qquad 4.19$$

where n_C is defined as a function of n_I to ensure a steady-state ($\dot{I} = \dot{Q} = 0$) concentration gradient (γ) in accordance with published data (Barrett *et al*. 2009).

$$\gamma = 0.5 \tag{4.20}$$

$$n_C = \frac{n_I}{V_q}\left(\frac{1}{\gamma} - 1\right) \tag{4.21}$$

The saturation of insulin clearance α_I is defined as a population constant 0.0017 L/mU that implies half maximal insulin clearance occurs at I=588 mU/L (Thorsteinsson 1990; Thorsteinsson *et al*. 1986).

4.3.3 Glucose-insulin pharmaco-dynamics

The glucose model uses a representation of total glucose in a single compartment model. The model defines the rate of glucose decay with both insulin dependent and independent clearance. The model is defined:

$$\dot{G} = -p_G(G - G_B) - SI(GQ - G_BQ_B) + \frac{P_X}{V_G} \tag{4.22}$$

where: G is the glucose concentration [mmol/L]; p_G is the glucose dependent glucose clearance [1/min]; SI is the insulin sensitivity [L/mU/min]; P_X is the glucose bolus [mmol/min]; V_G is the glucose distribution volume [L] and the 'B' subscript denotes the basal concentrations of the respective species.

The typical use of the DISST model sets p_G as a constant (0.004 1/min) and identifies SI and V_G as variable parameters (Lotz *et al*. 2010).

The development of the DISST model and a full justification was presented in the doctoral thesis of Dr Thomas Lotz (Lotz 2007) and abbreviated in (Lotz *et al*. 2010). It will not be repeated here for brevity. However an electronic copy is available (Lotz 2007). A comprehensive comparison between the DISST model the established Minimal-Model that is typically used in insulin sensitivity tests is presented in Chapter 7.

The entirety of the DISST model is summarised graphically in Figure 4.01.
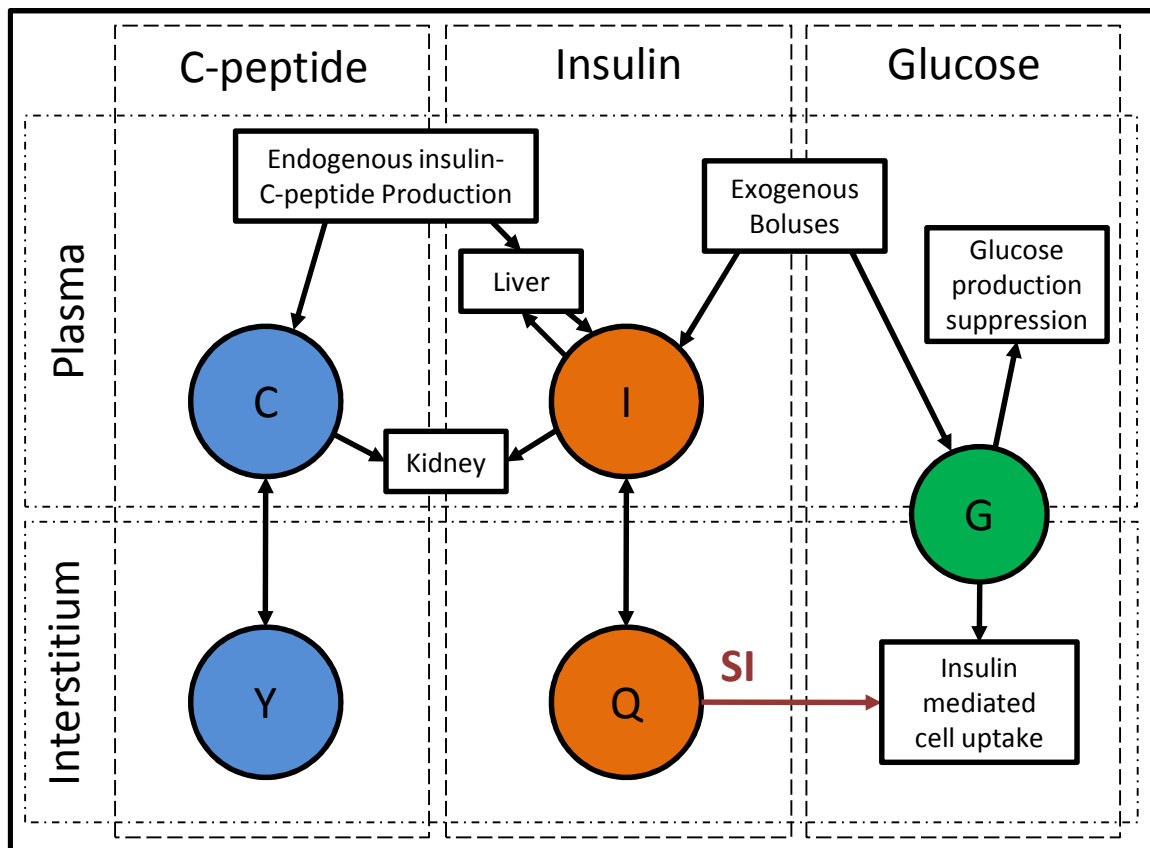
Figure 4.01. Physiological model for the PK and PDs of C-peptide, insulin and glucose. Note that plasma and interstitial glucose is modelled as a single compartment due the fast glucose transition between these compartments.

4.4 Identification of the pilot cohort model parameters

The pilot investigation was exhaustively described in Lotz *et al.* (Lotz *et al.* 2010), but will be repeated here in lesser detail as it is important prior background for the developments of the DISST tests presented in this thesis. Furthermore, many investigations presented later in this thesis use the pilot data and refer to the pilot outcomes.

4.4.1 Study design and participants

The pilot investigation of the DISST test sought to measure the repeatability of the DISST tests within participants at varied and consistent bolus doses. The study was conducted in two parts. The first part measured the inter-dose, within subject repeatability of the DISST. Part 2 measured the intra-dose, within subject repeatability.

Participants were sought to encompass the anatomical characteristics of a general western adult population. Although predefined guidelines were not set, care was taken to ensure that a range of BMI, ages and diabetic statuses were tested. Table 4.01 defines the characteristics of the study cohort.

Participant	Sex	Age [yrs]	Weight [kg]	BMI [kg/m²]	Fasting glucose [mmol/L]	Fasting insulin [mU/L]	NGT *IFG* **T2D***
1	f	57	89	33.9	5.8*	30.8	*IFG*
2	f	59	67	25.5	5.9*	1.4	*IFG*
3	f	59	87	39.2	4.7	12.5	NGT
4	f	21	78	25.2	5	5.2	NGT
5	m	41	76	21.7	4	0.5	NGT
6	f	45	76	25.4	4.1	1.7	NGT
7	m	55	73	24.1	4.5	4.4	NGT
8	f	51	67	27.2	4.3	1.4	NGT
9	f	35	66	24	4.8	6.6	NGT
10	f	30	50	19.5	4.2	3.2	NGT
11	f	55	85	30.1	6.8	9.2	**T2D**
12	m	60	76	23.7	4.4	3.2	NGT
13	f	48	91	33.4	5.2	9.5	NGT
14	f	41	111	41.3	4.5	3.9	NGT
15	m	29	84	25.9	5.1	2.5	NGT
16	m	49	105	35.1	6.3*	16.6	*IFG*
17	f	25	60	25.3	4.5	3	NGT
18	m	22	65	21.5	4.1	1.9	NGT
Q_1	m/f 6/12	30	67	24	4.3	1.9	14 NGT
Q_2		46.5	76	25.5	4.6	3.6	3 IFG
Q_3		55	87	33.4	5.2	9.2	1 T2D

Table 4.01. Anatomical description of the pilot investigation study cohort. (* IFG was diagnosed using a cut-off value of 5.56 mmol/L)

There were three dosing regimens used during the two-part study. The low dose included a 5g dose of glucose, with a 0.5U dose of insulin. The medium dose was 10g glucose with 1U insulin, and the high dose used 20 grams of glucose and 2U insulin. Participants of Part 1 had at least one test at the medium dose with at least one test at either low or high dosing. Participants of Part 2 received two or more tests at either low dose or medium dose. Table 4.02 lists those participants

who received which specific doses and thus, and for which parts of the study they provided results.

Participant	Tests			Part	
	5g 0.5U	10g 1U	20g 2U	1	2
1		1	1	X	
2		1	1	X	
3		3			X
4	1	1		X	
5‡		5	1	X	X
6		2	1	X	
7	1	1		X	
8	1	1		X	
9		1	1	X	
10	2	1		X	X
11	2	1		X	X
12*		1			
13		3			X
14		2	1	X	X
15	2	1		X	X
16	2	1		X	X
17†		1	1		
18*		1			
totals	11	28	7	13	8

Table 4.02. Participant test doses. (*Participants 12 and 18 only undertook 1 test and thus could not provide evidence of repeatability. † The medium dose test of this participant mistakenly used a 0.5U bolus of insulin, invalidating their results for analysis. ‡ Three of this participant's medium dose tests were omitted from the study to reduce the effect of this individual on the cohort's outcomes.)

Individuals were intended to take tests within a few days of one another. However, significant delays occurred between the tests of some participants. All participants signed informed consent prior to each test. Ethical approval was obtained from the Upper South A Regional Ethics Committee. All tests were completed using the protocol defined in Section 4.2 with the dosing schedules defined in Table 4.02

Blood samples were assayed at the 'bedside' for glucose using the C8000 enzymatic glucose hexokinase assay (Abbott Labs, Abbot Park, Illinois). Samples were then spun and frozen for later insulin and C-peptide assays. The electrochemiluminoessescence (ELICA) immunoassay (Roche Diagnostics, Mannheim, Germany) was used to assay the insulin and C-peptide concentrations.

4.4.2 Parameter identification

Participant-specific parameter values were identified from the DISST pilot investigation data using the integral method (Hann *et al*. 2005a; Lotz 2007; Lotz *et al*. 2010). Detailed identification processes are described (Lotz *et al*. 2010).

Initially, the C-peptide model was de-convolved using a linear interpolation of the plasma C-peptide data (C_{interp}) (Lotz *et al*. 2009). Equation 4.02 can thus be solved analytically for *Y(t)*.

$$Y_t = k_1 \int_0^t C_{interp} e^{-k_2(t-\tau)} \, d\tau \qquad\qquad 4.02a$$

This term was incorporated into the integral formulation of Equation 4.01:

$$C_{t1} - C_{t0} = k_1 k_2 \int_{t0}^{t1} \int_0^\tau C_{interp} e^{-k_2(t-\tau)} \, d\tau dt - (k_1 + k_3) \int_{t0}^{t1} C dt + \int_{t0}^{t1} \varphi \frac{U_N}{V_P} dt$$

$$4.01a$$

Equation 4.01a was rearranged for the participant's endogenous insulin production response to the test stimulus, U_N.

$$\varphi \frac{U_N(t_1) - U_N(t_0)}{V_P} = C_{t1} - C_{t0} - k_1 k_2 \int_{t0}^{t1} \int_0^\tau C_{interp} e^{-k_2(t-\tau)} \, d\tau dt + (k_1 + k_3) \int_{t0}^{t1} C dt$$

$$4.01b$$

Thus, U_N was defined using Equation 4.01b at a 1-minute resolution between *t*=0 and *t*=end.

Secondly, the participant's insulin concentration response to the test stimulus was defined using the physiological model of Equations 4.15 and 4.16, the *a-priori* parameter values defined in Equations 4.17 to 4.21, and the U_N profile identified with Equation 4.01b.

To estimate minute-wise insulin profiles, insulin data was interpolated between $t=0$ and $t=15$ when the insulin bolus is administered. From this point, a double exponential decay was defined using the insulin data from 10-minutes post-bolus, and a 'false' insulin concentration value was used to model the bolus-induced concentration increase, in a least squares approach. This process produced a minute-wise profile of plasma insulin (I). The corresponding interstitial insulin concentration was identified using the analytical solution of Equation 4.16:

$$Q_i = e^{-\int_0^i n_C + \frac{n_I}{V_Q}} \left(Q_0 + \frac{n_I}{V_Q} \int_0^i e^{\int_0^i n_C + \frac{n_I}{V_Q}} I\right) \qquad 4.16a$$

Thus, profiles for both plasma and interstitial insulin responses to the test stimulus were defined. The integral method (Hann et $al.$ 2005b) was used identify participant-specific hepatic clearance (n_L) and extraction (x_L) values. The integral formulation of Equation 4.15 was separated into the coefficients of the known and unknown parameters:

$$n_L \underbrace{\int_{t0}^{t1} \frac{I}{1 + a_I I}}_{CN} + x_L \underbrace{\int_{t0}^{t1} -U_N}_{CX} = \underbrace{I_{t1} - I_{t0} - \int_{t0}^{t1} [n_K I - \frac{n_I}{V_P}(I - Q) + \frac{U_X}{V_P}]}_{C} \qquad 4.23$$

These coefficients were identified over a series of consecutive 2 minute periods that began at $t=0$ ($[t_0, t_1, t_2, \ldots, t_{end}]=[0, 2, 4, \ldots, end]$). This approach allowed the generation of a matrix equation in terms of n_L and x_L to be formed:

$$\begin{bmatrix} CN_{t0}^{t1} & CX_{t0}^{t1} \\ CN_{t1}^{t2} & CX_{t1}^{t2} \\ CN_{t2}^{t3} & CX_{t2}^{t3} \\ \vdots & \vdots \\ CN_{t_{end-1}}^{t_{end}} & CX_{t_{end-1}}^{t_{end}} \end{bmatrix} \begin{bmatrix} n_L \\ x_L \end{bmatrix} = \begin{bmatrix} C_{t0}^{t1} \\ C_{t1}^{t2} \\ C_{t2}^{t3} \\ \vdots \\ C_{t_{end-1}}^{t_{end}} \end{bmatrix} \qquad 4.23a$$

Thus, n_L and x_L were able to be constrained as necessary and identified using linear least square approach. The plasma and interstitial insulin concentration profiles were re-simulated using the physiological model with the identified hepatic clearance parameters.

The physiological simulation of interstitial insulin was then used with the glucose data and the integral method (Hann et $al.$ 2005b) to identify participant-specific values for insulin sensitivity and glucose distribution.

Initially a 'false' data value was added immediately after the glucose bolus. The glucose concentration of the point was identified by dividing the bolus content by a function of the participant's body-weight and adding the value to the basal glucose concentration. The glucose assays that were taken in the period ten minutes after the glucose bolus are affected by noise and were subsequently ignored. A linear interpolation was then used as an estimate of the glucose response to the test stimulus. The integral formulation of Equation 4.22 was rearranged and separated into the coefficients of the known and unknown parameters. The coefficients of the glucose model parameters were evaluated over periods that begin at 2-minute intervals from $t=0$ and end at the end of the test. Thus, a second matrix formulation was defined in terms of SI and V_G.

$$SI \underbrace{\int_{t0}^{t1} GQ - G_b Q_b}_{CS} + \frac{1}{V_G} \underbrace{\int_{t0}^{t1} P_X}_{CV_G} = \underbrace{G_{t1} - G_{t0} - \int_{t0}^{t1} p_G(G - G_b)}_{C} \qquad 4.24$$

The coefficients of the glucose model were evaluated at periods which begin at 2 minute intervals from $t=0$, and end at the end of the test. These periods were chosen to maximise the impact of the latter stages of the test on the identified insulin sensitivity value (Lotz 2007; Lotz et al. 2010). A matrix formulation was then defined in terms of SI and V_G.

$$\begin{bmatrix} CS_{t0}^{t_{end}} & CV_{G t0}^{t_{end}} \\ CS_{t1}^{t_{end}} & CV_{G t1}^{t_{end}} \\ CS_{t2}^{t_{end}} & CV_{G t2}^{t_{end}} \\ \vdots & \vdots \\ CS_{t_{end-1}}^{t_{end}} & CV_{G t_{end-1}}^{t_{end}} \end{bmatrix} \begin{bmatrix} SI \\ V_G \end{bmatrix} = \begin{bmatrix} C_{t0}^{t_{end}} \\ C_{t1}^{t_{end}} \\ C_{t2}^{t_{end}} \\ \vdots \\ C_{t_{end}}^{t_{end}} \end{bmatrix} \qquad 4.24a$$

Bounds were placed on the value of V_G to reduce the effect that incomplete mixing might have on the sensitivity term. The values of V_G is limited to within 12 to 25% of the participant's bodyweight, per published data (Lotz 2007; Lotz et al. 2010). Evaluation of Equation 4.24a yielded participant-specific values for SI and V_G.

4.4.3 Study outcomes - Part 1 inter-dose repeatability

Figure 4.02 and Tables 4.03 and 4.04 show the dose dependence of the insulin sensitivity and production parameters identified in the DISST test. The endogenous insulin production profile

was separated into the basal (U_B), first (U_1) and second (U_2) phases of insulin production. The first phase of insulin production was defined as the average pre-hepatic insulin output above the basal rate during the period of time 10 minutes after the glucose bolus. The second phase production was defined as the average pre-hepatic insulin production during the 20 minutes after the first-phase period. Note that the values presented Lotz *et al*. provide cumulative values in units of pmol rather than average values in units of mU/min presented here (Lotz *et al*. 2010).

The discrepancy between Part 1 participant's test results were calculated with Equation 4.25.

$$\Delta = \frac{\overline{M_{higher}} - \overline{M_{lower}}}{\overline{M_{lower}}}(*100) \qquad 4.25$$

The higher dose tests measured slightly reduced insulin sensitivity than lower dose tests within the same individual. The mean reduction observed in participants who underwent the medium and low dose tests was 3.5 % (SD 25.1), while the reduction observed in those of the underwent the high and medium tests was 6% (SD 18.3%). Overall, the lower dose tests observed a 5% reduction in identified sensitivity than the high dose tests undertaken by the same participants. This outcome is almost certainly a result of the saturation effect of insulin, which becomes less efficient at higher concentrations (Docherty et al. 2010; Natali et al. 2000; Prigeon et al. 1996).

The first phase production of insulin was an average of 68.4% (SD 66.3%) larger during the higher dose tests than for the lower dose tests of the same participants. The increased first phase production observed in the low-medium subgroup was 78.1% (SD 71.7%), which was greater than the increase observed in the medium-high dose subgroup 54.9% (SD 63.3%). This result implies that although first phase production is proportional on the magnitude of the bolus, the amount that can be produced may be a limiting factor for some participants.

Subject	Dose	S_I $[10^{-4}\text{L/mU/min}]$	ΔS_I [%]	U_B [mU/min]	U_1 [mU/min]	ΔU_1 [%]	U_2 [mU/min]	ΔU_2 [%]
4	low	13.39		19.6	22.1		25.1	
	med	16.49	23.1	20.9	25.4	14.8	29.1	15.8
7	low	19.33		24.9	41.9		23.6	
	med	18.06	-6.6	24.7	78.6	87.5	30.8	30.5
8	low	18.64		11.4	38.0		10.2	
	med	13.61	-27.0	12.8	83.3	119.1	16.3	60.7
10	low	43.73		13.7	48.0		13.0	
	low	29.19		15.6	52.0		16.4	
	med	17.40	-52.3	13.5	62.8	25.8	30.8	109.0
11	low	6.88		36.2	20.2		54.9	
	low	5.75		42.2	18.8		51.6	
	med	6.73	6.5	33.9	22.7	16.2	49.1	-7.9
15	low	8.28		20.0	40.0		24.1	
	low	8.99		22.1	36.6		30.8	
	med	7.39	-14.4	20.8	64.8	69.4	34.3	25.2
16	low	3.27		62.7	24.5		101.6	
	low	3.16		57.0	12.3		82.1	
	med	3.17	-1.4	66.2	57.8	213.8	128.8	40.2
mean			-3.5			78.1		39.1
SD			25.1			71.7		37.4

Table 4.03. Dose dependence of insulin sensitivity and production characteristics of participants who underwent DISST tests with the low and medium doses during the DISST pilot investigation.

Second phase insulin production was also a function of test dosing. The higher dose tests on average stimulated a 40.0% (SD 29.5%) greater second phase response. Unlike the first phase response, this result was consistent between low-medium and medium-high subgroups, further strengthening the case for a limitation on the first phase capability of test participants.

Figure 4.02 (upper right) shows that the basal insulin production rate was not dependent on the test dose, as expected. A reduction of 1.3% (SD 14.6%) was measured in the lower dose tests compared to the higher dose test of the same participants. This outcome was expected and serves to validate the findings of the first and second phase production profiles identified.

Subject	Dose	S_I [10^{-4}L/mU/min]	ΔS_I [%]	U_B [mU/min]	U_1 [mU/min]	ΔU_1 [%]	U_2 [mU/min]	ΔU_2 [%]
1	med	3.13		70.9	39.3		96.7	
	high	2.69	-14.1	68.9	102.7	161.2	138.1	42.7
2	med	19.47		8.9	17.5		15.4	
	high	13.43	-31.0	12.0	22.5	28.8	23.9	55.7
5	med	26.45		14.3	41.1		18.1	
	med	19.97		11.1	34.9		13.5	
	high	25.07	8.0	10.9	56.4	48.5	20.0	26.3
6	med	14.84		17.0	38.8		30.5	
	high	12.83	-13.6	17.2	55.5	42.9	48.0	57.5
14	med	11.70		21.1	69.7		31.7	
	med	11.65		25.8	73.9		38.6	
	high	14.12	20.9	19.1	66.7	-7.1	41.8	19.0
mean			-6.0			54.9		40.2
SD			18.3			63.3		17.2

Table 4.04. Dose dependence of insulin sensitivity and production characteristics of participants who underwent DISST tests with the medium and high doses during the DISST pilot investigation.
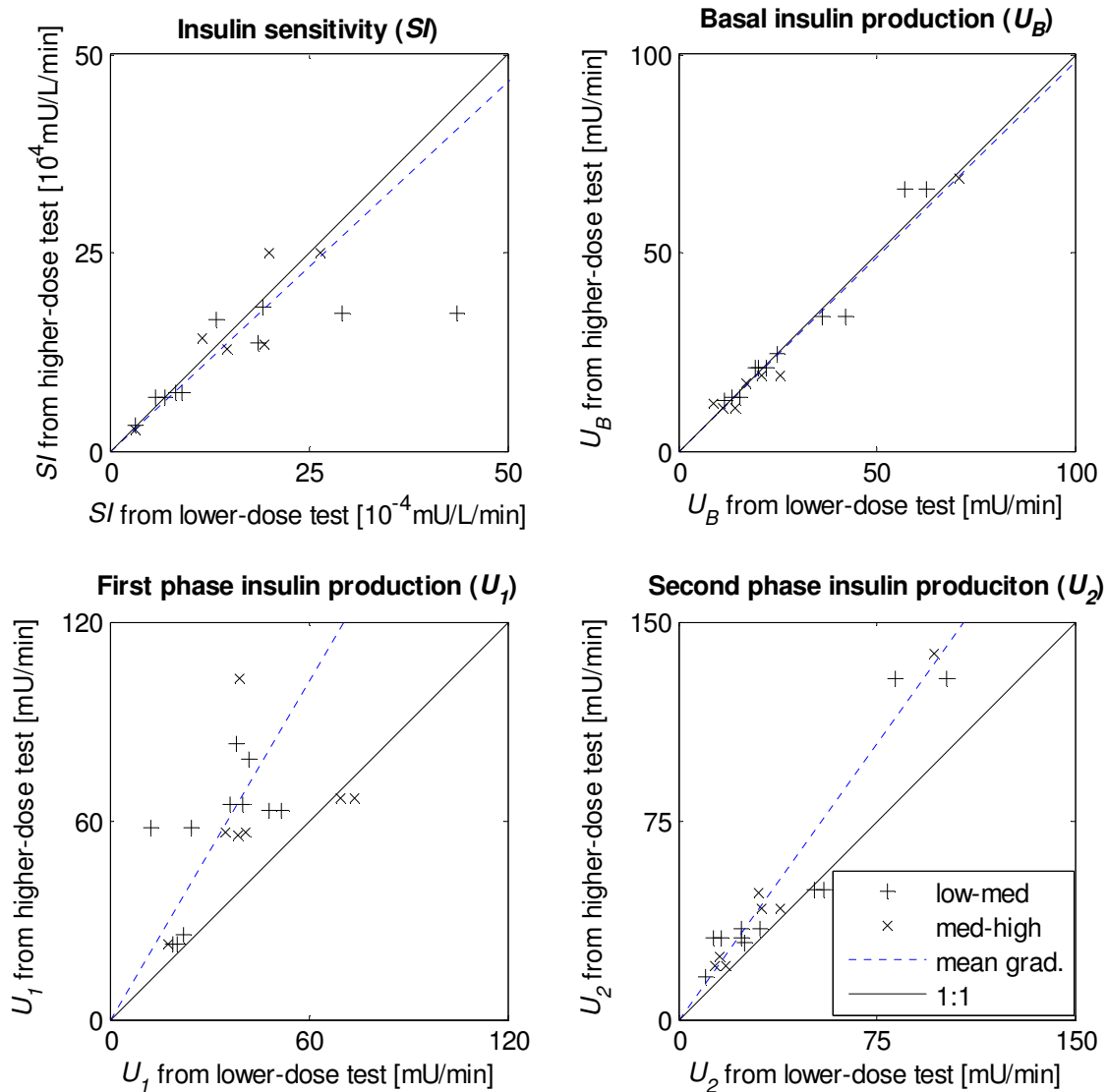
Figure 4.02. The effect of dose changes in the outcomes of the DISST test metrics of insulin sensitivity and production.

Part 1 of the pilot investigation sought to evaluate the ability of the model and *a-priori* identified parameters to produce consistent results across varying test doses. The mean variation in insulin sensitivity was very small, although the standard deviation of this variation was relatively large. Thus, the model did not produce a biased result based on dose, and the variation observed is a function of the variations in the individual participant's physiology across tests.

Short-term erratic changes in insulin sensitivity can be affected by a number of factors including stress and illness (Greisen *et al.* 2001; Hollenbeck & Reaven 1987; Van den Berghe *et al.* 2006; Zierler 1999), sleep deprivation (Davidson *et al.* 1987; Van Cauter *et al.* 1997), menstrual cycle (Trout *et al.* 2007), time of day (Van Cauter *et al.* 1997), recent exercise and diet (Borghouts & Keizer 2000; Nishida *et al.* 2004; O'Gorman *et al.* 2006; Zierler 1999). It is likely that a

culmination of these factors and assay error caused the moderate intra-participant variation observed in this pilot trial cohort.

As expected, first phase insulin production was proportional to dose. The proportional effect is a key aspect to models that have sought to develop the Eaton *et al.* and Van Cauter *et al.* based models (Eaton *et al.* 1980; Van Cauter *et al.* 1992) that the DISST uses. Such models use two parameters that link insulin production to changes in glucose concentration and glucose concentration above basal. However, to accurately fit the measured C-peptide data, such models require so-called 'potentiation-profiles' (Ferrannini & Mari 2004) that have very limited physiological basis and are thus barely clinically irrelevant data-fitting terms. The utility of potentiation profiles is essentially limited to making a model fit the Van Cauter model-based results. Thus, this type of model is not used for the DISST test to provide a cosmetic façade for the endogenous insulin production profile.

4.4.4 Study outcomes - Part 2 intra-dose repeatability

Part 2 of the pilot investigation of the DISST test measured the variations observed across tests by participants when the same dose is used for each test. Table 4.05 shows the changes in insulin sensitivity and production observed in participants of Part 2 of the DISST pilot investigation. These results thus quantify the repeatability of the test.

Intra-participant variation of insulin sensitivity and production was defined as the maximum absolute deviation from the mean participant value using Equation 4.26.

$$\Delta = max_i \left| \frac{M_i - \bar{M}}{\bar{M}} \right| \qquad\qquad 4.26$$

The mean difference in insulin sensitivity with a consistent dose was 11.3% (SD 9.0%). Although this value is larger than the mean variation measured in Part 1 it must be remembered that Part 1 measured model bias as a result of differing doses. The low mean variation was due to the even parameter spread in both positive and negative directions caused by the precision of the model. The variation in Part 2 was constrained to positive values, and thus was larger. The intra-participant standard deviation in insulin sensitivity observed in Part 2 was significantly lower than that observed in Part 1 indicating a more accurate and repeatable intra-participant result overall.

Both U_1 and U_2 were more repeatable in Part 2 of the DISST pilot investigation. This can be predominantly attributed the proportional insulin production response to the content of the glucose bolus.

Subject	Dose	S_I [10^{-4}L/mU/min]	ΔS_I [%]	U_B [mU/min]	U_1 [mU/min]	ΔU_1 [%]	U_2 [mU/min]	ΔU_2 [%]
10	low	43.73		13.7	48.0		13.0	
	low	29.19	19.9	15.6	52.0	4.0	16.4	11.5
11	low	6.88		36.2	20.2		54.9	
	low	5.75	8.9	42.2	18.8	3.4	51.6	3.1
15	low	8.28		20.0	40.0		24.1	
	low	8.99	4.1	22.1	36.6	4.5	30.8	12.2
16	low	3.27		62.7	24.5		101.6	
	low	3.16	1.7	57.0	12.3	33.1	82.1	10.6
3	med	10.18		34.1	120.8		45.8	
	med	8.59		38.8	142.4		50.0	
	med	7.37	16.8	43.2	131.6	8.2	61.4	17.2
5	med	26.45		14.3	41.1		18.1	
	med	19.97	14.0	11.1	34.9	8.1	13.5	14.3
13	med	16.31		35.6	45.4		54.8	
	med	13.51		36.2	54.5		59.3	6.4
	med	21.20	24.7	34.1	59.4	11.9	53.2	
14	med	11.70		21.1	69.7		31.7	
	med	11.65	0.2	25.7	73.9	2.9	38.6	9.8
mean			11.3			9.5		10.6
SD			9.0			10.0		4.4

Table 4.05. Intra-dose variability of insulin sensitivity and production values for participants of Part 2 of the DISST pilot investigation.

4.4.5 Overall outcomes of the DISST pilot study

The DISST is unique amongst the established tests presented in Table 2.02, as it accurately quantifies both insulin sensitivity and endogenous insulin production. Most importantly, it is the only test that can accurately and concurrently quantify all of the characteristics of the pathogenesis of type 2 diabetes represented in Figure 1.03.

Furthermore, the model allows a much less clinically intensive protocol than most of the non-fasting tests. The repeatability of the tests is very reasonable given the particularly low clinical intensity of the protocol and the ad-hoc nature of the pilot investigation.

4.5 *In-silico* investigation of the DISST variability

An *in-silico* Monte-Carlo investigation was undertaken to compare the effect of assay error on the DISST, EIC and HOMA metrics (Lotz *et al*. 2008). *In-silico* investigations are typically computer simulations that model the behaviour of an indicative 'virtual' cohort to a test stimulus.

The Monte Carlo analysis of the DISST test used the clinical data from 148 EIC tests undertaken by 74 participants during an dietary and exercise based intervention investigation (McAuley *et al*. 2002). EIC data was used to identify participant-specific values for insulin sensitivity (SI) and insulin clearance (n_L). Insulin production is suppressed during the EIC (Argoud *et al*. 1987; Liljenquist *et al*. 1978), and thus participant specific values could not be identified using EIC data. Thus, first and second phase insulin production values were assumed as functions of body surface area for each participant (Lotz *et al*. 2008). The basal insulin production rate was derived using a function of the basal rate and clearance of insulin. First pass extraction was randomly defined between a range of 50-95%. Endogenous glucose production (EGP) was assumed to be suppressed by between 25% and 75%.

These test-specific parameter values were used to simulate C-peptide, insulin and glucose responses to the DISST test stimulus. The low-dose, medium-dose and high-dose DISST protocols were used with each set of model parameters. These simulations were 'sampled' at the time specified by the protocol to present a paired 'synthetic' data set for each EIC test undertaken. Each simulated data set was then identified 500 times, with each iteration adding new, normally distributed assay error to the synthetic DISST data to assess its realistic impact. The magnitude of the assay error used was in accordance with reported assay errors (glucose ~2%, insulin ~3%, C-peptide ~4%) (Devreese & Leroux-Roels 1993; Roche 2004,2005). EIC derived insulin sensitivity values and HOMA were also identified using the same reported assay errors for comparison.

The coefficient of variation (CV) of the DISST was dependent on the test doses. The low-dose, medium-dose and high-dose tests produced CV values of 6.9%, 4.5% and 3.6%, respectively. The EIC CV was 3.3%, while the HOMA CV was over 10%. These values for EIC and HOMA CV are low compared to the clinically derived values presented in Table 2.02. However, it must

be considered that the clinically derived values incorporate daily changes in participant physiology and thus should potentially, be greater than the variation caused solely by assay error as assessed in this *in-silico* analysis.

The *in-silico* analysis produced EIC and DISST results that correlated at R=0.91. However, when equality of units was enforced, the correlation increased to R=0.98. These values thus represent theoretical maximum correlations that might be found in a clinical validation trial assuming assay errors is the only variation between tests (i.e. no daily physiological variation).

Chapter 5. A geometric method for *a-priori* model identifiability analyses

A novel method for the evaluation of model identifiability using integrals instead of derivatives was developed during the DISST investigations. Contrary to established methods, an appraisal of the model parameters in the presence of assay error is provided by this novel approach. The method is presented here to explain how model or protocol selection can affect parameter identification, and aids the justification of the DISST modelling strategy. Furthermore, this chapter highlights and explains the issues frequently encountered during insulin sensitivity identification with established models in comparison to the robust DISST model. The method has been published (Docherty *et al.* 2011a).

5.1 Motivation

Approaches for the analysis of model identifiability typically assume continuous perfect input data (Bellman & Åström 1970; Bellu *et al.* 2007; Pohjanpalo 1978). However, these methods can produce false assurances of identifiability as they rely on idealised assumptions. The limitation of discrete data that is subject to assay error causes parameter trade-off in many cases (Caumo *et al.* 1999; Erichsen *et al.* 2004; Pillonetto *et al.* 2002; Quon *et al.* 1994b). Hence, there is often a limitation on the identified metrics clinical value. Thus, not only should a model be checked for identifiability in the classical, ideal *a-priori* sense, but the susceptibility of a model's parameters to mutual interference should also be tested. The latter point should be potentially more critical for models using clinical data with assay error.

For example, the Minimal Model of insulin sensitivity (Bergman *et al.* 1979) has been shown to be identifiable using typical methods (Audoly *et al.* 2001; Audoly *et al.* 1998; Chin & Chappell). However, with discrete data subject to assay error, parameter identification has sometimes failed (Erichsen *et al.* 2004; McDonald *et al.* 2000; Pillonetto *et al.* 2002), particularly for insulin resistant individuals for whom accuracy is most clinically valuable. Numerous Bayesian techniques have had success in limiting this failure (Cobelli *et al.* 1999; Denti *et al.* 2009; Pillonetto *et al.* 2003; Pillonetto *et al.* 2002), but they tend to force the parameters to diverge away from their true least square values, limiting the relevance of the model and exaggerating the influence of population trends on the parameter values derived from individual test. Thus, widespread clinical application of these models has been limited by the ambiguity of results.

This chapter presents a novel geometric method for identifiability analysis that incorporates consideration of assay error. Furthermore, the method highlights areas for potential improvements to protocols and sampling times that would improve practical identifiability. At this stage of development, the method is limited to linear and non-linear first-order models that allow a separation of model parameters. However, this class of model is typical of those found in pharmacokinetic (PK) and pharmaco dynamic (PD) modelling and thus cover a wide range of models in the field.

5.2 Proposed method description and study design

The proposed method was evaluated *in-silico* using clinically validated models of insulin kinetics and a DISST model of insulin mediated and glucose clearance. The method was evaluated on its ability to predict the coefficients of variation of identified parameters in a Monte Carlo analysis.

The analysis used random assay error to simulate real clinical data, and thus simulate the variability in the identified parameter spread expected in a clinical study.

5.2.1 Proposed method process

To evaluate identifiability of a model, the integral formulations of the variable parameters' coefficients are evaluated using an estimated response to the test stimulus. Thus, the method cannot be used in complete ignorance of the expected behaviour of the test participant. In particular, the approximate shape of the species concentrations as a result of the test protocol must be known. This is a reasonable assumption in almost all PK/PD studies. Particularly in well-understood areas like insulin-glucose metabolic systems.

The specific steps are illustrated using the general function in Equation 5.01.

$$\dot{X} = f(X, Y, C, D, a, b) \qquad\qquad 5.01$$

where: X is a measurable concentration; Y is dependent concentration in a remote compartment; C and D are known input vectors or scalars; and a and b are scalar variable model parameters to be identified.

1. Rearrange the governing equation to create a first order differential equation with

linearised parameters in terms of *a-priori*, constant and measurable concentration terms

$$\dot{X} = af_1(X, Y, c_i) + bf_2(X, Y, c_i) + d_i \qquad\qquad 5.01a$$

2. Derive the integral formulation of this governing equation

$$X - X_0 = a \int f_1(X, Y, c_i) + b \int f_2(X, Y, c_i) + d_i \qquad\qquad 5.01b$$

3. Evaluate the integral of the coefficients of each parameter between 0 and each proposed sample time using an assumed participant response to the test stimulus.

$$\tilde{a} = \int_0^i f_1(X, Y, c_i) \qquad \tilde{b} = \int_0^i f_2(X, Y, c_i) \qquad\qquad 5.02$$

where: *i* is each measured sample time after the first.

4. Divide the resulting values by their respective means to normalise the coefficients. (i.e. to ensure the mean parameter coefficient value for both parameters is 1).

$$\breve{a} = \tilde{a}/\bar{\tilde{a}} \qquad\qquad \breve{b} = \tilde{b}/\bar{\tilde{b}} \qquad\qquad 5.03$$

5. Subtract one set of coefficients from the other and define the 2-norm of the result ($\|\Delta\|_2$).

$$\|\Delta\|_2 = \left\|\breve{a} - \breve{b}\right\|_2 \qquad\qquad 5.04$$

6. Any distinction at all between the coefficients would imply identifiability (i.e. if $\|\Delta\|_2 \neq 0$). In reality, the effect of assay error on parameter identification is inversely proportional to the magnitude of this distinction, and proportional to the magnitude of any assay error (ε):

$$Variability = \mu \frac{\varepsilon}{\|\Delta\|_2} \qquad\qquad 5.05$$

where: μ is a proportionality factor that incorporates factors such as the relative contribution of the parameter to the derivative of the relevant species concentration in the governing differential equation, and the absolute magnitude of the noise at the sampled times in relation to the relative magnitude of the parameter coefficient.

Thus, the method cannot accurately predict the coefficient of variation that a rigorous Monte Carlo analysis may find. However, it can predict the change in the coefficient of variation that might be observed when changes are made to the test sampling or stimulus protocols.

5.2.2 The DISST model

The physiological models for insulin and glucose used by the DISST were used to evaluate the proposed *a-priori* identifiability analysis. The model is presented in Section 4.3 (Equations 4.15, 4.16 and 4.22) and is repeated here for ease of reading.

$$\dot{I} = -n_K I - n_L \frac{I}{1 + \alpha_I I} + \frac{n_I}{V_P}(Q - I) + \frac{U_X}{V_P} + (1 - x_L)\frac{U_N}{V_P} \qquad 4.15$$

$$\dot{Q} = \frac{n_I}{V_Q}(I - Q) - n_C Q \qquad 4.16$$

$$\dot{G} = -p_G(G - G_B) - SI(GQ - G_B Q_B) + \frac{P_X}{V_G} \qquad 4.22$$

where: all terms are defined in Section 4.3

5.2.3 Participants

Parameter values from two participants of the pilot investigation of the DISST (Lotz *et al.* 2010) were used to generate *in-silico* simulated data to construct and demonstrate the method proposed here. *In-silico* data was used in this analysis because it easily allows protocols to be changed to illustrate the impact on identifiability. Equally, varying levels of noise can be added to illustrate its impact. The participant characteristics are summarised in Table 5.01 and represent extremities of the range of cases encountered in typical research studies of insulin sensitivity. One of the

participants is a healthy and active normo-glucose tolerant (NGT) individual while the other is a sedentary individual with impaired glucose tolerance (IGT) and suspected un-diagnosed type 2 diabetes.

Glucose tolerance	Sex	Age	BMI	U_N* [mU/min]			n_L [1/min]	x_L [1]	V_G [L]	SI [10^{-4}L/mU/min]
				U_B	U_1	U_2				
NGT	M	22	21.5	15.5	283.9	5.6	0.218	0.797	9.8	20.95
IGT	F	57	33.9	70.9	143.6	92.5	0.064	0.822	13.4	2.24

Table 5.01. Anatomical and identified parameter values (using the iterative integral method) of two participants of the DISST pilot investigation, Subjects 18 and 1, respectively. (* in contrast to the typical deconvolution approach (Section 4.4.2) U_B, U_1 and U_2 rates were identified by the iterative integral method as a 3-step function)

5.2.4 Simulated test protocol

The simulated protocol is similar to the DISST test; a 10g glucose bolus was administered at t=7.5 and a 1U insulin bolus was administered at t=17.5. The test duration was 60 minutes with a five-minute sampling frequency. The U_N profile was defined as a step function with three stages including basal, first and second phase production rates. The first phase of insulin production had a five-minute duration and began with the glucose bolus. Simulations of plasma and interstitial insulin were completed using Equations 4.01 and 4.02, the parameter estimation equations from Van Cauter *et al*. (Van Cauter *et al*. 1992), n_L and x_L values from Table 5.01 and an a_I value of 0.001 L/mU. Glucose was simulated using Equation 4.22 and the plasma and interstitial insulin profiles are obtained by evaluating Equations 4.15 and 4.16. Parameter identification for the cases tested was performed using the iterative integral method described in Section 4.4.

5.2.5 Analysis

A series of variable parameter selection and sampling scenarios were analysed using the DISST model in Monte Carlo analyses. The analysis processes are summarised:

- Clinically measured physiological parameters of the NGT participant presented in Table 5.01 were used to define simulated responses to the DISST test protocol described in Section 4.2. The IGT participant was included in the analysis from Section 5.2.2.

- Samples were obtained from the simulated profiles at the defined times. Each iteration of the Monte Carlo analysis added normally distributed assay error (magnitude of the error is defined below).

- For each analysis, 100 iterations were used with parameter identification performed using the iterative integral method.

- Each scenario measured the mean value of the parameters identified in the Monte Carlo simulation normalised by the simulation value from Table 5.01, and the coefficient of variation (CV) of each identified parameter. (Thus, perfect convergence is defined as a mean of 1 and a CV of 0.) The CV value is the paramount indicator of parameter trade off during identification and thus was compared to the $\varepsilon/\|\Delta\|_2$ value defined in Section 5.2.1. A large CV value indicates that in the presence of assay error, the identified model parameters have encountered trade-off during identification.

- Finally, values are obtained for μ to linearise Equation 5.05 for each of the combinations of parameters tested.

5.2.6 Derivative algebra method for determining model identifiability

5.2.6.1 Structural identifiability of the insulin model

The traditional and established method of evaluating model identifiability converts the governing equations into expressions of *a-priori* terms, model parameters, and the observed data. The ultimate aim is to prove that each model parameter is a coefficient of a unique function of the observed data, *a-priori* values, the derivatives of observed or *a-priori* data or combinations of terms observed or *a-priori* terms. Using the algebraic derivative approach of (Ritt 1950) and refined in (Bellu *et al.* 2007) the identifiability of the model can be confirmed. The *a-priori* inputs are (U_N, U_X) are considered the most important coefficients, followed by their derivatives $(\dot{U}_N, \dot{U}_X)$. The observed species (denoted Y, in this case $Y=I$) is considered the next most important, followed by its derivatives $(\dot{Y}, \ddot{Y})$. The species of the governing equations follow (I, Q) and their derivatives $(\dot{I}, \dot{Q})$ are considered the most deleterious terms. The aim is to have as many parameters as functions of U_N or U_X as possible, and have none of the model parameters in terms of $I, Q, \dot{I}$ or $\dot{Q}$. This ranking of terms is summarised below:

$$[U_N < U_X < \dot{U}_N < \dot{U}_X < Y < \dot{Y} < \ddot{Y} < I < Q < \dot{I} < \dot{Q}]$$

Equations 4.15 was rearranged in terms of Y and substituted into Equation 4.16 (to minimise un-necessary complexity $\acute{x}_L = (1 - x_L)/V_P$)). Equation 4.16a was considered the "Characteristic Equation" of the model as all species are either observed or known *a-priori* inputs.

$$\dot{Y} + n_K Y + n_L \frac{Y}{1 + \alpha Y} + \frac{n_I}{V_P}(Y - Q) - \frac{U_X}{V_P} - \acute{x}_L(U_N) = 0 \qquad \text{4.15a}$$

$$-\frac{n_I^2}{V_P V_Q} Y + \left(n_C + \frac{n_I}{V_Q}\right)\left[\dot{Y} + n_K Y + n_L \frac{Y}{1 + \alpha_I Y} + \frac{n_I}{V_P} Y - \frac{U_X}{V_P} - \acute{x}_L(U_N)\right] \dots$$

$$\text{4.16a}$$

$$\dots + \ddot{Y} + n_K \dot{Y} + n_L \frac{\dot{Y}}{1 + \alpha_I \dot{Y}} + \frac{n_I}{V_P}\dot{Y} - \frac{\dot{U}_X}{V_P} - \acute{x}_L(\dot{U}_N) = 0$$

if: $Y = I$

Thus, the model terms in the Characteristic Equation had the following coefficients:

$$\dot{Y}: (n_C + n_K + \frac{n_I}{V_P} + \frac{n_I}{V_Q}) \qquad \text{4.16a-I} \qquad\qquad Y: \left(n_K + \frac{n_I}{V_P}\right)\left(n_C + \frac{n_I}{V_Q}\right) - \frac{n_I^2}{V_P V_Q} \qquad \text{4.16a-II}$$

$$\frac{\dot{Y}}{1 + \alpha_I \dot{Y}} : n_L \qquad \text{4.16a-III} \qquad\qquad \frac{Y}{1 + \alpha_I Y} : n_L(n_C + \frac{n_I}{V_Q}) \qquad \text{4.16a-IV}$$

$$\dot{U}_X : \frac{-1}{V_P} \qquad \text{4.16a-V} \qquad\qquad U_X : \frac{-1}{V_P}(n_C + \frac{n_I}{V_Q}) \qquad \text{4.16a-VI}$$

$$\dot{U}_N : -\acute{x}_L \qquad \begin{matrix}\text{4.16a-}\\\text{VII}\end{matrix} \qquad\qquad U_N : -\acute{x}_L(n_C + \frac{n_I}{V_Q}) \qquad \begin{matrix}\text{4.16a-}\\\text{VIII}\end{matrix}$$

Using arbitrary values for $U_N, U_X, \dot{U}_N, \dot{U}_X, Y$ and $\dot{Y}$ and assuming a non-zero α_I value in Equations 4.16a-I, III-VIII provided a unique solution for each model parameter (n_K, n_I, n_L, n_C, V_P, V_Q, x_L). Thus, global identifiability was confirmed by the traditional derivative based method for the model parameters of the characteristic equation (4.16a). Thus, according the established derivative-based method, the parameters of Equations 4.15 and 4.16 are also *a-priori* identifiable.

5.2.6.2 Structural identifiability of the glucose model

The structural identifiability of the glucose model is considerably less complex than the insulin kinetic model. In particular, the characteristic set was defined with a single equation:

$$\dot{G} + p_G(G - G_B) + SI(GQ - G_B Q_B) - \frac{P_X}{V_G} \qquad \text{4.22a}$$

Thus the relevant coefficients were defined:

$$G: p_G \qquad\qquad GQ: SI$$
$$P_X: 1/V_G \qquad\qquad Q_B: G_B SI$$

Simple observation confirmed that p_G, SI, G_B and V_G are globally identifiable using derivative-based identifiability analysis.

It is important to note that this is an accepted traditional form of model analysis. It is ideal in that it assumes perfect (zero assay error) continuous measurement. Hence, these results imply globally unique, identifiability of the DISST insulin PK and glucose PD models, but only under these clinically un-realistic assumptions.

5.3 Analysis of the DISST insulin PK models

5.3.1 Justification of the iterative integral method

When the sampled 0% assay error, *in-silico* data from the NGT participant was used in the iterative integral method to identify n_L, n_K, n_I/V_P(lumped), x_L and V_P as variable parameters, convergence to the simulation values occured as shown in Figure 5.01. This result confirmed the traditional identifiability analysis of Equations 4.16a and 4.15a in Section 5.2.6. However, when 1% normally distributed noise was added to the simulated data, parameter values did not converge to simulation values. When the sample noise was increased to 3.5%, which is more indicative of the actual magnitude of measurement noise encountered clinically, parameter convergence accuracy was significantly compromised. Hence, despite proven (no noise) structural identifiability using well accepted methods, the addition of assay error or noise yielded corrupted or potentially unidentifiable results.
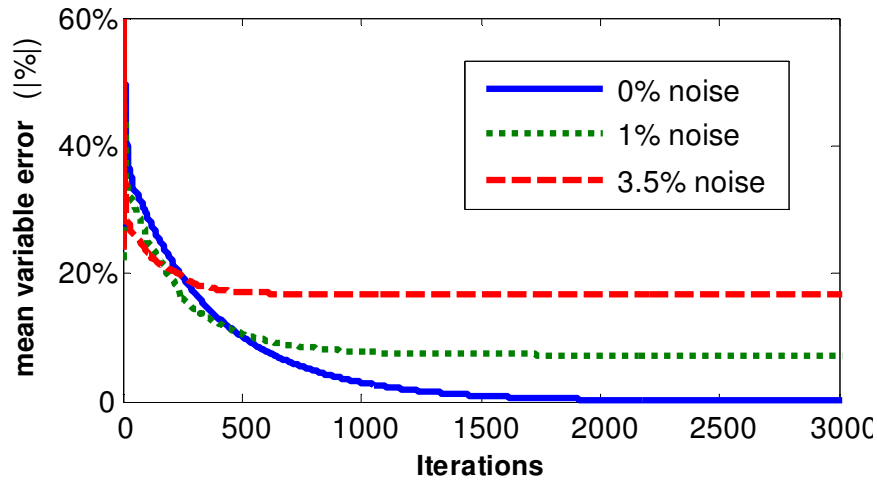
Figure 5.01. Mean absolute percentage error between the simulation and identified parameters in the presence of 0%, 1% and 3.5% random assay error, with respect to iterations of the iterative integral identification approach.

5.3.2 Hepatic and renal clearance rate identification

To understand why the addition of noise degraded the quality of identification, the case of interference between n_K and n_L was tested. In this analysis, all parameters of Equations 4.15 and 4.16 were set as constants except for only n_L and n_K, which were identified parameters. From the analyses in Sections 5.2.6 and 5.3.1, parameter value convergence was assured for the noiseless case. However, for the 1% and 3.5% noise cases, parameter interference caused considerable parameter divergence in the value of the identified parameters compared to the actual values used *in-silico*. The Monte Carlo analysis described in Section 5.2.5 was used to evaluate these two parameters alone in the presence of assay error or noise, and the results were shown in Table 5.02.

The matrix equation used by the iterative integral method was in the form:

$$-n_k \int_0^i I - n_L \int_0^i \frac{I}{1 + \alpha_I I} = I_i - I_0 + \frac{n_I}{Vp} \int_0^i (I - Q) - (1 - x_L) \int_0^i U_N + \int_0^i \frac{U_X}{V_P} \qquad 5.06$$

where: i = 5, 10, 15, … ,60 minutes, matching sampling times.

Thus, the magnitude of the $\|\Delta\|_2$ term was identified for this model and sampling protocol as:

$$\|\Delta\|_2 = \left\| \frac{\int_0^i I}{mean\left(\int_0^i I\right)} - \frac{\int_0^i \frac{I}{1+\alpha_I I}}{mean\left(\int_0^i \frac{I}{1+\alpha_I I}\right)} \right\|_2 = 0.032$$

However, Table 5.02 shows that if α_I was increased significantly to 0.05 L/mU (50x), an arbitrarily chosen value that is not necessarily physiologically representative (Thorsteinsson 1990), parameter convergence is more stable. The $\|\Delta\|_2$ term was re-evaluated with the exaggerated α_I value.

$$\|\Delta\|_2 = \left\| \frac{\int_0^i I}{mean\left(\int_0^i I\right)} - \frac{\int_0^i \frac{I}{1+\alpha_I I}}{mean\left(\int_0^i \frac{I}{1+\alpha_I I}\right)} \right\|_2 = 0.464$$

Thus, the parameters identified with the exaggerated α_I term were hypothesised to have approximately 15 times smaller variability than those identified with the accepted α_I value. Table 5.02 shows the effect of the α_I distinction on the identified parameter values.

Noise	0%		1%		3.5%	
Saturation	α_I=0.001	α_I=0.05	α_I=0.001	α_I=0.05	α_I=0.001	α_I=0.05
n_K	1 (0)	1 (0)	0.952 (29.7%)	0.999 (1.7%)	0.946 (83.6%)	1.004 (5.9%)
n_L	1 (0)	1 (0)	1.035 (21.4%)	1.001 (0.8%)	1.041 (59.5%)	1.000 (2.8%)

Table 5.02. Normalised parameter variation (mean/true value, (CV)) when n_K and n_L are identified parameters.

Table 5.02 shows the distinction between the effects of noise on identified parameter values when the α_I term was changed. Although the 0% noise case indicates that the parameters are uniquely identifiable, at 1% noise the variation in the identified values limits their clinical viability. At 3.5% noise, which may be expected in a real clinical setting, the parameters are effectively no longer uniquely identifiable. The very large CVs of the identified parameter values illustrate this point.

However, when the α_I term was significantly increased, unique identifiability was once again possible, even with 3.5% noise. The mean ratio of variation caused by the disparate α_I values was approximately 1:20. This ratio is larger than the ratio predicted by the method (1:15 =

0.032:0.464), but still represents a positive outcome in terms of predicting the relative magnitude of the change using the value of $\|\Delta\|_2$. The reason for this outcome can be viewed graphically by the increased contrast between integral formulations of the parameter coefficients. This contrast is shown in Figure 5.02.
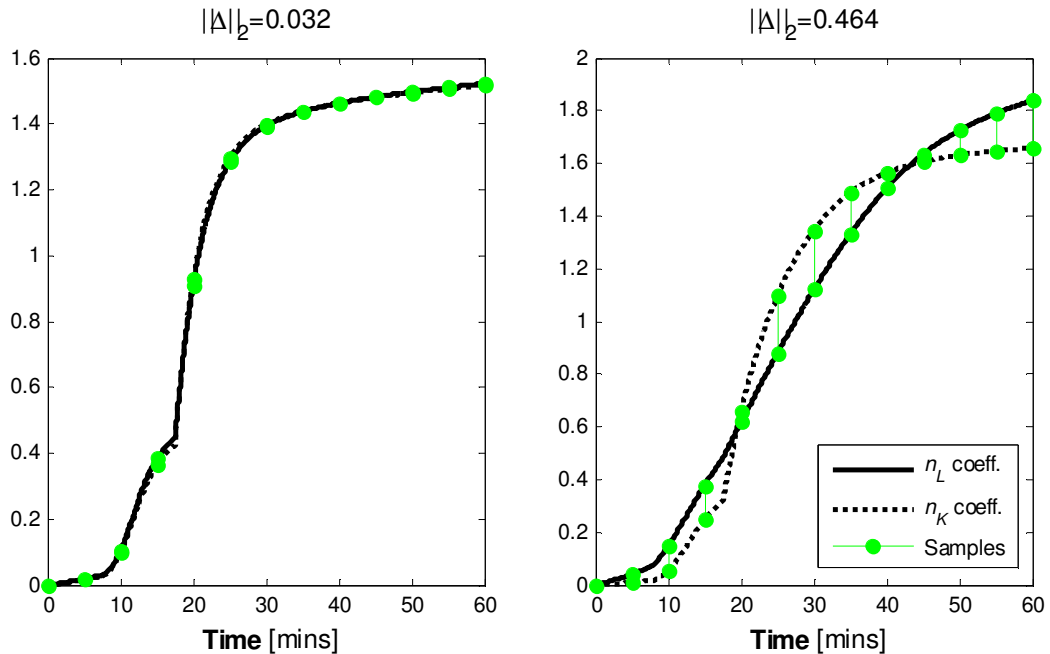
Figure 5.02. Contrasts between the coefficients of hepatic and renal clearance rates with disparate saturation values at the defined sample times.

The difference between the curves Figure 5.02 at the sample times indicated the identifiability of the model parameters in this two-parameter case. Thus, when the saturation term was increased, the integral formulations of the parameter coefficients were more distinct and identifiability was increased. Despite the positive findings of the typical, derivative based identifiability analysis, a realistic saturation value of 0.001 L/mU caused n_K and n_L to become uniquely un-identifiable in a real clinical setting. This outcome may be considered an elementary finding that should have been inferred with a quick observation of Equation 4.15. However, it points to a failing of typical *a-priori* identifiability tests that this approach can negate with a quick graphical analysis.

The findings of this analysis also show that the functional effects of n_L and n_K on insulin concentration in Equation 4.15 are so similar that there would be a negligible effect if the terms were combined. As such, analysis of the DISST model in Chapter 5 will use a combined n_L and n_K term (n_T) without the saturation term, which is negligible except at extremely high insulin concentrations. Equation 4.15 was thus redefined:

$$\dot{I} = -n_T I - \frac{n_I}{V_P}(I - Q) + (1 - x_L)\frac{U_N}{V_P} + \frac{U_X}{V_P} \qquad\qquad 5.07$$

5.3.3 Plasma insulin distribution volume and insulin clearance identifiability

The proposed method can also explain why n_T and V_P are only identifiable in the presence of noise with intelligent positioning of samples. To identify n_T and V_P the form of the governing matrix equation was defined with the variable parameters on the LHS and the known terms on the RHS:

$$\overline{V_P} \int_0^i (n_I(Q - I) + U_x) - n_T \int_0^i I = I_i - I_0 - (1 - x_L)\int_0^i \frac{U_N}{V_P} \qquad\qquad 5.08$$

where: $\overline{V_P}=1/V_P$ (U_N/V_P was obtained via C-peptide data and then multiplied by V_P, thus, as V_P was assumed unknown in this case. However in this case, the U_N/V_P must be used directly in RHS of the equation.)

As with the n_K and n_L analysis, the 0% noise case exactly reproduced the simulation values. Figure 5.03 shows the coefficients of the two parameters for three different sampling protocols. In this case, Protocol 1 used the 5 minutely sampling defined in Section 5.2.4. However, Protocol 2 used samples at t=0, 15, 20, and 60, and Protocol 3 used samples at t=0, 5, 45, and 60 minutes. Thus, Protocol 1 required 13 samples, while Protocols 2 and 3 only required four samples, which was a significant reduction (~70%).

The $\|\Delta\|_2$ terms were defined for each of these protocols (Table 5.03) and the distinction of the parameter coefficients that yield these values are displayed graphically in Figure 5.03. The $\|\Delta\|_2$ values indicated that Protocol 3 was comparatively unable to reproduce the simulation values because the samples occur at points where the integrals of the parameter coefficients were effectively equal. Contrary to the expected result, which was that parameter identification is best with the frequently sampled Protocol 1, the method predicted that the sparsely sampled Protocol 2 would be more accurate. Protocol 2 was expected to have better identifiability than Protocol 3 despite the equal number of samples by merit of the placement of the samples in relation to the features of the integral of the parameter coefficients.

	$\|\Delta\|_2$
Protocol 1	0.731
Protocol 2	0.990
Protocol 3	0.045

Table 5.03. The value of the $\|\Delta\|_2$ term for the sampling protocols defined.

Table 5.04 shows the parameter convergence and variablitiy for the exact same model with the three different sampling protocols.
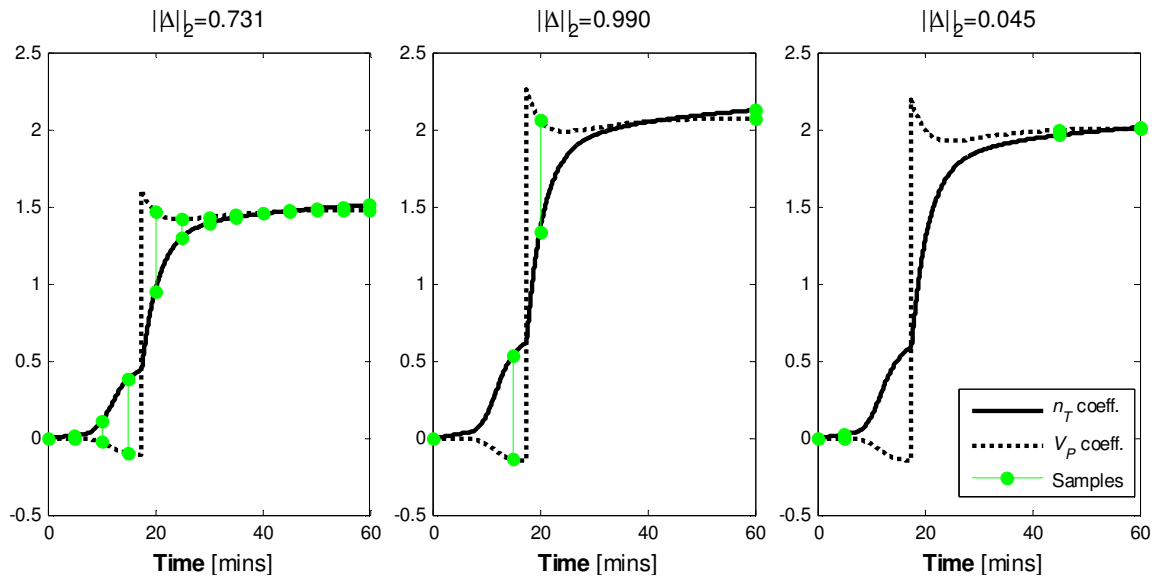
Figure 5.03. Effect of the sampling times on the observability of the difference between the integral of the coefficients of the parameters in Equation 5.08. The $\|\Delta\|_2$ value is also shown for each sampling case.

Noise	1%			3.5%		
Protocol	1	2	3	1	2	3
n_T	1 (0.4%)	1 (0.3%)	0.973 (0.2%)	1.003 (1.2%)	1.001 (1.4%)	0.958 (4.5%)
V_P	1.001 (1.4%)	0.995 (1.2%)	1.231 (17.5%)	0.999 (4.7%)	0.995 (5.1%)	1.190 (46.3%)

Table 5.04. The distinction between the stability of the identified parameters

It was evident that although Protocols 2 and 3 contain the same number of samples, the resolution of the identified parameters was considerably reduced in Protocol 3. In effect, $\overline{V_P}$ was un-identifiable with Protocol 3. It bears reiterating that this result occurs because of the lack of

distinction in the coefficients of the parameters at the sample times as indicated in Table 5.03 and Figure 5.03.

Thus, the method predicted the poor performance of the third Protocol, while it predicted much lower variability for both Protocols 1 and 2. However, it also suggests that Protocol 2 would improve slightly upon Protocol 1, which was not the case as both protocols performed equally in terms of parameter identifiability. It is suspected that the equality of variance is an artefact of the normalisation as a function of mean coefficient at the sample value, artificially lowering the magnitude of the $\|\Delta\|_2$ terms in Protocol 1.

Importantly, these findings highlight the inefficiency and extreme clinical burden and intensity of frequent sampling in contrast to well-positioned and infrequent sample timing. More specifically, Protocol 1 used nine more samples (225%) than Protocol 2 with significant added clinical intensity and assay cost (225% more!) for absolutely no information gain. This outcome was successfully predicted and easily illustrated by the integral-based identifiability analysis method presented here.

5.4 Analysis of the DISST glucose PD model

Equation 4.22 was used in the analysis of identifiability of terms frequently used to model the PDs of insulin and glucose. All analyses in this section simulated insulin concentration profiles for the plasma and interstitium only once in each Monte Carlo analysis. Thus for clarity and simplicity, it was assumed that insulin is not subject to assay error in Section 5.4. Furthermore, while glucose assay error from a blood gas analyser is approximately 2%, errors of 1% and 3.5% were used for consistency with Section 5.3. As such, the resultant coefficients of variation should not be considered fully applicable clinically, but merely as an indication of parameter trade-off during identification for a range of clinically valid assay errors. The Monte Carlo analysis method with the NGT participant described in Section 5.2.3 was repeated for the glucose PD model. The IGT participant was used in tandem from Section 5.4.2.

5.4.1 Insulin sensitivity and distribution volume

Use of the DISST model typically entails the identification of SI and V_G in Equation 4.22 (Docherty *et al*. 2009; Lotz *et al*. 2008; Lotz *et al*. 2010). Figure 5.04 and Table 5.05 indicate that these parameters are uniquely identifiable in the presence of measurement noise given a

surprisingly small number of data points. Three sampling protocols were used in this analysis. Specifically, Protocol 1 used the 5-minute sampling resolution described in Section 5.2.4, while Protocols 2 and 3 used equally spaced 10 and 20-minute resolutions, respectively.
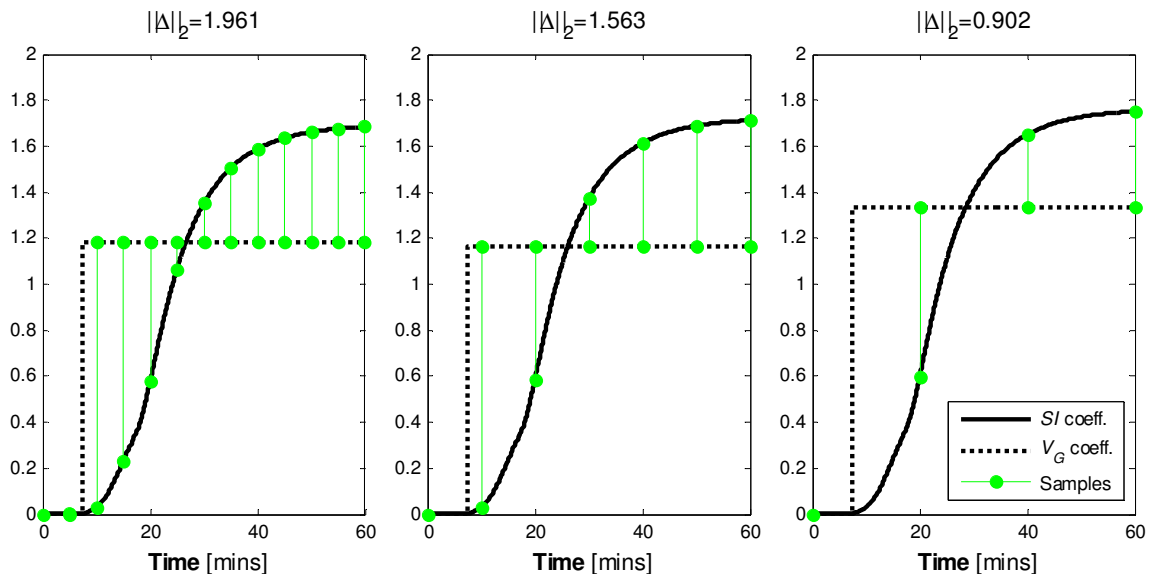
Figure 5.04. Distinction between the integral of the coefficients of the parameters of Equation 4.22 at different sampling protocols.

Table 5.05 shows that parameter stability is generally very high even in a sparsely sampled data set with a relatively high level of noise. This result was expected due to the relatively large difference in the coefficient integrals shown in Figure 5.04 for each of the sampling protocols. Thus, like the case of V_P and n_T, intelligent sample timing was proven to significantly reduce clinical burden and study cost with negligible loss of information. Furthermore, the method presented in this chapter successfully predicted and clearly illustrated this outcome.

Noise	1%			3.5%		
Protocol	1	2	3	1	2	3
SI	1.001 (0.7%)	1.002 (0.8%)	1 (1.6%)	1.002 (2.8%)	1.002 (3.3%)	1.010 (5.4%)
V_G	1 (1.2%)	1 (1.4%)	1.001 (2.9%)	0.998 (4.6%)	1.001 (4.9%)	0.990 (8.7%)

Table 5.05. Glucose PD identified parameter variation.

5.4.2 Insulin sensitivity and glucose dependent decay

Model-based studies of insulin sensitivity frequently identify p_G in addition to SI and V_G as variable parameters when using the Minimal Model (Bergman *et al*. 1979) or similar. However, numerous identifiability issues arise using the Minimal Model and the non-linear least squares identification methods. It has been reported that these issues can be exacerbated for insulin resistant (IR) individuals (Pillonetto *et al*. 2002; Quon *et al*. 1994b). Thus, the second, IGT participant defined in Table 5.01 was also analysed.

Some insight into the parameter trade-off during identification of dynamic test data can be seen in Figure 5.05, which contrasts the integral formulations of the parameter coefficients based on glucose tolerance status. The contrasting shape of the integral formulations of the parameter coefficients is best observed in the p_G coefficient.

The p_G coefficient is the only term in Equation 4.22 that could possibly have a negative coefficient. Thus, the integral of the coefficient can form a convex shape that contrasts well with the coefficient of SI, as seen for the NGT participant in Figure 5.05. However, the negative coefficient of p_G can only occur when the participant's glucose concentration goes below the basal concentration. Thus, as only NGT participants achieve such concentration reductions in typical dynamic insulin sensitivity tests, the parameter identifiability of IR participants is impaired in comparison.

In particular, Figure 5.05(right) shows minimal difference and a much smaller $\|\Delta\|_2$ value for this IGT individual indicating increasing potential for parameter trade-off in the identification process and an increasing potential loss of effective identifiability. As mentioned, this limitation of the Minimal Model has been well reported, but not explained in the literature until now.

Table 5.06 shows the identified parameter variation when the 60 minute 10-minute sampling protocol was used. It was apparent that the insulin resistant individual's parameter identifiability was much lower than the NGT participant despite the identical PD model, test protocol and identification process. Table 5.06 highlights this result, as well as the increasing loss of identifiability as assay error increases. These outcomes are in accordance to previously observed findings and the proposed method's prediction.
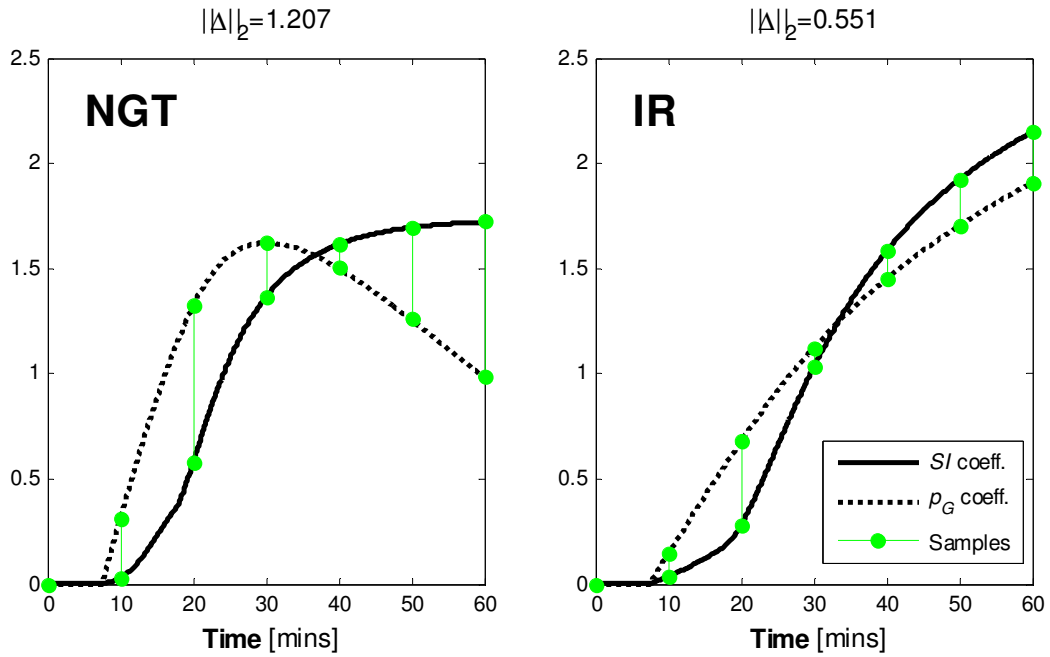
Figure 5.05. The disparity between the integral formulations of the coefficients of *SI* and p_G based on glucose tolerance status.

Noise	1%		3.5%	
Participant	NGT	IR	NGT	IR
SI	1 (0.8%)	1.001 (16.1%)	0.998 (3.2%)	0.876 (44.9%)
p_G	1.009 (32.0%)	0.959 (75.6%)	1.109 (80.2%)	1.573 (124.4%)

Table 5.06. Normalised mean values of *SI* and p_G and their coefficients of variation.

5.4.3 A hypothetical protocol to enable p_G identification in dynamic tests

To forcibly improve identifiability of the parameters describing the IR individual, the clinical protocol used in the analysis could be altered based using the proposed method to guide the alteration. After an initial observation of the effect of the insulin bolus on glucose concentration, more insulin could be introduced to ensure that the resistant test participants' glucose concentration is maintained at approximately 0.5 mmol/L below the basal concentration. Such a protocol may include an extension of the protocol described in Section 5.2.4 wherein a period of slight hypoglycaemia is achieved for each participant with a series of participant-specific insulin boluses or infusions administered with feedback control.

However, to allow a fair comparison between the variability of the model parameters of the proposed protocol and the protocol used in Section 5.4.2 the sampling regimen and test duration was maintained. Thus, the additional insulin was administered as a bolus at t=32.5 minutes to test this scenario *in-silico*. In a clinical setting, the magnitude of the bolus would be participant-specific and dependent on the glucose concentration response alone, as glucose can be assayed in real time.

Note that this task would be very difficult and potentially dangerous in a regular clinical setting but is useful in this analysis. In particular, the amount of insulin required would vary between participants, and must be estimated in ignorance of endogenous insulin production or clearance. This lack of knowledge may cause a high incidence of potentially harmful hypoglycaemia. Hence, this protocol is only mooted to illustrate the ability of the method, and in reality, the introduction of additional insulin would be applied slowly so that safety could be assured. Hence, although the method proposed here is not necessarily safe enough for clinical application, it is illustrative of a new approach of IVGTT protocols, which may increase the identifiability of Minimal Model parameters in the IR subgroup.

For the case of the particularly resistant IGT participant presented here, a 3U bolus at t=32.5 minutes was required to reduce glucose sufficiently. The proposed protocol altered the shapes of the integral of the parameter coefficients for the IGT individual. In doing so, it was hypothesised that it would increase the distinction between these curves to avoid the similarity seen in Figure 5.05(right) to ensure identifiability. The $\|\Delta\|_2$ value obtained for the IGT participant and the updated protocol indicates that the identified parameter variability would be approximately halved. Figure 5.06 and Table 5.07 show the results.
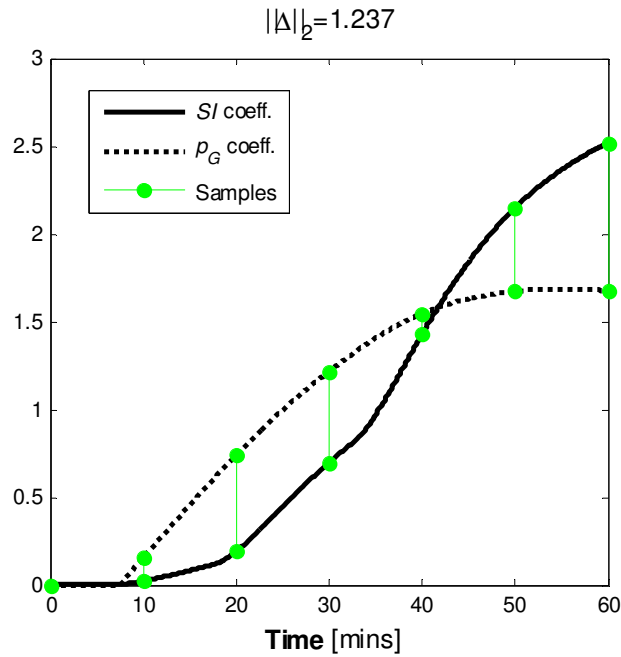
Figure 5.06. Effect of the alteration of the protocol on the parameter coefficients of the insulin resistant individual.

Noise	1%	3.5%
SI	1.002 (2.9%)	0.996 (7.0%)
ΔSI	+0.001 **(-13.2%)**	+0.120 **(-37.9%)**
p_G	0.989 (36.9%)	0.992 (96.9%)
Δp_G	+0.030 **(-38.7%)**	-0.581 **(-27.5%)**

Table 5.07. Parameter variability and (CV) from the hypothetical protocol. The bold values are indicative of the change afforded by the added insulin bolus. (ΔSI and Δp_G show the change between these values and those from the same individual presented in Section 5.4.2)

Although the very large, inhibitive CV values for some of the parameters indicate that the particular proposed protocol could not be used clinically, the parameter variability decreased in the order predicted by the presented identifiability method. Furthermore, the hypothetical protocol and *in-silico* analysis presented has confirmed the reasons for the poor parameter identification observed in many clinical studies that utilise these two competing model parameters (Pillonetto *et al.* 2002; Quon *et al.* 1994b). In addition, it has demonstrated a serious limitation of traditional identifiability methods, which provide a false confirmation of identifiability in ignorance of probable participant behaviour or protocol design.

The distinction of the parameter coefficients of the IGT participant during the modified protocol was similar to the distinction of the sensitive participant of the standard protocol. Thus, the identifiability of the parameters is compared across these tests. Table 5.08 shows that the difference in parameter identifiability was minimal.

Noise	1%	3.5%
ΔSI	+0.002 (+2.1%)	-0.002 (+4.8%)
Δp_G	-0.020 (+4.9%)	-0.117 (+16.9%)

Table 5.08. The difference between the mean values and coefficients of variation of the IR participant of the modified protocol and the NGT participant of the standard protocol.

The protocol presented in this section would be virtually impossible to apply clinically in the 60-minute duration, as it was described here. However, this analysis was limited by the need for a consistent duration and sampling regimen to Section 5.4.2. More importantly, this analysis shows that the method could thus be used to define similar protocols that could yield data that enables unique identification of these parameters.

More practically, if protocols like the one proposed here for IR individuals are pursued, they would most likely require two sections in a longer test. The first section would involve the protocol defined in Section 5.2.4, and would be followed immediately by an infusion of insulin designed to safely bring the participant's glucose concentration to 0.5 mmol/L below the basal level. Robust results would be most assured if the participant's glucose concentration was maintained slightly below basal for approximately 30 minutes, and thus, the protocol would most likely require about 2 hours. However, a stable result in terms of both SI and p_G would be generally assured.

5.4.4 Removal of redundant points

The $t=40$ minute samples in Figure 5.05(left) and $t=30$ in Figure 5.05(right) show virtually no distinction between the coefficients of either profile. Thus, according to the theory presented, it should provide no added benefit to the identification process. To test this hypothesis, the analysis of Section 5.4.2 was repeated with these samples removed. The identified values and the differences between this analysis and that of Section 5.4.2 are given in Table 5.09, while the distinctions in the coefficients are shown in shown in Figure 5.07.
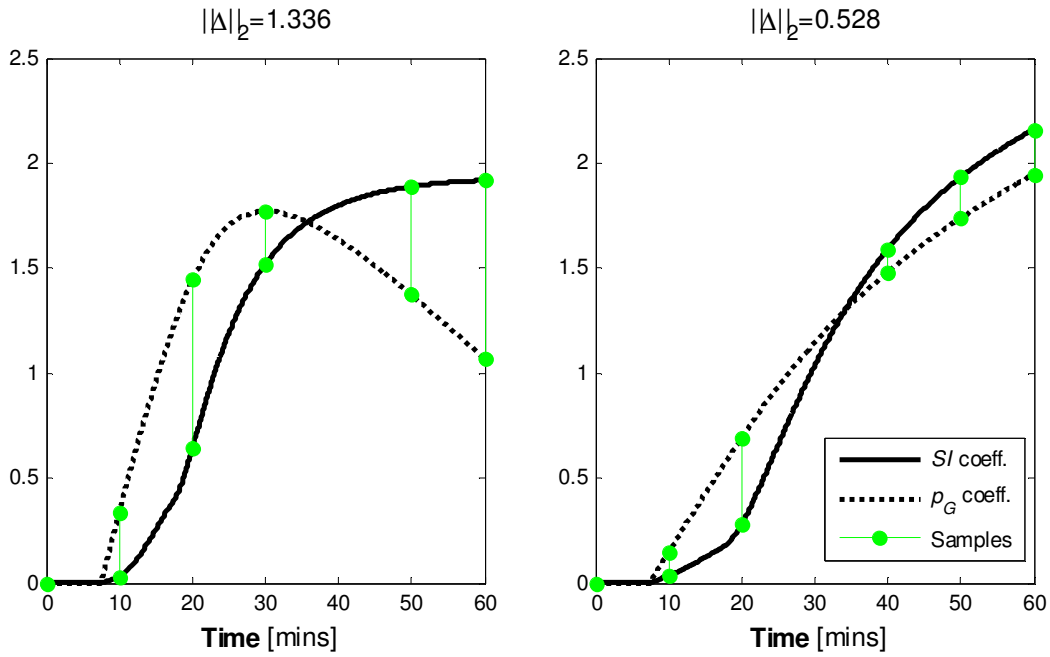
Figure 5.07. The coefficient comparison in an alternative sampling protocols which omits samples at t=40 and 30 minutes for the NGT and IGT participants, respectively, which according to Figure 5.05 have a negligible value due to the small differences.

Noise	1%		3.5%	
Participant	NGT	IR	NGT	IR
SI	0.999 (1.0%)	0.960 (18.2%)	1 (2.9%)	0.852 (49.6%)
ΔSI	-0.001 **(+0.2%)**	-0.041 **(+2.1%)**	+0.002 **(-0.3%)**	-0.024 **(+4.7%)**
p_G	1.019 (36.0%)	1.152 (69.3%)	1.400 (76.2%)	1.231 (115.6%)
Δp_G	+0.010 **(+4.0%)**	+0.193 **(-0.3%)**	+0.311 **(-4.0%)**	+0.342 **(-8.8%)**

Table 5.09. Normalised mean values of identified parameters and their coefficients of variation with the omission of assumed negligible data points. The bold values are most indicative of the changes in parameter identifiability. (ΔSI and Δp_G is the change in values between this analysis and Section 5.4.2)

The findings of this analysis imply that the omission of the samples that were assumed redundant, or of little value, did in fact, have little effect on the outcome of the identification process. Most changes were very small and only those for the particularly un-stable model parameters showed any noticeable changes. Hence, it is clear that this method could be used to define sample times and protocols for maximum identifiability and accuracy while minimising clinical burden.

5.5 The value of μ

The value of μ in Equation 5.05 could be used to enable prediction of the probable variability in the identified parameters in a Monte Carlo simulation. Thus, the effects of protocol changes on parameter identifiability could be predicted without the need for numerous Monte Carlo simulations. To identify the value of μ, linear relationships between the CV values obtained and the $\varepsilon/\|\Delta\|_2$ values were defined. As noiseless identifiability of all models has been shown. Thus, the y-intercept can be assumed zero, and the μ value was identified using Equation 5.09:

$$\mu = \frac{1}{N} \sum \frac{CV}{\varepsilon/\|\Delta\|_2} \qquad\qquad 5.09$$

Figure 5.08 shows the adherence of μ to linear relationships while Table 5.10 shows the value of μ for the different identified parameters.

Analysis	Parameter	μ
Section 5.3.2	n_L	0.560
	n_K	0.801
Section 5.3.3	n_T	0.081
	V_P	0.683
Section 5.4.1	SI	1.483
	V_G	2.408
Sections 5.4.2 to 5.4.4 (incl.)	SI	6.435
	p_G	26.00

Table 5.10. Values for μ from the various analyses
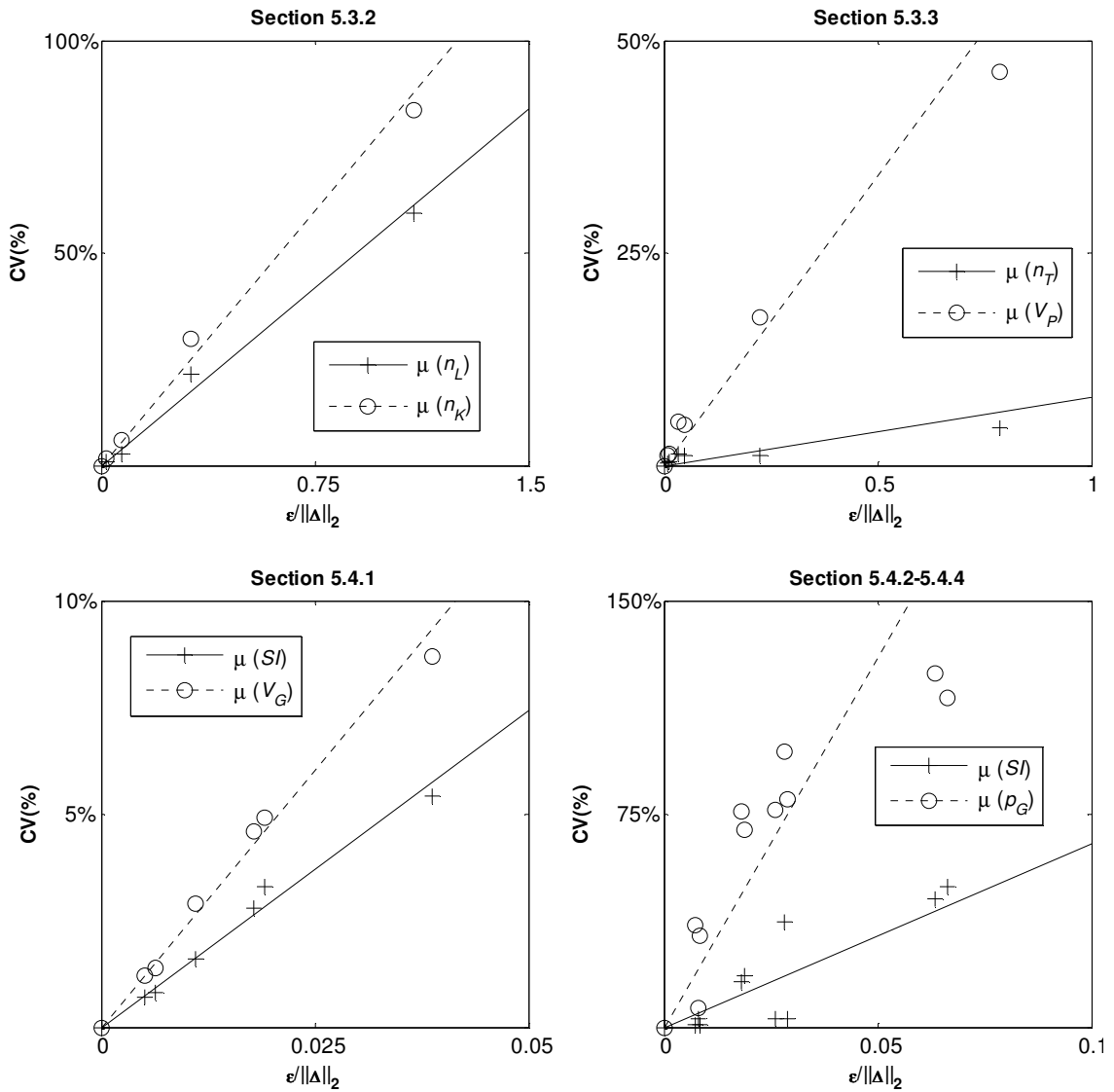
Figure 5.08. Comparison between CV values and the $\varepsilon/\|\Delta\|_2$ term to provide parameter specific values for μ.

It can be observed that no single value for μ could be applied across all models and that different model parameters were considerably more susceptible to the distinction of the parameter coefficients. However, the general adherence to the linear relationships observed in most examples implied that the form of Equation 5.05 was accurate for this purpose, with the possible exception of *SI* in Sections 5.4.2 to 5.4.4.

5.6 Implications of the geometric identifiability analysis

The method presented was able to predict and explain the parameter identifiability behaviour exhibited in models of insulin kinetics and insulin/glucose dynamics. This capability is in direct contrast to the traditional, well-accepted, derivative-based identifiability analysis (Audoly *et al.* 2001; Audoly *et al.* 1998; Bellu *et al.* 2007; Chin & Chappell 2010; Pohjanpalo 1978) that can only provide a confirmation of an infinitesimal distinction of the model parameters in the governing equations. In addition to the ability to predict parameter identifiability, the method was shown to be able to aid sample selection and explain the non-identifiability of the p_G term when IR participants undertake common dynamic insulin sensitivity tests. It was also used to derive and justify a novel dynamic protocol to make this parameter more identifiable in this subgroup.

However, the method does have some notable limitations. In particular, it has only been demonstrated here in cases identifying two parameters. In reality, many models utilise more than two parameters to describe physiological kinetics and seek to identify all at once. It is expected that the proposed method will still be capable of predicting the identifiability of such models. However, more care must be taken to construct the coefficient integral formulations, as combinations of parameters may come into conflict. This task would require the contrast between the most deleterious combination of integral coefficients to be measured. However, this point was not explored in this study, as the goal was to introduce this overall approach, and only a limited number of parameters are involved.

Only cases with lineariable model parameters in terms of measured species were analysed. In reality, some model parameters are intrinsically linked and this method will not work. An example of linked parameters would be insulin sensitivity and insulin effect saturation which requires a Michaelis-Menten formulation (Docherty et al. 2010; Lin et al. 2011). In addition, some parameters effect remote, un-measured concentrations or masses and are thus not able to be identified with this method. For example, the n_C term in Equation 4.16 could not be evaluated for identifiability using this method without the inclusion of measurements of interstitial insulin (Sjostrand *et al.* 1999), which are difficult to clinically obtain, resulting in large errors. As such, this method should not replace the traditional identifiability analysis, but be used in tandem with it to produce both theoretical and practical investigations of identifiability.

Furthermore, the method assumes that an expected range of parameter values is known prior to the commencement of the clinical study. This knowledge is important, as the method requires that species simulations are available to define the coefficients of the parameters. However, in most cases, the researcher will be able to obtain an indication of the likely range of parameter

values in a cohort from a brief literature search, and likely test outcomes could be cases that can be safely evaluated prior to clinical testing.

There is also an assumption that the model captures all of the dynamics of the system perfectly. In reality, no model can provide such accuracy. In particular, Figures 5.04 to 5.07 show a sample taken at $t=10$ for glucose. Practically, this sample may be affected heavily by mixing-induced error (Caumo *et al*. 1999; De Gaetano & Arino 2000; Edsberg *et al*. 1987). Although the method presented indicates that this is a valuable sample, if it is used in the glucose pharmacodynamic model of Equation 4.22, the resultant parameters will be overly influenced by the un-modelled mixing effect (Lotz 2007).

Although the method has limitations and potential for improvement, it can currently provide valuable information at a study design stage, as well as valuable identifiability information not available from typical methods. It can differentiate between the applicability of different dynamic tests based on cohorts, and also help to define optimal sample timing and frequency. In particular, it explained the observation of poor Minimal Model parameter convergence in insulin resistant participants that has been widely reported without clearly defining the cause. Thus, despite the method's limitations, it should still be used in the design stage of a study to ensure that the resultant clinical data can provide usable results, and time and money is not wasted.

Finally, the method has highlighted the limitation of discrete binary identifiability analyses as providing potentially misleading assurances of model parameter identifiability in real clinical applications. Importantly, the method has shown that identifiability is instead a continuous, analogous artefact of sample timing and the distinction between parameter coefficients.

5.7 Future work

It seems likely that some of limitations of the method could be overcome.

Initially, the case of three or more parameters could be potentially resolved with matrix algebra. The most deleterious combination of coefficients could be identified and used to quantify the contribution of μ. For example, consider three parameters a, b and c. The coefficient integrals of a, b and c (f_a, f_b and f_c, respectively) could be entered into matrix equations (Equations 5.10) to find the smallest possible distinction between a combination of parameters using differing parameter coefficient contributions (A, B, C).

$$[f_a \quad f_b]\begin{bmatrix} A \\ B \end{bmatrix} = [f_c] \qquad \text{or } [f_a \quad f_c]\begin{bmatrix} A \\ C \end{bmatrix} = [f_b] \qquad \text{or } [f_b \quad f_c]\begin{bmatrix} B \\ C \end{bmatrix} = [f_a]$$

$$5.10$$

Equation 5.05 could be evaluated with the combination of 5.10 that produces the lowest value of Equation 5.11.

$$min\|\Delta\|_2 = \Sigma\left([f_i \quad f_j]\begin{bmatrix} I \\ J \end{bmatrix} - [f_k]\right) \qquad 5.11$$

where: $min\|\Delta\|_2$ is the minimum distinction i, j and k can equal any combination of a, b or c.

However, this possibility has not been tested yet, and remains to be done.

Secondly, μ could potentially be defined prior to any Monte Carlo analyses if the nature of the samples contribution to the final variation is accurately defined. To define this value *a-priori*, the expected parameter values of indicative cases must be included in the initial analysis in some way. p_G, V_G, V_P and n_K all had a much higher variability than their paired parameters. This effect is likely to be an artefact of their reduced effect on their governing equations. Furthermore, the placement and distribution of error must be evaluated. Proportionally distributed error was placed on the *in-silico* data in accordance with assay manufacturer recommendations. However, the identification methods aim to minimise the absolute error. Improvement in predictability may also be possible with the refinement of the contribution of different species. This error was most apparent in the analysis of n_T and V_P.

Finally, the current form of the method can be used to asses many models, and is not necessarily limited to the field of physiology. Any model that has two parameters in terms of measureable species can be evaluated with the current method. In regards to insulin sensitivity, the method has explained the poor resolution of the Minimal Model for insulin resistant participants, and was used to simulate a new protocol which improved identifiability. The protocol was limited by the need to allow a fair comparison between tests, and thus could be improved significantly and identification of the Minimal Model in all types of participants could be achieved without the need for Bayesian techniques.

PART THREE: Clinical validation and applications

Chapter 6. DISST validation study

This chapter describes the validation investigation of the DISST that clinically measured the equivalence between the DISST and the euglycaemic clamp (EIC) metrics of insulin sensitivity. This investigation have been published by McAuley *et al.* (McAuley *et al.* 2011).

6.1 Study design

Clinical validations of new tests are usually completed by performing the test in a representative cohort using both the proposed method and the existing gold-standard method, and comparing the results. Thus, to validate the DISST test's ability to quantify insulin sensitivity, a clinical study measured the equivalence between the DISST values of insulin sensitivity and the values produced by the euglycaemic clamp. The participants also undertook the OGTT, as it is the simple diagnostic standard test. Ethics Approval for this study was granted by the Upper South Island Regional Ethics Committee B.

6.2 Participants

Fifty subjects were recruited under strict guidelines that ensured the study cohort's metabolic, anatomic and ethnic distribution was representative of the wider New Zealand community. Each participant undertook the DISST, the EIC and OGTT protocols within an 8-day period. The order of the participant's tests was assigned from a predetermined randomized list based on their order of recruitment. Insulin sensitivity metrics were derived from the DISST, EIC and OGTT data. Furthermore, the OGTT was used to determine and diagnose normal glucose tolerance, impaired glucose tolerance, or type 2 diabetes based on accepted clinical guidelines (ADA 2006,2010). Although sufficient data was obtained from the OGTT to identify a number of surrogate sensitivity metrics, only the Matsuda index (Matsuda & DeFronzo 1999) was derived. Upon the morning of their first test each participant was weighed, had their height measured and completed a brief questionnaire concerning family history of type 2 diabetes and personal medical history.

The method proposed by Hauschke (Hauschke *et al*. 1999) used the CV values of the DISST and EIC tests from the Lotz *et al*. Monte Carlo investigation (Lotz *et al*. 2008) to define the number of

participants required to adequately power the validation study. Thus, 50 participants were required for the validation study to have a power of 0.9.

The 50 participants were passively recruited from the Christchurch region of New Zealand in accordance with the conditions of the approved ethical consent. Participants were recruited using flyers and newspaper advertisements. Upon expression of interest, potential participants were given an information sheet that defined the clinical procedures and risks, and listed the benefits of the study. Participants signed informed consent prior to the commencement of any clinical procedure. Figure 6.01 shows examples of the flyers and advertisements.
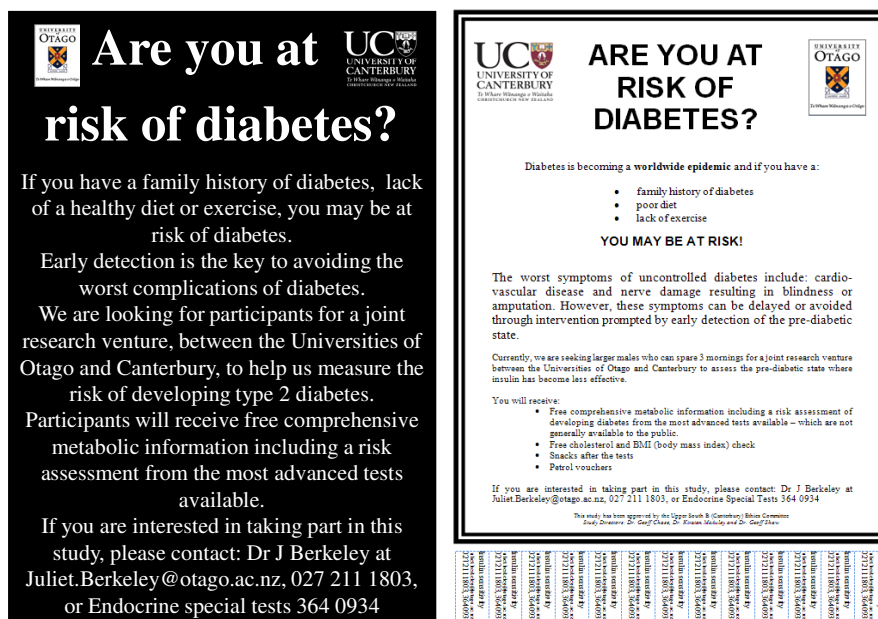
Figure 6.01. Newspaper advertisement for participants (L) and a flyer specifically targeting obese males (R).

To ensure that the study cohort was representative of the wider population, six subgroups were defined that ensured anatomical diversity. The subgroups were defined: five lean males (BMI<25), five lean females, 10 overweight males (BMI>25, <30), 10 overweight females, 10 obese males (BMI>30) and 10 obese females. The age range was 18 to 70 years and participants were omitted if they had any diagnosed major disease or illness (including known diabetes). The participant characteristics are listed in Table 6.01a-c, covering obese, overweight and lean participants respectively. Cohort characteristics are summarised in Table 6.01d. In total, the validation cohort included 33 New Zealand Europeans, 5 individuals with Maori decent, 2 Caucasian Australians, 1 Irish and 3 continental Europeans, 2 from the middle east, 1 Caucasian and 1 Hawaiian American, and a Korean.

Participant	Sex	Age [yrs]	Weight [kg]	BMI [kg/m²]	Fasting glucose [mmol/L]	Fasting insulin [mU/L]	NGT IGT* T2D*
6	F	46	84.3	30.7	4.5	5.9	NGT
12	F	45	76.5	30.4	5.1	11.0	NGT
15	F	35	78.3	30.1	4.7	8.6	NGT
18	F	28	125.3	45.6	4.8	23.2	NGT
19	F	45	110.4	42.4	4.8*	15.1	T2D
21	F	46	86.5	34.2	5.4	9.5	NGT
22	F	37	92.35	32.4	4.8	22.6	NGT
34	F	37	77.1	32.3	4.4	8.1	NGT
36	F	42	209.6	64.9	4.5	23.6	NGT
45	F	50	150.2	55.4	4.7	7.9	NGT
17	M	56	98.9	32.2	3.9	1.6	NGT
27	M	43	110.4	36.4	4.5	9.5	NGT
37	M	30	134.8	39.8	4.0	34.0	NGT
38	M	36	99.6	33.2	4.6	7.8	NGT
40	M	67	110.1	38.9	5.2	22.8	NGT
42	M	65	93.9	30.1	4.8*	7.8	IGT
44	M	42	101.2	35.3	4.7	22.8	NGT
47	M	69	112.9	35.1	5.3	12.8	NGT
48	M	65	115.9	36.4	5.2*	14.5	IGT
50	M	28	120.3	33.4	4.8	9.6	NGT
Q_1	10 M 10 F	36.5	89.4	32.3	4.5	8.0	17 NGT
Q_2		44	105.6	34.7	4.8	10.4	2 IGT
Q_3		53	118.1	39.4	5.0	22.7	1 T2D

Table 6.01a. Obese participants of the DISST validation study. (* according to the 2hr OGTT glucose criteria of the ADA)

Participant	Sex	Age [yrs]	Weight [kg]	BMI [kg/m²]	Fasting glucose [mmol/L]	Fasting insulin [mU/L]	NGT IGT* T2D*
9	F	60	66.3	25.8	3.7	3.7	NGT
10	F	54	66.4	26	4.2	5.5	NGT
24	F	60	65.3	25.1	4.8*	8.0	IGT
25	F	60	67.0	27.2	3.8	2.2	NGT
32	F	47	73.8	28.3	4.3	7.2.0	NGT
35	F	50	92.8	29.9	5.2	10.4	NGT
39	F	50	66.1	27.5	4.6	5.0	NGT
43	F	42	83.1	29.3	4.2	9.5.0	NGT
46	F	20	85.0	29.8	4.3	9.5	NGT
49	F	43	86.9	28.8	4.9	14.0	NGT
2	M	27	85.9	28.5	4.5	4.3	NGT
3	M	31	86.9	26.6	4.8	4.5	NGT
4	M	33	94.9	28.9	5.3	7.5	NGT
13	M	50	89.4	25.7	4.6	9.7	NGT
14	M	36	78.0	26.6	5.1	5.9.0	NGT
16	M	24	84.1	26.1	4.6	8.8	NGT
23	M	40	91.5	28	4.1	7.1	NGT
26	M	22	80.6	26.3	4.4	9.7	NGT
31	M	23	81.6	27.5	4.3	4.6	NGT
41	M	47	87.6	27.7	4.3*	17.0	IGT
Q₁	10 M 10 F	29	70.4	26.2	4.2	4.8	18 NGT 2 IGT
Q₂		42.5	83.6	27.5	4.5	7.4	
Q₃		50	87.3	28.7	4.8	9.6	

Table 6.01b. Overweight participants of the DISST validation study. (* according to the 2hr OGTT glucose criteria of the ADA)

Participant	Sex	Age [yrs]	Weight [kg]	BMI [kg/m²]	Fasting glucose [mmol/L]	Fasting insulin [mU/L]	NGT IGT* T2D*
5	F	34	62.4	21.3	4.2	3.9	NGT
7	F	45	59.0	22.0	4.8	5.2	NGT
11	F	29	55.5	19.8	4.6	13.7	NGT
20	F	25	63.2	22.4	4.4	4.5	NGT
33	F	27	66.8	24	4.4	2.6	NGT
1	M	21	62.3	19	3.8	5.3	NGT
8	M	29	72.0	21.3	4.3	3.6	NGT
28	M	30	56.9	19.1	4.1	2.9	NGT
29	M	25	73.2	23.2	4.4	2.3	NGT
30	M	25	77.0	22.7	3.6	1.4	NGT
Q_1		25	59.0	19.8	4.1	2.6	
Q_2	5 M 5 F	28	62.8	21.7	4.3	3.8	10 NGT
Q_3		30	72.0	22.7	4.4	5.2	

Table 6.01c. Lean participants of the DISST validation study cohort. (* according to the 2hr OGTT glucose criteria of the ADA)

Participant	Sex	Age [yrs]	Weight [kg]	BMI [kg/m²]	Fasting glucose [mmol/L]	Fasting insulin [mU/L]	NGT IGT* T2D*
Q_1		29	72.0	25.8	4.3	4.6	45 NGT
Q_2	25 M 25 F	41	84.7	28.7	4.6	8.0	4 IGT
Q_3		50	98.9	33.2	4.8	11.2	1 T2D

Table 6.01d. Summary quartiles of the whole validation study cohort. (* according to the 2hr OGTT glucose criteria of the ADA)

6.3 Test protocols

6.3.1 DISST protocol

Participants reported to the place of testing in the morning after an over-night fast and were seated in a relaxed position for the duration of the test. A cannula was placed the antecubital fossa (large vein in the inner elbow). A three-way tap was connected to the cannula to facilitate the extraction of 'dead-space' during sampling and enable flushing of boluses. A fasting blood sample was obtained followed by a 10g glucose bolus (50% dextrose, although after Participant 20, 25% dextrose was used to avoid the incidence of pain caused by the concentration of the glucose). Further samples were taken at 5 and 10 minutes after the glucose bolus. A 1U insulin bolus (actrapid, measured out using an insulin pen) was administered immediately after this sample. Further samples were taken 10 and 20 minutes after the insulin bolus. Due to the occasional difficulty in maintaining a free flowing cannula, some blood samples were not taken precisely at the time defined by the protocol. The timing of samples in a dynamic test is critical to the accuracy of the resultant metrics. As such, a timer devise was used to record the actual times of samples and boluses.

A purpose built timer program was installed on a palm-held computer with a purpose-built software package installed. The program displayed a large countdown timer for each scheduled sample. The clinician recorded the actual times (at 1-second resolution) that the samples and boluses were performed by pressing a single button. The software was encoded in visual basic studio (.NET).

6.3.2 EIC protocol

Participants attended the place of testing in the morning after an over-night fast and were seated in a relaxed position for the duration of the test. Two cannula were placed in the antecubital fossa (vein in the inner elbow) and retrogradly (toward fingers) into any vein in the dorsum (back) of the hand. The retrograde direction of the dorsum cannula is designed to gain proximity to arterialized blood. Participants placed their hands into a heated-hand-box (Figure 6.02) that was designed to re-circulate warmed air around the back of their hand. The re-circulated air was temperature controlled at 60°C ±1°C although some participants requested a lower temperature of 50°C. This heating was intended to mobilize the arterial blood and ensure the samples obtained

from the second cannula more representative of the interstitial regions of the body. The cannula in the arm was attached to glucose and insulin feeds. The glucose infusion was a solution with 25% dextrose. A 50 ml syringe was made up of 49 ml of saline, 1 ml of the participants own blood and an amount of insulin defined by Equation 6.01. (The participant's blood reduced the binding of insulin to the syringe wall.)
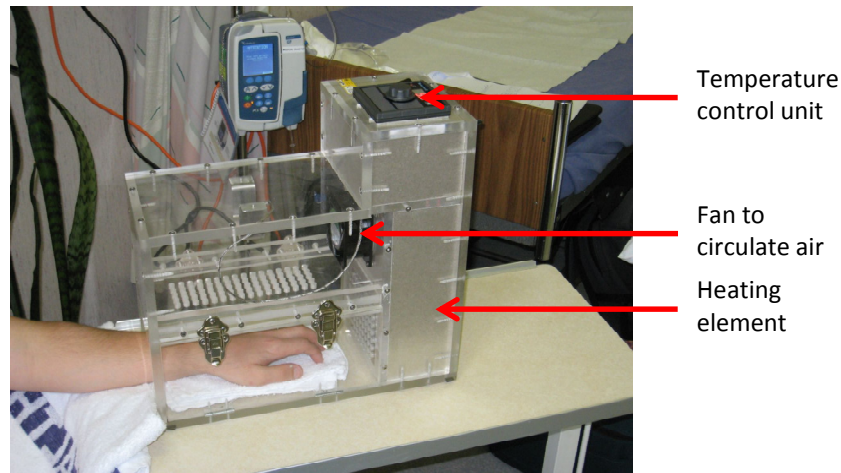
Figure 6.02. Heated hand box

$$Insulin(units) = 8 * BSA = \frac{8}{60} * \sqrt{BW(kg) * height(m)} \qquad 6.01$$

Both infusions were applied with rate-controlled pumps. Initially, a basal fasting blood sample was obtained and assayed for glucose, and then frozen and stored for later insulin assay. If the participants fasting glucose was between 4 to 5 mmol/L their target glucose was equal to their fasting glucose. However, if it was outside this range, a value of 4.5 mmol/L was used.

A priming insulin solution infusion rate of 80mL/hr was started at t=0 minutes. At t=2 minutes this was reduced to 40 mL/hr, and at t=4 minutes it was finally reduced to 20 ml/hr for the remainder of the trial. This infusion rate was designed to increase and maintain the participant's insulin concentration at approximately 100 mU/L in order to suppress the participant's endogenous insulin and glucose production (DeFronzo *et al*. 1979). The glucose infusion started at t=4 minutes and usually started at an ml/hr rate equal to the participants weight in kilograms. Blood samples were taken at 5-minute intervals and assayed at the 'bedside' for glucose. Feedback control was used to maintain the participants' blood glucose concentration at the predetermined target concentration by varying the glucose infusion rate.

The blood samples taken at t=60, 80, 100, 120 and 140 minutes were spun and frozen for later insulin assays. The maximum test duration was 150 minutes. All assayed glucose concentrations and glucose infusion rates were recorded.

6.3.3 OGTT protocol

Participants attended the place of testing on the morning after an overnight fast. Participants remained in a relaxed, seated position for the duration of the test. A cannula was placed in the antecubital fossa (large vein in the inner elbow) to enable blood samples. A fasting sample was taken and was followed closely by the oral consumption of the glucose drink. The drink contained 75 grams of glucose, was lightly carbonated, and had an overall volume of 250 ml. Further blood samples were taken at t=30, 60 and 120 minutes after the basal sample. All samples were assayed for glucose at the bedside, spun and frozen for later insulin assays.

Current medical practice guidelines state that a 120-minute glucose assay greater than 11.1 mmol/L can be used to diagnose diabetes, while 120-minute values over 7.8 mmol/L imply impaired glucose tolerance (ADA 2006,2010).

6.3.4 Assay techniques

Glucose assays were analyzed using YSI 2300 stat plus Glucose and L-Lactate analyzer using whole blood. These were converted to plasma glucose, using Equation 6.02. Samples for insulin and C-peptide were separated immediately and frozen. Measurements of insulin were undertaken by the Endolab, Canterbury Health Laboratories using Roche Elecsys® after PEG precipitation of immunoglobulins (Roche Diagnostics, Mannheim, Germany). 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 (Assmann *et al*. 1983).

$$G_{plasma} = \frac{G_{wholebloodglucose}}{2.4 \times 10^{-3} * haematocrit(\%)} \qquad 6.02$$

6.4 Parameter identification methods

6.4.1 DISST parameter identification

Glucose, insulin and C-peptide data obtained during the DISST tests were analysed using the same methods used to identify the model parameters of the pilot investigation. Thus, the methods described in Section 4.4 are generally relevant to this investigation. However, due to the increased timing resolution and reduced number of samples two changes to the identification process were necessary. The method was adapted to analyse data in terms of seconds, not minutes. Furthermore, there was not sufficient data to produce a unique double exponential estimation of the insulin decay. Thus, a single exponential with an offset, positive steady-state condition was used as an approximation.

The EIC metric of insulin sensitivity is typically presented in units of 10^{-2}mg·kg^{-1}·(mU/L)$^{-1}$·min^{-1}. Thus, the DISST *SI* metrics were transformed to gain equivalence. Equation 6.03 was used to transform the identified DISST value into EIC units. The equation converts the DISST *SI* from mmol/L to mg/kg and also plasma to interstitial insulin concentrations.

where: $$ISI_{DISST} = SI_{DISST} * \frac{V_G}{BW} * 18000 * \gamma \qquad\qquad 6.03$$

Where: V_G is the identified glucose distribution volume [L], *BW* is the participant bodyweight [kg], and γ is the steadystate ratio between plasma and interstitial insulin (0.5) (Barrett *et al.* 2009).

6.4.2 EIC insulin sensitivity identification

EIC metrics are generally reported as the rate of glucose uptake normalised by bodyweight (*M*) with the plasma insulin concentration reported at a cohort level. Other studies report the normalised rate of glucose uptake divided by the plasma insulin concentration (*I*) at an individual level. The latter method is considered more accurate. Further accuracy can be achieved by modifying *M*/*I* by a space correction (*SC*) which measures the change in glucose concentration during the identification period of the clamp protocol.

For this validation study, *M*/*I* with *SC* (Equation 6.04 and 6.05) was used with measurements from the final 40 minutes of each trial to define *ISI*. The mean glucose infusion rate over this

period is calculated and divided by the participant's body weight and plasma insulin assays to produce an *M/I* value. The EIC insulin sensitivity index (*ISI_{40}*) is calculated:

$$ISI_{40} = 100\frac{M_{40}}{\overline{I_{40}}} + SC_{40} = 100\frac{P_{X40}}{\overline{I_{40}}*BW} + SC_{40} \qquad 6.04$$

where:

$$SC_{40} = \Delta G_{40}\frac{1.9}{2.2} \qquad 6.05$$

where: M_{40} and P_{X40} are the normalised and average rates of glucose infusion over the final 40 minutes of the test [mg·kg^{-1}·min^{-1}] and [mg/min], respectively; $\overline{I_{40}}$ is the mean insulin value during the final 40 minutes of the test [mU/L]; SC_{40} is the space correction that accounts for the change in glucose during the identification period of the test [10^{-2}*mg·kg^{-1}·(mU/L)$^{-1}$·min^{-1}] and *BW* is the participants bodyweight [kg].

6.4.3 OGTT surrogate sensitivity identification

Data from the OGTT was used to define the Matsuda and HOMA indices of insulin sensitivity (Equations 2.04 and 2.09, respectively) and define the participant's diabetic status (Tables 6.01a-d).

$$ISI_{Matsuda} = \frac{10000}{\sqrt{G_B I_B \bar{G}\bar{I}}} \qquad 2.04$$

$$HOMA_{SI} = \frac{22.5}{G_B I_B} \qquad 2.09$$

6.5 Study outcomes

6.5.1 Comparison across metrics

Figures 6.03, 6.04 and 6.05 show the correlation, Bland-Altman and ROC curve representations of the equivalence between *ISI_{DISST}* and *ISI_{40}*, respectively. The ROC curve was evaluated with a cut-off value of 6.94×10^{-2} mg·kg^{-1}·(mU/L)$^{-1}$·min^{-1} (10^{-2} mg·kg^{-1}·(pmol/L)$^{-1}$·min^{-1}) which defines a possible threshold of insulin resistance.
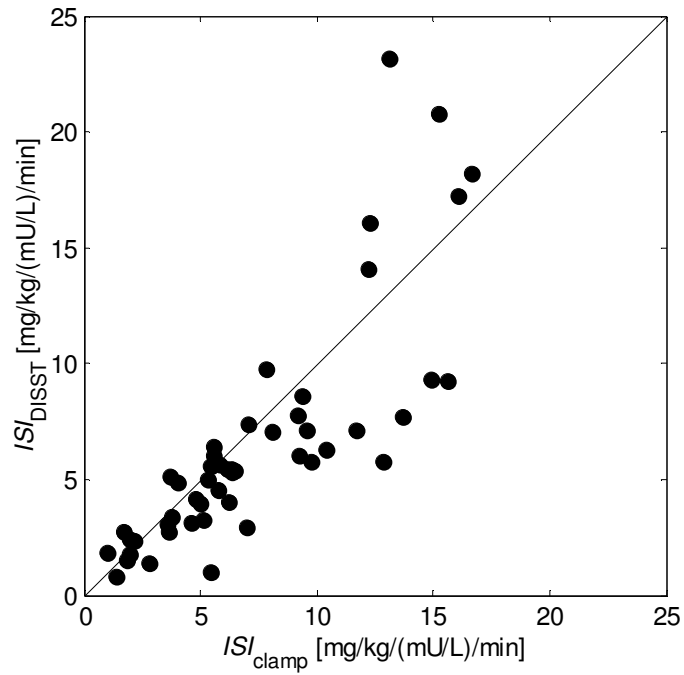
Figure 6.03. Correlation between the DISST and EIC insulin sensitivity values (N=50). (The diagonal line is the 1:1 line)
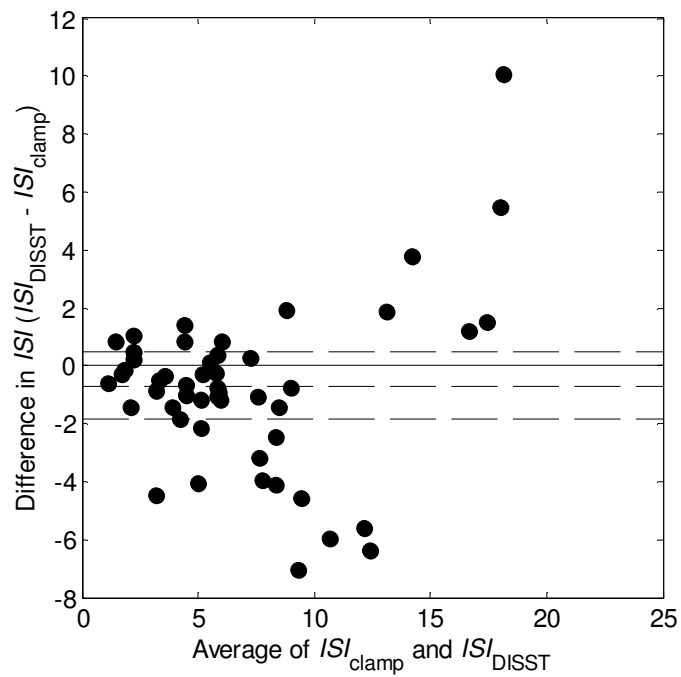
Figure 6.04. Bland Altman plot of the DISST and EIC sensitivity values.
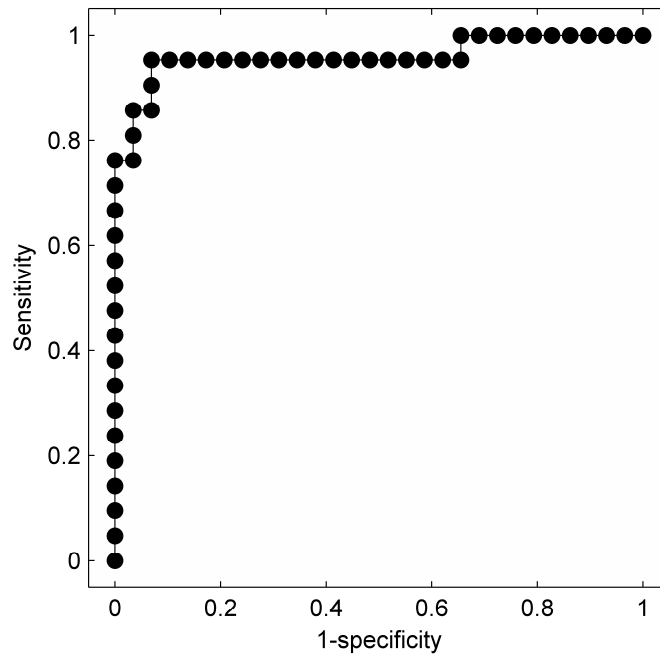
Figure 6.05. ROC curve using a diagnostic cut off value of $6.94 \times 10^{-2} mg \cdot kg^{-1} \cdot (mU/L)^{-1} \cdot min^{-1}$ on the EIC test

The correlation between the DISST and EIC in Figure 6.03 was R=0.82. This is considered a particularly strong correlation for such tests of a clinical nature. The Bland-Altman plot in Figure 6.04 showed a median sensitivity shift between metrics of -10.6% (IQR -26.8% to 7.0%). The ROC curve in Figure 6.05 contains a relatively large area (c=0.96), which indicates a particularly strong ability of the DISST protocol to predict the same diagnosis of insulin resistance that the would predict EIC predicts. This result is an artefact of the increased equivalence between the test metrics values around the arbitrarily chosen cut-off value for the ROC analysis.

Figures 6.06 and 6.07 and Table 6.02 show the performances of the Matsuda and HOMA metrics for insulin sensitivity in comparison to the EIC and DISST test values. The HOMA and Matsuda results were within the ranges reported in the literature (Table 2.02). In particular, the Matsuda results were close to the top of the reported range.

	EIC R (c-ROC)	Matsuda R (c-ROC)	HOMA R (c-ROC)
DISST	0.82 (0.96)	0.56 (0.93)	0.56 (0.83)
HOMA	0.60 (0.92)	0.93 (0.99)	-
Matsuda	0.74 (0.95)	-	-

Table 6.02. Pearson correlations and c-ROC values between the sensitivity metrics derived during the DISST validation study
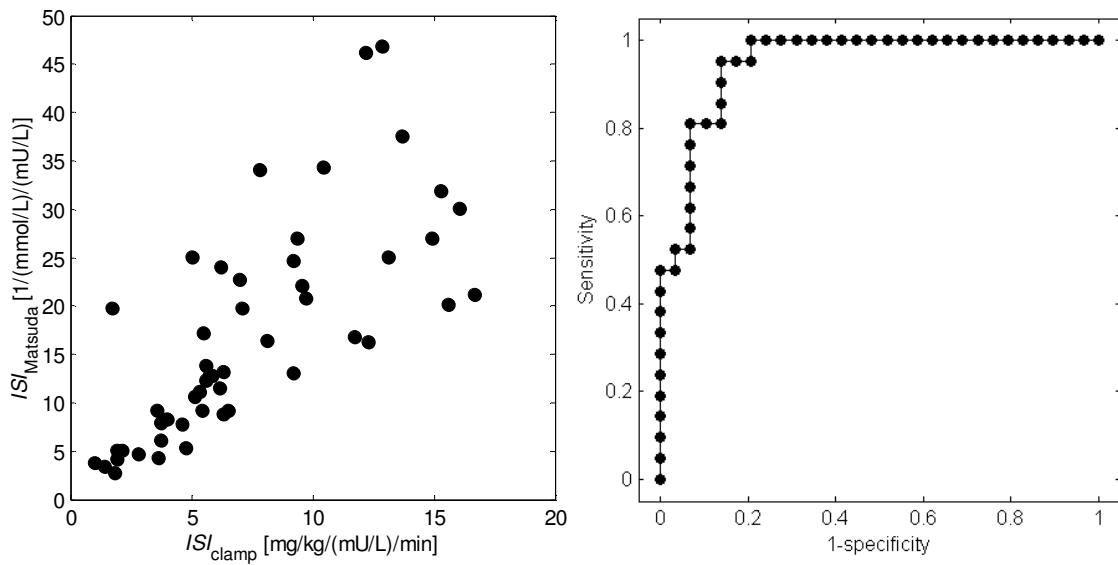
Figure 6.06. Comparison between the Matsuda index and EIC sensitivity values
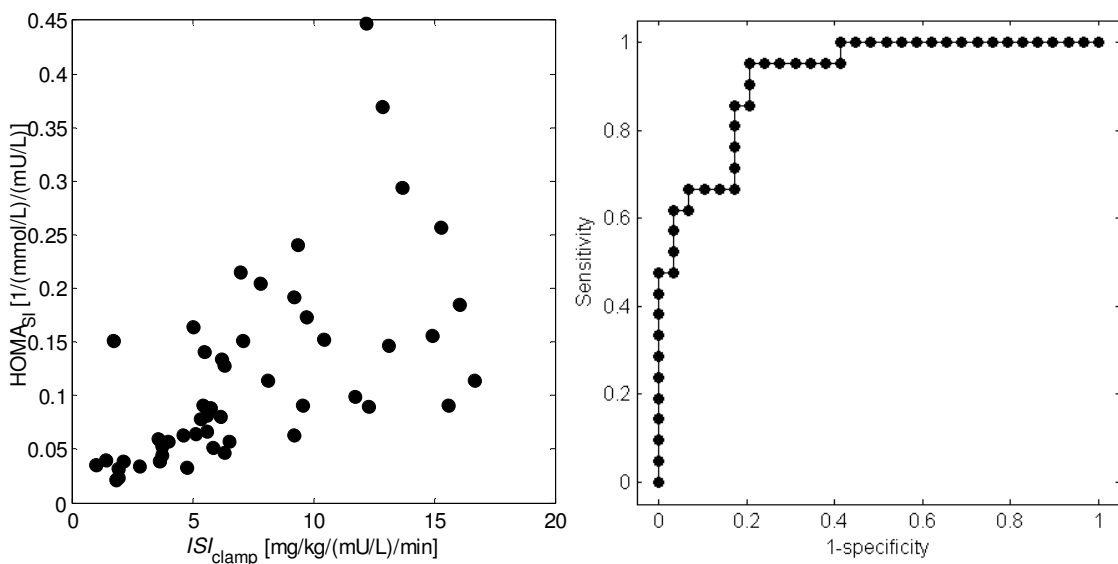
Figure 6.07. Comparison between the HOMA sensitivity index ($1/HOMA_{IR}$) and the EIC insulin sensitivity index.

6.5.2 Grouped participant results

The grouped results of the 50 DISST validation cohort participants are presented in Tables 6.03 to 6.05. These tables show the ability of each metric to discriminate between groups. Table 6.03 looks at insulin sensitivity. Table 6.04 makes the same comparison using insulin production metrics. Finally, Table 6.05 uses other clinically relevant blood analytes. The p-values are defined with Student's t-test between the lean and obese subgroups.

Insulin sensitivity values from the analysed tests

		total (N=50)	Lean (N=10)	Overweight (N=20)	Obese (N=20)	NGT (N=45)	IGT (N=5)	$p_{Lean-Obese}$
SI_{DISST} [10^{-4}·L·mU^{-1}·min^{-1}]	Q_1	4.95	6.17	6.58	3.16	5.44	2.34	$<10^{-3}$
	Q_2	7.67	13.99	8.13	5.06	8.00	4.14	
	Q_3	10.25	21.67	10.17	7.83	10.78	6.27	
ISI_{EIC} [10^{-2}·mg^{-1}·kg^{-1}·(mU/L)$^{-1}$·min^{-1}]	Q_1	4.01	9.73	5.46	1.95	4.74	1.30	$<10^{-5}$
	Q_2	6.19	14.02	6.40	4.17	6.34	3.61	
	Q_3	9.73	15.61	9.29	6.02	10.75	5.55	
$ISI_{Matsuda}$ [10^{-4}·(mmol/L)$^{-1}$·(mU/L)$^{-1}$]	Q_1	8.26	21.19	9.22	4.89	9.07	3.72	$<10^{-3}$
	Q_2	13.50	25.06	15.08	9.01	16.33	4.35	
	Q_3	24.01	30.05	24.35	12.58	24.78	14.41	
$HOMA_{SI}$ [22.5·(mmol/L)$^{-1}$·(pmol/L)$^{-1}$]	Q_1	0.36	1.02	0.42	0.26	0.40	0.26	0.0014
	Q_2	0.63	1.17	0.66	0.39	0.63	0.28	
	Q_3	1.06	1.49	1.02	0.55	1.10	0.64	

Table 6.03. Quartiles of insulin sensitivity values from the DISST validation cohort.

Table 6.03 shows that all of the sensitivity metrics obtained during the DISST validation study adequately discriminated between the lean and obese subgroups as expected. The low resolution of the HOMA metric caused the metric to have the least discriminatory power, while the normalisation of the EIC metric by participant bodyweight may have enhanced the p-value.

The basal and second phase insulin production showed a significant distinction between the lean and obese subgroups in Table 6.04. This could be expected by the overall metabolic trends of the subgroups. In particular, the more sensitive, lean participants would not require as much insulin to clear the standardised glucose stimulus used in the DISST test. The first phase insulin production metric did not show significant discriminatory power across subgroups. The hepatic insulin clearance rate also showed a significant distinction between subgroups. However, the first pass extraction was not group dependent. As expected, the trends showed that insulin production and clearance rates tended to cause more insulin to be available for the less sensitive, obese individuals. In contrast, sensitive participants tended to produce less insulin, and clear insulin relatively fast. This inter-dependence of insulin sensitivity, production and clearance rates are a key aspect in the development of the DISTq identification process described later in Section 8.2.

Insulin production and clearance during the DISST test

		total (N=50)	Lean (N=10)	Overweight (N=20)	Obese (N=20)	NGT (N=45)	IGT (N=5)	$p_{Lean-Obese}$
U_B [mU/min]	Q_1	21.5	16.6	22.8	34.7	21.1	32.4	$<10^{-4}$
	Q_2	28.8	17.3	26.9	46.8	27.6	38.2	
	Q_3	41.3	19.9	30.9	55.0	36.4	50.5	
U_1 [mU]	Q_1	580	384	643	568	582	477	0.16
	Q_2	775	556	816.7	725	798	655	
	Q_3	1101	909	1123	1311	1110	981.5	
U_2 [mU]	Q_1	539	350	591	945	483	954	$<10^{-4}$
	Q_2	902	458	790	1332	868	1276	
	Q_3	1310	700	1088	1911	1304	2039	
U_{Total} [mU]	Q_1	1721	1177	1826	2087	1712	1874	$<10^{-3}$
	Q_2	2197	1401	2175	2875	2183	2636	
	Q_3	2864	1874	2618	3516	2819	3810	
n_L [1/min]	Q_1	0.073	0.083	0.091	0.067	0.075	0.065	0.004
	Q_2	0.104	0.135	0.114	0.086	0.108	0.086	
	Q_3	0.130	0.18	0.131	0.107	0.134	0.095	
x_L [1]	Q_1	0.624	0.685	0.624	0.621	0.623	0.626	0.31
	Q_2	0.674	0.716	0.659	0.649	0.663	0.688	
	Q_3	0.739	0.755	0.716	0.744	0.734	0.793	

Table 6.04. Insulin production and clearance parameters identified during the DISST validation study.

The blood analytes reported in Table 6.05 all also showed the expected trends. High cholesterol and triglycerides concentrations are considered unhealthy (Amato *et al*. 2010), and were highest in the obese subgroup. HDL cholesterol was lowest in the obese group, and is considered more healthy in high concentrations.

		Total (N=50)	Lean (N=10)	Overweight (N=20)	Obese (N=20)	NGT (N=45)	IGT (N=5)	$p_{Lean-Obese}$
HDL-C [mmol/L]	Q_1	1.0	1.2	1.1	1.0	1.1	0.9	0.019
	Q_2	1.2	1.4	1.3	1.2	1.3	1.0	
	Q_3	1.4	1.6	1.5	1.2	1.5	1.1	
Chol [mmol/L]	Q_1	4.3	3.6	4.5	4.7	4.4	3.0	0.014
	Q_2	5.1	4.0	5.2	5.1	5.0	5.5	
	Q_3	5.5	4.6	5.9	5.6	5.4	5.9	
TAG [mmol/L]	Q_1	0.8	0.6	0.8	1.0	0.8	1.0	0.016
	Q_2	1.0	0.8	1.0	1.3	1.0	1.2	
	Q_3	1.4	0.9	1.5	1.6	1.4	2.7	

Table 6.05. Cholesterol and triglyceride blood analytes from the DISST validation study cohort.

6.5.3 Individual results

The benefits of capturing the endogenous insulin production profile are best illustrated via individual results. Thus, nine illustrative cases are presented. Participants 48, 19, 40, 37 and 36 had the lowest insulin sensitivity scores of 1.006 to 1.942×10^{-2} mg·kg^{-1}·(pmol/L)$^{-1}$·min^{-1} according to the EIC. While the EIC indicates that these are all resistant individuals, no information is given as to the nature of the participant's resistance as the EIC cannot differentiate the β-cell function of these individuals. The DISST test responses of these participants are shown in Figures 6.08-6.12 in order of EIC sensitivity (1.006 to 1.942, respectively).
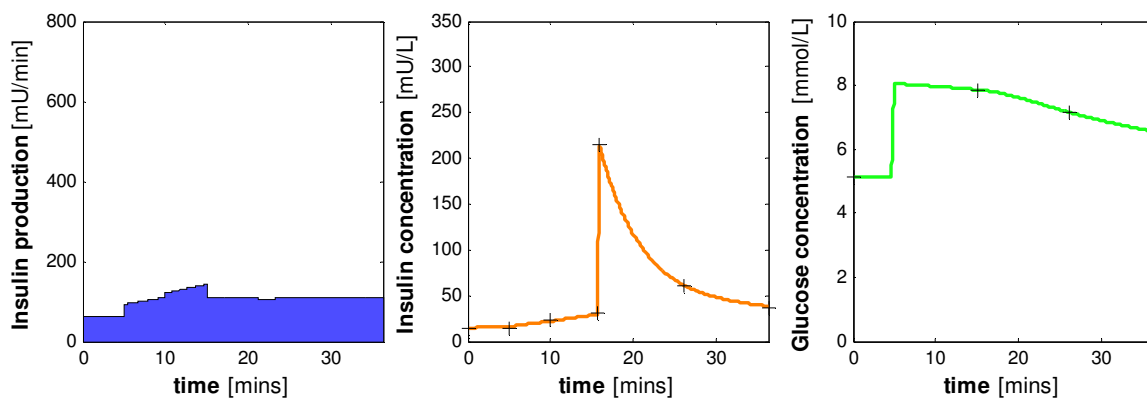
Figure 6.08. DISST test response of Participant 48
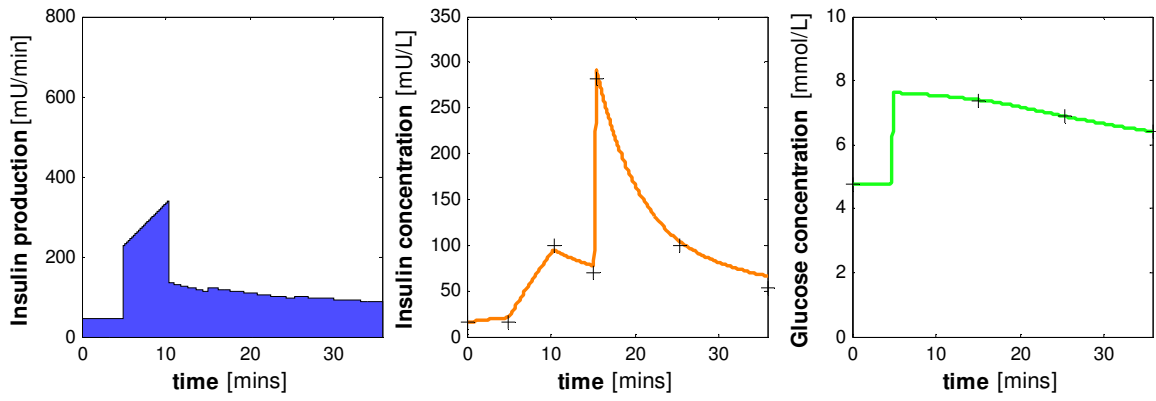
Figure 6.09. DISST test response of Participant 19
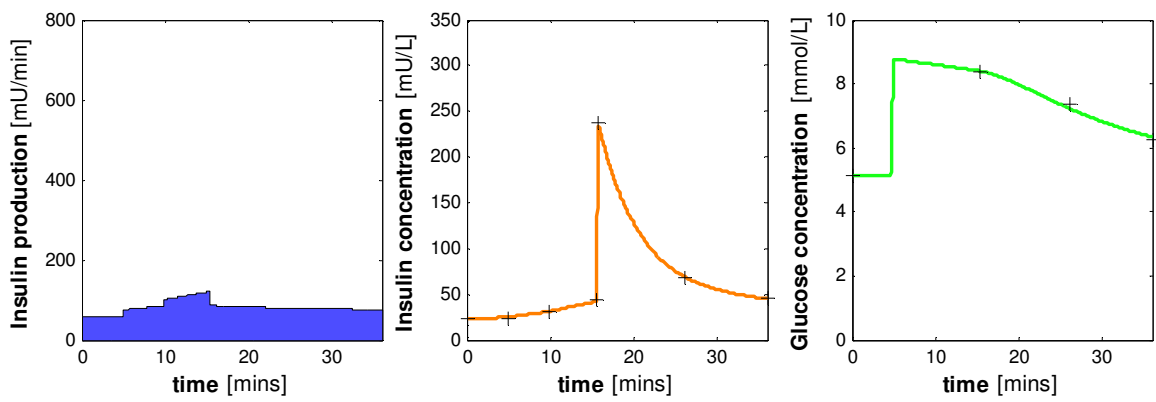
Figure 6.10. DISST test response of Participant 40
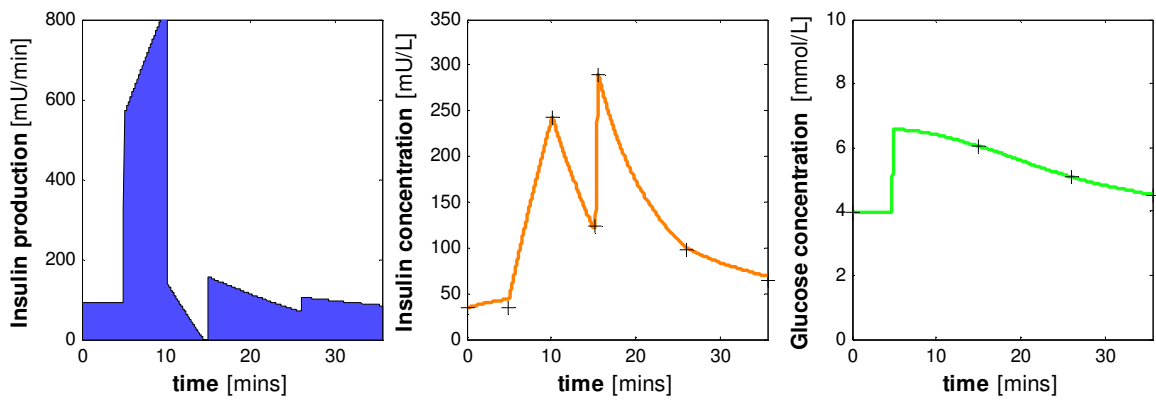
Figure 6.11. DISST test response of Participant 37
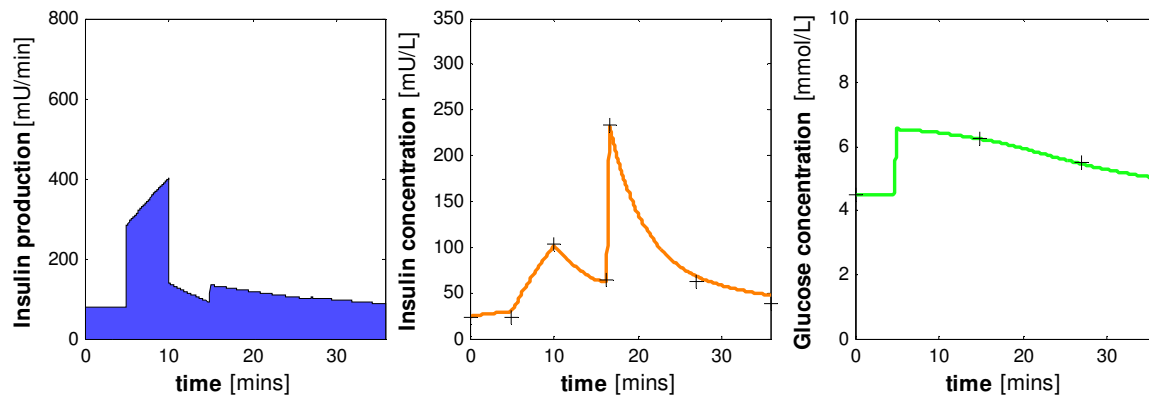
Figure 6.12. DISST test response of Participant 36

Figures 6.08-6.12 show how the physiology of the five cases can be contrasted. While the glucose concentrations of Participants 40 and 48 is increased there was no corresponding increase in the rate of insulin production and the glucose concentration of these participants did not return to basal within the duration of the test. Participants 19 and 36 exhibited a reasonable increase in insulin production with an elevated second phase of production. The first phase of insulin production of Participant 37 was very large and managed to bring the glucose concentration back to basal within the duration of the test despite the participant's significant insulin resistance. Thus, these participants had vastly differing physiological conditions and should potentially undergo differing interventional measures.

Four further cases (Participants 28, 46, 3 and 8) are presented in Figures 6.13 to 6.16. While the first two are interesting cases, the second two are indicative of normal and athletic individuals. Participant 28 had type 2 diabetes, but underwent a particularly extreme and intensive self-imposed intervention. According to the OGTT test taken during the validation study, the participant managed to reverse the diagnosis of diabetes. Participant 46 volunteered to participate in the validation study because she was concerned about the effect that a copious consumption of highly caffeinated, sugary energy drinks had on her metabolism. Her self-reported consumption was in the order of 5+ per day (approximately 1.5 litres per day). Participant 3 is representative of healthy, but relatively sedentary, individuals who undertake moderate amounts of exercise. Participant 8 is representative of individuals who live active lifestyles and regularly exercise vigorously.
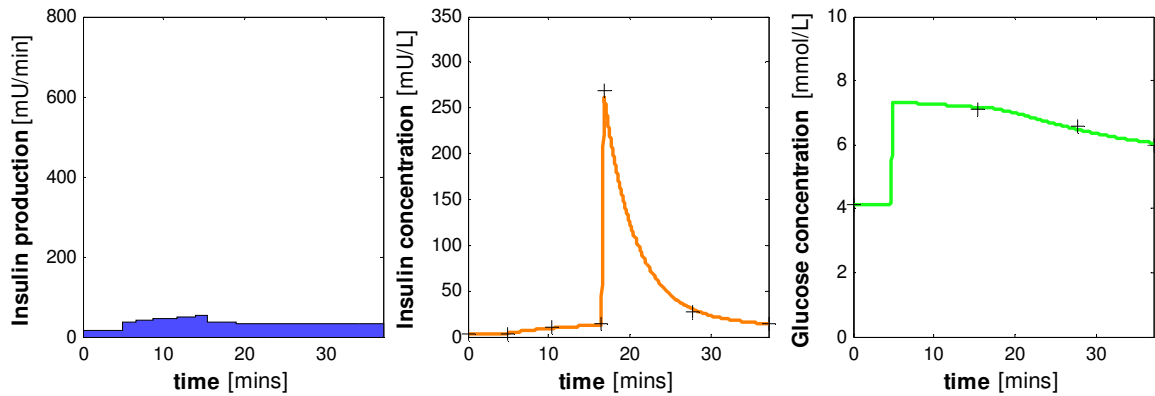
Figure 6.13. DISST test response of Participant 28

The most noticeable feature of the test response of Participant 28 was the significantly reduced insulin production rate. This result indicates that the damage to the participants β-cell mass was not reversed when the diabetic diagnosis was reversed. The participant's basal glucose was low as a result of their intensive exercise and diet regimen. However, the exogenous insulin did not have a significant effect on the participant's glucose concentration, indicating moderate insulin resistance. This resistance would not be picked up by a fasting glucose assay and the relatively low basal insulin would result in a false "healthy" result from the HOMA. In contrast to this participant's DISST response, the participant's glucose consumption rate increased significantly during the latter stages of the EIC test, resulting in a relatively high EIC derived insulin sensitivity for this individual $7.01 \times 10^{-2} \mathrm{mg \cdot kg^{-1} \cdot (mU/L)^{-1} min^{-1}}$ (~50th percentile). Thus, both of the most frequently used insulin sensitivity tests completely misinterpreted this participant's physiological condition.
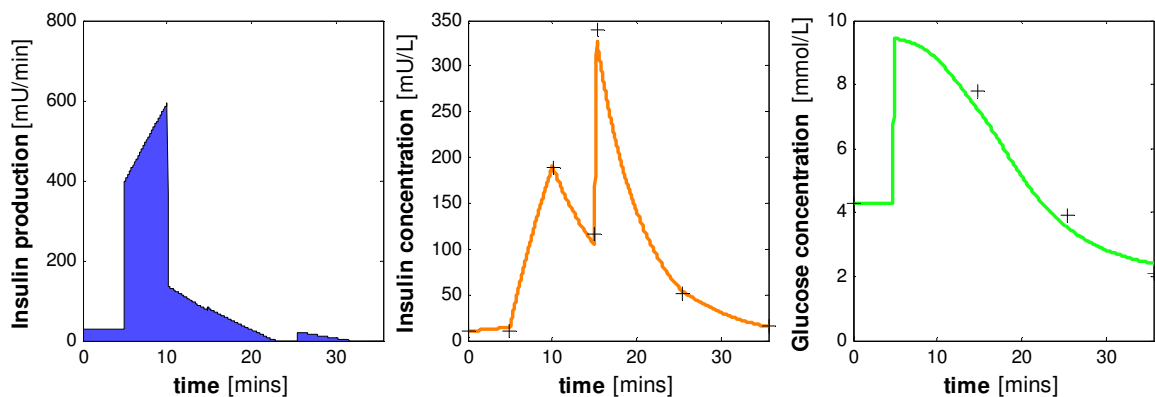
Figure 6.14. DISST test response of Participant 46

It seems that Participant 46 has a first phase of insulin production that has adapted to cope with the frequent ingestion of sugary energy drinks. The rapid increase in blood glucose due to the glucose bolus was detected by the pancreas and the subsequent excessive insulin release was almost sufficient to prompt hypoglycaemia in the participant. Nevertheless, the insulin sensitivity

values of this individual were relatively consistent between the DISST and the EIC (7.77 and 9.16×10^{-2} mg·kg^{-1}·(mU/L)$^{-1}$·min^{-1}, respectively).
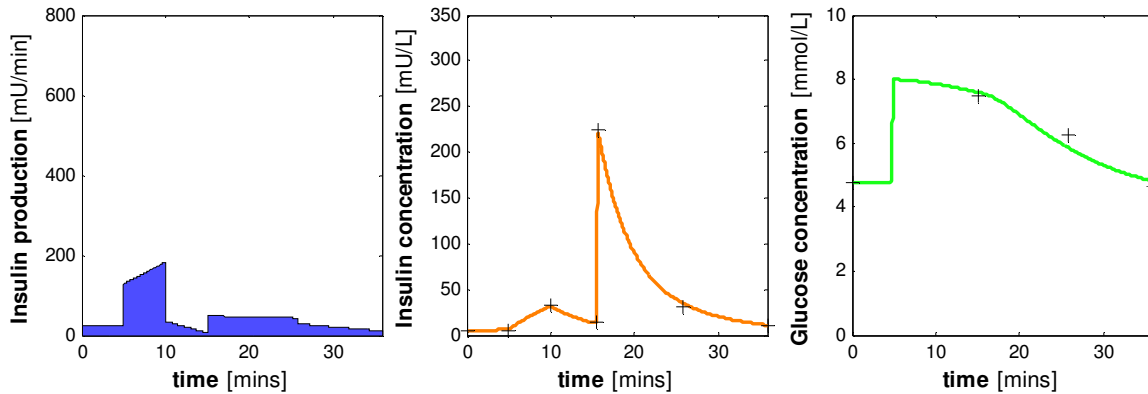
Figure 6.15. DISST test response of Participant 3.

Participant 3 is indicative of most relatively healthy, yet sedentary, individuals in the western world. The glucose concentration tends to return to basal, while insulin production is moderate. This participant is still in the healthy NGT range of Figure 1.03. They are thus a typical NGT result.
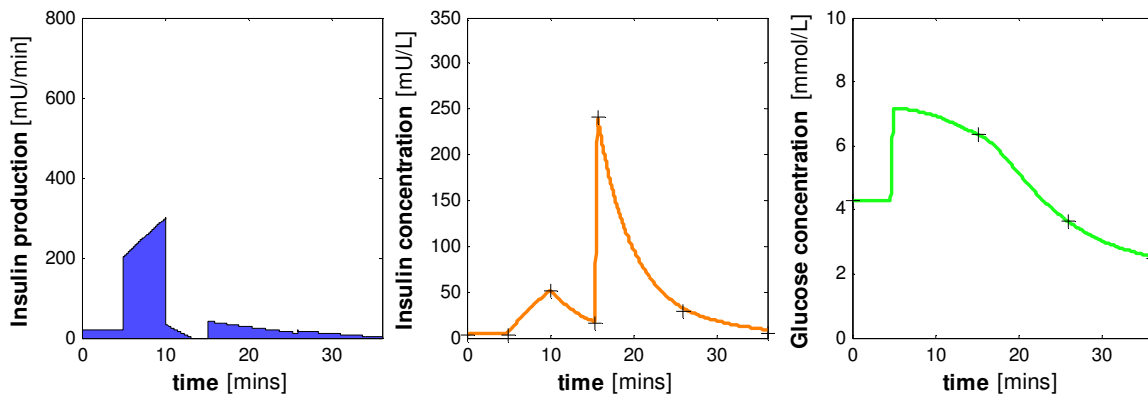
Figure 6.16. DISST test response of Participant 8.

Particularly healthy individuals tend to respond quickly to changes in glucose, and produce a relatively large first phase insulin response to the bolus. Although the abnormal first phase production rates of Participants 37 and 46 (~3units) dwarf this response (1.1units), the case presented is slightly larger than the upper quartile for the validation cohort. The added exogenous insulin bolus was sufficient to reduce the participant glucose concentration close to hypoglycaemic levels. Hence, this participant displays very high insulin sensitivity. This participant's measured insulin sensitivity values were 18.2 and 16.7×10^{-2} mg·kg^{-1}·(mU/L)$^{-1}$·min^{-1} by the DISST and EIC, respectively.

Overall, insulin sensitivity in conjunction with second and basal insulin production rates are most representative of a participant's position on the pathogenesis of type 2 diabetes. The two latter cases (Participants 8 and 3) clearly show a range for which the gold standard tests work (3, 8) and those for which they are not suitably informative (28, 46). More generally, the DISST's unique delivery of both insulin sensitivity and first and second phase insulin production makes it a much more informative test with a greater ability to define a participant's stage on the pathogenesis of type 2 diabetes. The approximate position of the participant cases presented in this section are overlaid on Figure 1.03 and shown in Figure 6.17. Participant 28 is not included as the pathogenesis figure does not account for those that return from the far left. In terms of insulin sensitivity and glucose concentration, Participant 28 would likely be placed close to Participant 46. However, their insulin production capability was most similar to Participants 40 and 48.
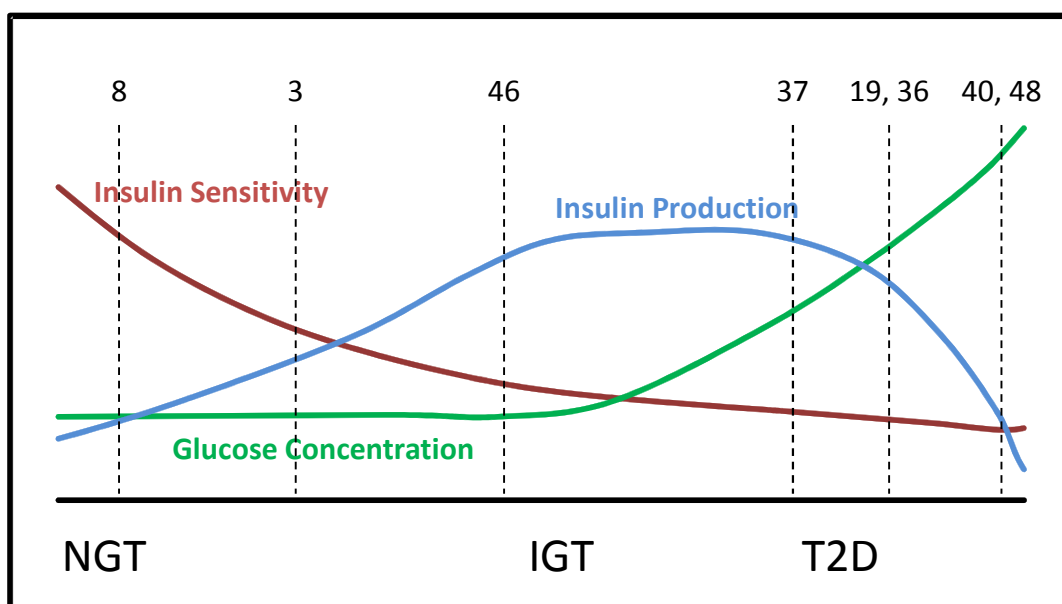
Figure 6.17. Approximate positions of the participants on the pathogenesis of type 2 diabetes.

6.6 Clinical implications of the DISST validation study

6.6.1 Comparison between the DISST and the EIC

The primary objective of the validation study was to assess the ability of the DISST test to replicate the sensitivity values of the euglycaemic clamp (the gold standard) to enable a low-cost, alternative test for clinical studies. The correlation of R=0.82 was close to the highest reported correlation for any of the tests presented in Table 2.02. Considering the significant reduction in

cost associated with the DISST test this is a very positive result. Furthermore, the c-ROC values between the DISST and the EIC implies a strong equivalence in terms of diagnosis, which is perhaps more clinically relevant.

The *in-silico* correlation of R=0.92 reported in Lotz *et al*. (Lotz *et al*. 2008) measured the effect of assay error on correlation and thus presents a 'best-case' scenario that perhaps could not be expected in a clinical setting. In a clinical setting, consistency in participant physiology is not necessarily assured across tests. Numerous factors such as stress and illness (Greisen *et al*. 2001; Hollenbeck & Reaven 1987; Van den Berghe *et al*. 2006; Zierler 1999), sleep deprivation (Davidson *et al*. 1987; Van Cauter *et al*. 1997), menstrual cycle (Trout *et al*. 2007), time of day (Van Cauter *et al*. 1997), recent exercise and diet (Borghouts & Keizer 2000; Nishida *et al*. 2004; O'Gorman *et al*. 2006; Zierler 1999) can have daily effects on insulin sensitivity. Thus, shifts in participant physiology during the up to seven days between tests may have had a negative effect on the clinically measured correlation between the DISST and EIC.

The DISST presents a different type of insulin sensitivity to the EIC. The EIC seeks to suppress endogenous glucose production and measure the rate of glucose uptake to the periphery under insulin concentration that are known to reduce in efficiency due to saturation effects (Kolterman *et al.* 1980; Natali *et al.* 2000). In contrast, the DISST identification method does not take changes in endogenous glucose production into account. Contrary to many IM-IVGTT protocols, the doses used in the DISST protocol are relatively small and physiological in magnitude. Thus, it is assumed that the suppression of hepatic glucose secretion is minimal. However, any glucose production suppression effect that does occur will be incorporated into the DISST sensitivity metric. Thus, the DISST measures a whole body, rather than peripheral sensitivity. Despite the considerable difference between the DISST and the EIC protocols and parameter identification methods, the correlation and diagnostic equivalence of the test was very high.

In contrast to the EIC, the DISST provides a more complete observation of the participant's physiology. While the EIC was designed to measure peripheral insulin sensitivity alone, the DISST measures insulin sensitivity in tandem with insulin production. Both insulin sensitivity and production are critical to pinpoint a participant's position on the pathogenesis of type 2 diabetes.

With reference to Figure 1.03 and the cases discussed in Section 6.5.3, measurements of insulin sensitivity alone cannot differentiate accurately between insulin resistant individuals. In particular, the participant simulations presented in Figures 6.08 to 6.12 show very distinctive physiology despite relatively similar EIC sensitivity values. Participants 19, 37 and 36 have all retained relatively healthy endogenous insulin production capabilities despite considerable insulin

resistance. This result would imply that these individuals are further left on Figure 1.03 than Participants 40 and 48 whose insulin production capability seems to have waned. Thus, the treatment of Participants 19, 37 and 36 could potentially target reversal of the progression to diabetes, whereas the treatment of Participants 40 and 48 may aim to ameliorate symptoms caused by their metabolic condition, rather than the condition itself.

It is likely that Participant 28 did not mitigate the cause of diabetes; rather, he just managed to reverse the diagnosis and maintain a healthy glucose homeostasis through ongoing intensive lifestyle measures. In particular, it is evident that the participant's β-cell damage has not been reversed, which is clinically critical. The relatively high EIC score thus masks the participant's very low insulin production capability. If the participant were to discontinue the self-imposed intervention, it is likely that hyperglycaemia would occur.

The high rate of repeatability across tests is considered the predominant strength of the EIC. This consistency has earned the EIC gold standard status in the field of insulin sensitivity. The CV of the EIC has been reported in the range of 4-6% (DeFronzo *et al*. 1979; Mari *et al*. 2001; Monzillo & Hamdy 2003), while the DISST pilot investigation and Monte Carlo investigation estimate a CV of 7-10% (Lotz *et al*. 2008; Lotz *et al*. 2010). Furthermore, the EIC values for insulin sensitivity have been extensively studied and their predictive value for diabetes risk assessment has been well-reported (DeFronzo & Ferrannini 1991; Ferrannini 1997; Hanley *et al*. 2003; Zethelius *et al*. 2004). Thus, the EIC should remain the favourable test for investigations in which the most accurate and repeatable insulin sensitivity values alone are desired regardless of cost. However, when the high cost of the EIC is prohibitive the DISST should be considered as a sound lower-cost alternative.

The comprehensive information provided by the DISST implies that it may be more suitable than the EIC for appraisals of participant health. While the DISST can accurately define a participant's position on the pathogenesis of type 2 diabetes, the EIC can only define the participant's insulin sensitivity. This value can be used to evaluate a participant's risk of developing diabetes, but cannot accurately differentiate the physiology of insulin resistant participants, and thus does not provide complete information to guide therapeutic choices.

The EIC requires increased setup, running costs and increased clinical intensity. A heated-hand apparatus and two feed pumps are required. Two clinicians are usually required for the 4 to 5 hour protocol, and if the feedback rate is not well-controlled euglycaemia is not assured at the end of the test, negating the possibility of a result. In contrast, the DISST requires no heated-hand apparatus, one clinician, no feed pumps, and only a 30-45 minute protocol. The DISST requires

complex mathematical identification methods to be set up. However, once this task has been completed, it can be reused numerous times without added cost.

In terms of participant safety, the two tests are relatively similar. The 1U insulin bolus used in the DISST was sufficient to lower the glucose concentration of six participants below 3 mmol/L (including 2 just below 2.5 mmol/L). In these cases, the test clinician had to continue monitoring the participant's blood glucose content after the test, while administering food or glucose. Participants were not allowed to leave the place of testing until their blood glucose levels returned to safe levels. Conversely, if the glucose feed rate is sufficiently underestimated during the EIC, the participant's glucose concentration can drop rapidly. This behaviour can generally be remedied easily with quick adjustments to the glucose infusion. However, the participant's counter-regulatory responses are often triggered within this period resulting in considerable difficultly achieving euglycaemia during the test period. This occurred in 'practice trials' of the EIC prior to commencement of the validation study. Furthermore, if both insulin and glucose pumps are switched off simultaneously at the end of the EIC test, the latent insulin build-up in the participant will clear the participants remaining glucose and severe hypoglycaemia will occur.

In summary, ratified use of the DISST will result in an incidence of mild hypoglycaemia in some participants that must be monitored and mitigated by the clinician. Whereas, in-experience or careless application of the EIC protocol would almost certainly result in potentially harmful and severe hypoglycaemia.

6.6.2 Potential for the DISST to replace other tests

HOMA is a particularly inexpensive metric to obtain clinically, as it only requires a single fasting blood sample. The simple, low-cost metric is thus a popular choice if insulin sensitivity is a minor consideration of a study, or for risk assessment in a general practice setting. If a low-accuracy approximation of insulin sensitivity is all that is required, HOMA is the appropriate alternative and the DISST will not replace HOMA for this purpose. While the DISST is comparatively less expensive and intensive than most insulin sensitivity tests, HOMA is still less expensive and intensive.

HbA1c is becoming an increasingly used assay for the appraisal of a participant's ability to maintain safe glycaemic concentrations. The assay is simple to administer and does not require that the participant be fasted. It has recently been prescribed as a suitable method of diagnosis of type 2 diabetes (ADA 2010). HbA1c is a more suitable method of diagnosing diabetes than the DISST, as it directly measures the participant's exposure to daily glucose.

However, HbA1c is not an insulin sensitivity test and thus could not be used to define a participant's risk of developing type 2 diabetes. Nor can it quantify a participant's β-cell health. Blood glucose is the last species to be affected in the pathogenesis of type 2 diabetes. Thus, diagnosis of hyperglycaemia with the HbA1c assays will only recognise a reversible state in a very small period in the pathogenesis of type 2 diabetes and it thus has a very low value as a predictor of risk.

Longitudinal studies of glycaemic health could potentially use either test. While the DISST could measure the participant's changes in terms of insulin sensitivity and production, HbA1c could measure the effect of these changes on a participant's daily glucose. While the effects of intervention may be better measured with the DISST, the HbA1c assay may be preferable to measure the efficacy of insulin sensitizer and secretagogue drugs.

The DISST will not replace the OGTT for diagnosing type 2 diabetes. The worst symptoms of diabetes occur as a result of hyperglycaemia, which is typically a result of uncontrolled blood sugar. The OGTT measures the ability of the participant to clear a certain amount of glucose during a set time, which is indicative of daily glycaemic health. However, the OGTT cannot contrast the physiology of a participant with depleted β-cell function, but relatively high insulin sensitivity from a participant with low sensitivity, but normal β-cell function. Both participants may expect the same glycaemic levels during daily life, and the subsequent diabetes symptoms. Thus, although their conditions are distinct, their diagnosis should be the same. As the mechanism driving the reduction of glycaemic control is distinctive, the treatment should also be distinctive. The DISST allows an observation of whether the condition of such individuals an artefact of insufficient insulin production, or whether their condition is a result of insulin resistance. Thus, tailored treatment is enabled for these individuals due to the extra, clinically valuable information yielded by the DISST.

The DISST is considerably less expensive and intensive than most IVGTT or IM-IVGTT tests. The accuracy of the DISST in terms of intra-participant repeatability and EIC equivalence is in the upper range of reported values for the IM-IVGTT. Thus, the findings of this study and that of Lotz *et al*. (Lotz *et al*. 2010), imply that the DISST could make such dynamic intravenous tests obsolete.

The contrary argument is based on the ability of the IVGTT type tests to estimate the participant's glucose dependent glucose clearance (S_G - which is synonymous with p_G in the DISST model) in addition to insulin sensitivity when Bergman's Minimal Model is used (Bergman *et al*. 1979). This claim is eroded by the frequently reported cases of parameter identification in-stability (Caumo *et al*. 1999; Pillonetto *et al*. 2002). The trade-off between S_G and SI frequently results in

SI=0 test outcomes (Pillonetto *et al.* 2002; Quon *et al.* 1994b). Thus, the results of the test are discarded, and the arduous and expensive protocol was wasted. The utility of the S_G term is also limited; it has shown mild ability to detect diabetes risk. However, *SI* which is always identified in tandem is a stronger predictor of risk (Martin *et al.* 1992). In theory, S_G can moderate *SI* by limiting the effect of changes in the hepatic glucose balance on the measured insulin sensitivity. The low glucose dose of the DISST was designed to limit this perturbation and thus ameliorate the effects of using a population constant p_G value.

The model identifiability issue is not intrinsic to the IVGTT protocol; rather it is an artefact of the modelling strategy itself. The very high intensity of the frequently sampled IVGTT protocol is in an effort to provide sufficient data to differentiate between the comparable effects of the two glucose clearance terms. If other single-parameter models were used the IVGTT data would yield much more stable results. However, the outcomes of the DISST pilot and validation study as well as the geometric *a-priori* model identifiability analysis (Chapter 5) has shown that most IVGTT protocols produce much more data than is required to accurately identify single, or distinct parameter models and are thus unnecessarily intensive.

Furthermore, the endogenous insulin production values are almost never reported during IVGTT type tests because C-peptide assays are rarely obtained. Hence, there is no means of differentiating participants by their endogenous response to glucose stimulus. Thus, the DISST is a more informative test overall.

To summarise, the DISST is considerably less expensive than the most accurate tests at the cost of a very limited reduction in resolution. The test protocol is more intensive than the low cost alternatives, but provides a significantly improved result. The DISST offers a better compromise of intensity and accuracy than the established intermediary tests. Furthermore, the DISST provides participant-specific β-cell function and thus is unique in its ability to pinpoint a participant's status on the pathogenesis of type 2 diabetes. Figure 6.18 graphically summarises the approximate position of the DISST in relation to the established tests on axis of increasing error and intensity. The x-axis of Figure 6.18 is in terms of participant discomfort, clinical intensity, and assay cost, while the y-axis is in terms of correlation to the EIC and intra-participant repeatability of variability.
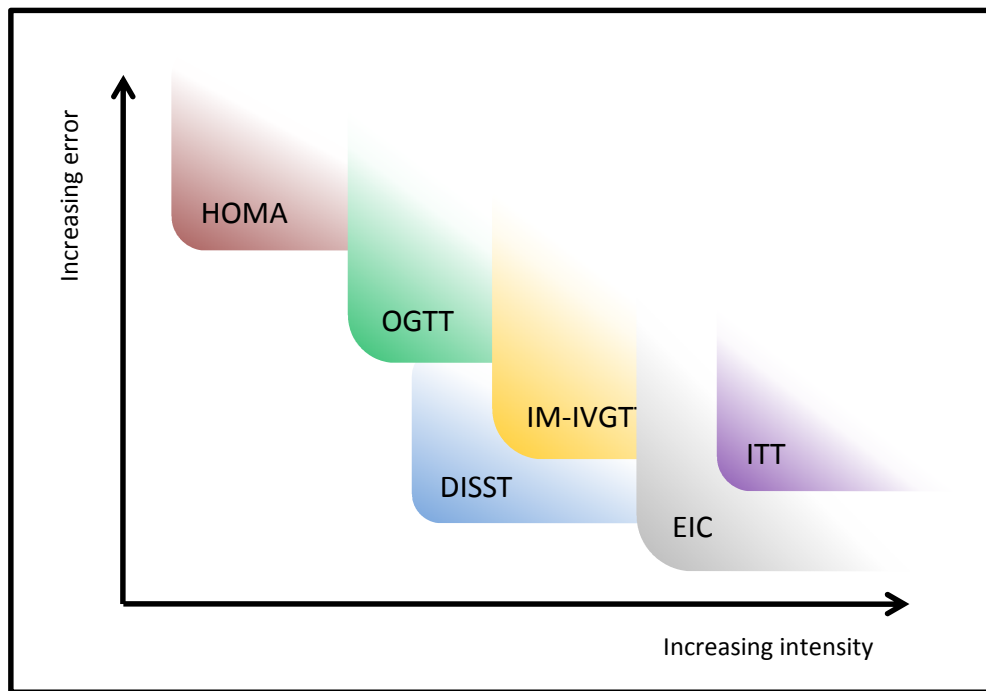
Figure 6.18. Visual representation of the position of the DISST test amongst the established tests.

6.6.3 Potential of the DISST and possible future investigation

In summary, the DISST model and protocol provided consistent participant results during the pilot investigation. The validation study compared the insulin sensitivity values of the DISST against those measured by the gold standard of insulin sensitivity testing, the euglycaemic clamp. The DISST insulin sensitivity vales are repeatable and have a strong equivalence to the EIC. This is despite the very low intensity of the test. Furthermore, the DISST is informative and unique in it's ability to accurately observe a participants' position on the pathogenesis of type 2 diabetes. Thus, the DISST is currently suitable for a number of applications:

- Clinical etiological investigations of type 2 diabetes.
- Evaluation of longitudinal dietary/exercise/medication intervention programmes in a clinical setting.
- For assessing or screening larger populations with a more rigorous metabolic appraisal than is often given through fasting measures.

Further investigation should be undertaken prior to recommendation of the DISST for wider use. Potential uses of the DISST requiring further study may include:

- It is very likely that the DISST is capable of type 2 diabetes risk assessment. The EIC test has been extensively proven to assess type 2 diabetes risk, and the DISST has been proven to be equivalent to the EIC. However, it remains to be explicitly proven that the DISST is an accurate risk assessor. Either this study must be undertaken clinically at significant expense, or data obtained from another study could be made available from which DISST results could be inferred through reduction of frequently sampled IM-IVGTT tests. Indeed, a study by Martin *et al.* achieved a positive risk prediction capability in a 10-year study using dynamic IVGTT tests with the Minimal Model (Martin *et al*. 1992). Perhaps mitigating the error introduced by the Minimal Model's parameter interference problem with the DISST model could increase the resolution of the risk assessment.

- The DISST could potentially be used to tailor and track treatment programmes of diabetic or pre-diabetic patients over time. However, a prior study of the efficacy of the DISST with respect to other available tests for monitoring treatment response should be undertaken.

Chapter 7. DISST and Minimal Model comparison

Chapter 7 compares the DISST model proposed by Lotz *et al*. (Lotz *et al*. 2010) to Bergman's Minimal Model (Bergman *et al*. 1979). The selection of the DISST model instead of the Minimal Model is an ongoing criticism of the DISST test. Thus, the suitability of the DISST model and parameters for modelling short duration dynamic test responses is compared to the suitability of the Minimal Model configuration.

7.1 Motivation

Bergman's Minimal Model of insulin/glucose pharmaco-dynamics has been established as the accepted model for the identification of insulin sensitivity from dynamic test data (Bergman *et al*. 1979; Ferrannini & Mari 1998; Pacini & Mari 2003). It is widely used in several forms of IVGTT tests.

The DISST test was purposely designed with a single parameter (*SI*) to model glucose decay, and was thus able to use a short duration, infrequently sampled protocol. Sections 5.5.1 and 5.5.2 show how this parameter selection favourably affected the parameter identifiability of the DISST model in comparison to the Minimal Model *in-silico*. However, the prevailing paradigm of researchers in the field of insulin sensitivity testing is that Minimal Model parameters should be identified from dynamic test data. Thus, in order to promote the use of the DISST model for sparsely sampled tests, the quality of outcomes of the DISST model must exceed the Minimal Model outcomes when relevant clinical data is used.

7.2 Analyses Methods

Initially, the Minimal Model parameters will be identified using the pilot cohort data and checked for repeatability. Secondly, the Minimal Model will be applied to the DISST data obtained during the DISST validation study and the equivalence between Minimal Model and EIC sensitivity values will be compared to the DISST equivalence. Finally, the changes observed by the Minimal

Model in an Atkin's diet intervention study will be compared to the expected outcomes, and the sensitivity values obtained from the DISST model.

7.2.1 Atkin's diet study design

The anatomical characteristics of the pilot and validation study cohorts are defined in Sections 4.5.1 and 6.2, respectively. Participants of the Atkins diet intervention are defined in Krebs *et al.* (Docherty et al. 2011d; Krebs et al. 2011), but will be listed in brief here. Inclusion criteria required established type 2 diabetes controlled with diet or anti-diabetic drugs. Exclusion criteria included recent weight loss or major illness. Fourteen participants were recruited. However, two pulled out of the study, the first due to exacerbation of a gallstone, while the second cited personal issues. Table 7.01 lists the characteristics of the Atkin's investigation cohort.

Subject	Age [yrs]	Weight [kg]	BMI [kg/m^2]	HbA1c [%]	G_B [mmol/L]
1	46	142.6	43.5	9.7	16.8
2	56	130.8	40.8	5.5	6.6
3	52	82.0	34.1	8.8	16.2
4	55	121.7	41.6	7.0	7.7
5	35	119.5	46.0	7.5	8.5
6	56	145.3	45.3	6.9	7.5
7	38	113.5	46.0	10.7	14.5
8	45	95.6	37.8	6.0	6.6
9	49	134.0	43.8	6.8	9.0
10	41	128.0	43.8	7.2	6.7
11	54	96.3	39.6	5.9	7.8
12	44	130.7	40.3	6.2	8.6
Q_1	42.5	104.9	40.0	6.1	7.3
Q_2	47.5	124.9	42.6	6.9	8.2
Q_3	54.5	132.4	44.6	7.8	10.4

Table 7.01. Characteristics of the participants of the Atkins dietary intervention study at week 0

Participants of the Atkin's diet underwent three dynamic insulin sensitivity tests at weeks 0, 12 and 24 of the dietary intervention. The sensitivity test protocol was a derivative of the frequently sampled IM-IVGTT designed by Ward *et al.* (Ward *et al.* 2001). A 0.2 g/kg glucose bolus was administered at t=0 and was followed by an insulin infusion that was designed to mimic the

endogenous insulin production reaction of healthy individuals. The glucose bolus was administered as a one-minute infusion of 50% dextrose. A two-minute insulin infusion began at t=2 minutes at a rate of 3.5 mU·kg^{-1}·min^{-1}. At t=7 minutes insulin infusion was resumed at 0.5 mU·kg^{-1}·min^{-1}, the rate was reduced to 0.25 mU·kg^{-1}·min^{-1} at t=17 minutes. Finally at t=50 minutes the infusion rate was reduced to 0.1 mU·kg^{-1}·min^{-1} where it stayed for the remainder of the protocol. Blood samples were obtained at t=-10, -5, -1, 0, 1, 2, 3, 4, 5, 6, 8, 10, 12.5, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 210, 240, 270 and 300 minutes (5 hours, 10 minutes). All samples were assayed for insulin and glucose.

7.2.2 Minimal Model parameter identification

The Minimal Model parameters were identified using the non-linear least-square Levenberg-Marquardt method (Marquardt 1963). Initial parameter estimates of S_G=0.01 1/min and SI=0.001 L·mU^{-1}·min^{-1} were used consistently here to ensure that the parameter identification method had the highest chance of accurate convergence. All re-simulations of the identified Minimal Model were checked to ensure adherence to measured assay values was achieved.

For the Minimal Model analysis of the DISST test data, the insulin profile was constructed via linear interpolation up until the bolus. The post bolus peak insulin concentration magnitude was estimated using the bolus content and volume of distribution obtained during the DISST identification. The post bolus concentration was modelled with a spline. This approach mitigated the error introduced by the sparse sampling on the linear interpolation, and thus is the best possible representation of the Minimal Model PKs of insulin from the data available. This method was more accurate than the standard linear interpolation typically employed by the Minimal Model (Bergman *et al*. 1979). Note that the DISST physiological model mitigated this error by fitting a modelled representation of the insulin PKs that includes insulin production dynamics, (which the Minimal Model almost never includes) to the measured data. Both the DISST and Minimal Model used linear interpolations of the insulin data obtained in the IM-IVGTT test used in the Atkins diet study.

7.3 Outcomes

7.3.1 Pilot study intra-participant repeatability

Table 7.02 presents low and medium dose DISST and Minimal Model insulin sensitivity values and their variations of the participants of Part 1 of the pilot investigation. Tables 7.02 to 7.04 are reproductions of Tables 4.03 to 4.05 in Sections 4.4.3 and 4.4.4 using only insulin sensitivity values.

		DISST model		Minimal Model			
		SI	ΔSI	S_G	ΔS_G	SI	ΔSI
Subject	Dose	[10^{-4}·L·mU^{-1}·min^{-1}]	[%]	[10^{-4}/min]	[%]	[10^{-4}·L·mU^{-1}·min^{-1}]	[%]
4	low	13.39		6*		1.38	
	med	16.49	23.1	6*	-	1.71	24.1
7	low	19.33		345.5		0.54	
	med	18.06	-6.6	6*	-	2.36	340.9
8	low	18.64		328.7		0.58	
	med	13.61	-27.0	600*	-	0.18	-68.8
10	low	43.73		6*		2.76	
	low	29.19		528.6		1.44	
	med	17.4	-52.3	421.4	-20.3	0.45	-78.4
11	low	6.88		306.5		0.05	
	low	5.75		370.7		0.05	
	med	6.73	6.5	193.8	-42.7	0.46	804.6
15	low	8.28		40.4		0.57	
	low	8.99		400		0.09	
	med	7.39	-14.4	181.7	-17.5	0.25	-24.5
16	low	3.27		73.6		0.05	
	low	3.16		6.01		0.48	
	med	3.17	-1.4	38.5	-3.3	0.36	37.3
mean			-3.5		-21		147.9
SD			25.1		16.3		322.5

Table 7.02. Dose dependence of parameters of the DISST and Minimal Models (* value reached bound and is thus not used in analysis).

The inter-dose repeatability of the Minimal Model parameters was significantly reduced in comparison to the DISST model. The S_G variation was large, but had very little bias. However, the Minimal Model insulin sensitivity bias was significant. The bias was shifted significantly by a few cases. However, Minimal Model parameter stability was generally very poor across all subjects.

Table 7.03 shows the DISST and Minimal Model parameter values from the pilot cohort participants who underwent the medium and high dose tests.

		DISST model		Minimal Model			
		SI	*ΔSI*	S_G	ΔS_G	*SI*	*ΔSI*
Subject	**Dose**	$[10^{-4}\text{L·mU}^{-1}\text{·min}^{-1}]$	[%]	$[10^{-4}/\text{min}]$	[%]	$[10^{-4}\text{L·mU}^{-1}\text{·min}^{-1}]$	[%]
2	med	19.47		7.03		0.45	
	high	13.43	-31	**6***	-	1.19	165.7
5	med	26.45		51.01		0.36	
	med	19.97		10.38		0.54	
	high	25.07	8	33.9	10.4	0.37	-18.5
6	med	14.84		6.03		0.3	
	high	12.83	-13.6	93.18	1444.0	0.26	-14.6
14	med	11.70		215.61		0.33	
	med	11.65		459.14		0.22	
	high	14.12	20.9	182.61	-45.9	0.3	6.3
mean			-6.0		348.5		26.7
SD			18.3		730.7		78.3

Table 7.03. Dose dependence of insulin sensitivity and production characteristics of participants who underwent DISST tests with the medium and high doses during the DISST pilot investigation.

In this case, a single outlier caused the high intra-participant variability in the S_G term. For the Minimal Model *SI* was quite stable for three out of the four participants. However, overall, the DISST test *SI* parameter was more repeatable.

Table 7.04 shows the intra-dose repeatability of the model parameters from Part 2 of the DISST pilot investigation. While the DISST *SI* variability obtained in Part 2 was relatively and consistently low, both Minimal Model parameters were considerably more variable. Closer inspection of Table 7.04 shows the parameter trade-off that occurs during the Minimal Model identification. While Subjects 3 and 16 had a higher S_G in the former tests, and a higher *SI* in the

latter tests, Subjects 5, 10, 14 and 15 had a high S_G in the latter test and a higher SI in the former test. Only Subjects 11 and 16, had relatively stable results and did not exhibit trade-off behaviour. Hence, there was a significant reduction in repeatability performance.

		DISST Model		Minimal Model			
Subject	**Dose**	SI $[10^{-4}L \cdot mU^{-1} \cdot min^{-1}]$	ΔSI [%]	S_G $[10^{-4}/min]$	ΔS_G [%]	SI $[10^{-4}L \cdot mU^{-1} \cdot min^{-1}]$	ΔSI [%]
10	low	43.73		**6***		2.76	
	low	29.19	19.9	528.6	-	1.44	31.2
11	low	6.88		370.7		0.05	
	low	5.75	8.9	306.5	9.5	0.05	4.3
15	low	8.28		40.4		0.57	
	low	8.99	4.1	400.0	81.7	0.09	72.1
16	low	3.27		73.6		0.05	
	low	3.16	1.7	6.0	84.9	0.48	81.9
3	med	10.18		569.7		0.19	
	med	8.59		480.7		0.12	
	med	7.37	16.8	177.8	39.2	0.29	44.2
5	med	26.45		10.4		0.54	
	med	19.97	14	51.0	66.2	0.23	39.8
13	med	16.31		401.2		0.2	
	med	13.51		351.2		0.23	
	med	21.2	24.7	506.5	20.7	0.3	21.7
14	med	11.7		215.6		0.33	
	med	11.65	0.2	459.1	36.1	0.22	19.3
mean			11.3		48.3		39.3
SD			9.0		29.6		26.5

Table 7.04. Intra-dose variability of the DISST and Minimal Model parameters from Part 2 of the DISST pilot cohort.

7.3.2 Validation study EIC correlation

The Minimal Model parameters were identified in the validation cohort DISST data to enable a comparison between the Minimal Model metrics and the *ISI* value from the EIC. Figures 7.01 and 7.02 show the equivalence of Minimal Model *SI* and S_G, respectively. Table 7.05 shows the correlation R-values and c-ROC values (using 6.94×10^{-2} mg·kg^{-1}·(mU/L)$^{-1}$·min^{-1} as a cut off value) of the figures shown.
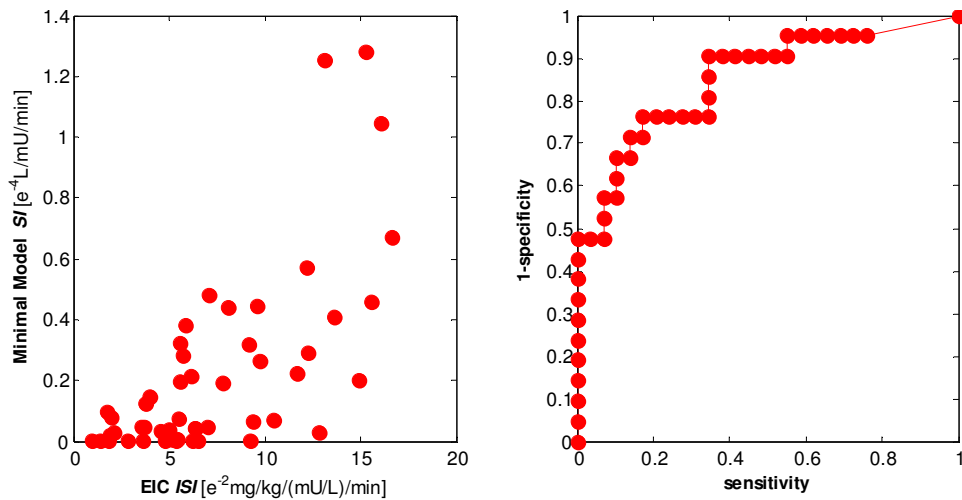
Figure 7.01. Correlation and ROC curve for the Minimal Model insulin sensitivity value.
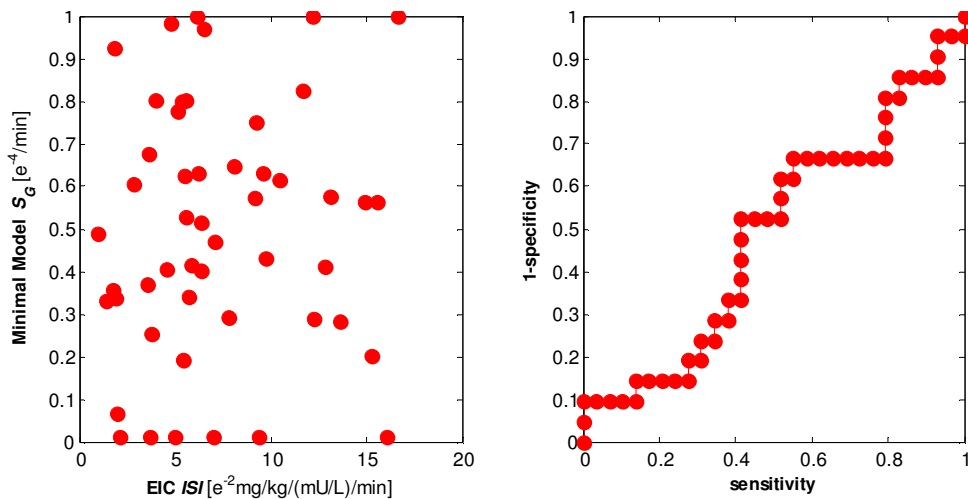
Figure 7.02. Correlation and ROC curve for the Minimal Model glucose dependent clearance term.

	DISST	Minimal Model	
	*SI**	S_G	*SI*
EIC correlation	0.82	0.08	0.68
c-ROC	0.96	0.49	0.85
DISST *SI* correlation	-	0.05	0.87

Table 7.05. Comparative statistics for the EIC equivalence of the parameters of the Minimal Model and the DISST test (* DISST values from Section 6.5.1)

The Minimal Model insulin sensitivity value correlated relatively well to the EIC considering the very sparse data obtained during the DISST test. Table 2.02 shows the reported values for IM-IVGTT to EIC correlations are in the range 0.70 to 0.89. Thus, the value of 0.68 obtained in this analysis was slightly below these reported values. However, as most IM-IVGTT test protocols are much more intensively sampled and have a much greater duration, identification of two partially distinct parameters is usually more stable. Thus, the result obtained was in accordance with expected values. Interestingly, the Minimal Model *SI* value correlated relatively well with the DISST *SI* value. This can probably be attributed to the equality of the metric units as well as identification data set.

The glucose dependent glucose clearance term (S_G) showed very poor equivalence to the EIC in terms of correlation and insulin resistance diagnosis. There were a significant number of cases of S_G identification reaching the bounds required for identification stability (18%).

A key outcome of this analysis was that the DISST model significantly outperformed the Minimal Model for the identification of insulin sensitivity with equivalence to the EIC using the relatively sparse data from the DISST protocol. The DISST model clearly avoided the parameter identification tradeoffs that obscured the Minimal Model parameters.

7.3.3 Atkins diet study descriptive capability

The Minimal Model was not designed for the infrequently sampled DISST protocol, as the Minimal Model requires relatively high-resolution data sets from clinically intense dynamic protocols to maximise identification stability. Identifying values from the low intensity and short duration DISST test may thus unfairly disadvantage the Minimal Model in comparison to the DISST model as the data sets were specifically designed for the DISST model. Thus, to fairly

appraise the Minimal Model parameter identification, frequently sampled IM-IVGTT test data was identified using both models and associated parameter identification methods.

The Minimal Model was identified in the data obtained during the arduous 5-hour IM-IVGTT protocol described in Section 7.2.1. The DISST model was not designed for such protocols, and as such is not well suited to describe the steady-state condition that is often achieved in the final stages of the test. The DISST model was specifically designed to capture short-term glucose decay. Thus, the DISST model parameters will be identified across the data between t=-10 and 60 minutes, with the data between t=0 and 10 ignored due to mixing effects. Note that insulin sensitivity is assumed to be constant over a test so using a shorter period is acceptable.

Table 7.06 shows the physiological changes observed in the Atkins cohort alongside the modelled insulin sensitivity changes from the DISST and Minimal Model. Table 7.07 summarises the proportional changes observed in the Atkins investigation and correlates the outcomes.

Subject	Weight [kg]			Basal Glucose [mmol/L]			DISST SI_{70} [10^{-4}L/mU/min]			MM SI_{310} [10^{-4}L/mU/min]		
	W 0	W 12	W 24	W 0	W 12	W 24	W 0	W 12	W 24	W 0	W 12	W 24
1	143	133	137	16.9	11.4	11.7	4.61	1.26	1.62	36.8	9.3	3.9
2	131	121	114	6.7	6.0	5.9	3.77	4.35	5.31	**0.0**	**0.0**	0.3
3	82	78	76	16.3	10.3	10.8	5.65	5.90	6.16	77.7	45.3	28.3
4	122	112	109	7.7	6.6	6.7	5.46	7.77	11.5	31.5	21.5	3.3
5	120	106	105	8.5	6.7	7.6	5.78	6.09	5.58	94.2	29.1	0.8
6	145	141	136	7.5	7.1	6.7	2.30	3.07	3.13	**0.0**	**0.0**	**0.0**
7	114	110	107	14.4	12.6	10.0	3.46	4.83	6.32	93.8	81.0	16.9
8	96	88	87	6.6	6.5	6.8	13.6	15.0	12.4	0.2	10.5	0.06
9	134	119	116	9.1	5.1	6.1	1.61	3.41	4.99	14.1	**0.0**	0.01
10	128	127	132	6.6	5.6	9.9	3.59	8.09	2.98	1.8	**0.0**	8.8
11	96	86	82	7.2	7.0	6.4	5.72	5.41	10.1	35.1	17.9	0.9
12	131	124	123	8.6	6.6	7.2	3.44	7.97	5.15	33.3	**0.0**	0.2

Table 7.06. Indicative physiological measurements and DISST and Minimal Model insulin sensitivity at weeks 0, 12 and 24.

	IQR			Correlation (ρ)	
	Q1	Q2	Q3	Δ DISST SI_{70}	Δ MM SI_{310}*
Δ **Weight** [%]	-11.6	-7.8	5.2	-0.16	0.11
Δ G_B [%]	-36.2	-14.6	-11.1	-0.30	0.41
Δ**DISST** SI_{70} [%]	0.4	29.6	56.9	-	-0.53
Δ**MM** SI_{310}* [%]	-196.9	-119.3	-49.0	-	-

Table 7.07. Comparative statistics of the physiological and sensitivity changes observed in the Atkins pilot investigation. (* Data from the MM *SI*=0 cases are ignored for the Minimal Model analysis.)

Note that while the Minimal Model insulin sensitivity values correlate positively, the changes presented by the model are contrary to the behaviour expected. In particular, as weight and basal glucose tended to decrease in the cohort, so does the Minimal Model insulin sensitivity. The expected correlation between sensitivity and weight/basal glucose change should be negative as shown by the DISST sensitivity value. Thus, the Minimal Model measured the opposite trend to that expected.

Compliance to the prescribed intervention was relatively good for the cohort, with the exception of Participant 10 who miss-interpreted the high protein diet to allow deep-fried fish and chips. As expected, this participant's basal glucose, weight and DISST modelled insulin sensitivity all worsened. In contrast, the Minimal Model measured an improvement in insulin sensitivity again failing to capture the expected change. All other participants achieved moderate improvements in basal glucose and bodyweight. With the exception of participants 1, 5 and 8 these participants also exhibited an improvement in DISST modelled insulin sensitivity. The Minimal Model insulin sensitivity value was consistently contrary to the expected behaviour.

7.4 Discussion

For each of the cases presented, the DISST modelled insulin sensitivity values produced more stable and reasonable results. The DISST tests conducted during the DISST pilot and validation studies provided insufficient data to allow a distinction between the S_G and *SI* Minimal Model terms. The novel *a-priori* identifiability method described in Chapter 5 predicted this outcome. The DISST test protocol used during the validation study was considerably sparser than typically used with the Minimal Model and bordered on non-identifiability. The S_G term in particular was not stable. Any small degree of un-modelled mixing or assay error manifest in the data set thus

has an amplified affect on the model parameters in the absence of further data that may reduce the error. Hence, the outcomes of the validation set were worse than that of the pilot set, which had a higher resolution.

In contrast, the single parameter of the DISST model was consistently stable during identification. The resulting DISST insulin sensitivity values were highly correlated to the EIC insulin sensitivity values, which are the gold standard in this field. Ultimately, these outcomes show that a low-duration and intensity protocol and a simple glucose PD model are sufficient to capture virtually the same insulin sensitivity information obtained during the EIC.

The insulin sensitivity test used in the Atkin's study should have provided data that is more suitable for the Minimal Method. However, the cohort participants were predominantly insulin resistant, for whom the Minimal Model has significant issues (Section 5.4.2). The reason for the identifiability failure of this cohort may be distinct from the reason that most IM-IVGTT studies fail. The protocol maintained an insulin infusion for the duration of the test. This allowed some of the participant's blood glucose concentrations to go below basal for a significant portion of the latter stages of the test. The S_G term of the Minimal Model always drives the simulated glucose concentration towards basal glucose and only the insulin action term (X) term is able to drive and maintain glucose below basal. Thus, simulated insulin action, synonymous in these models with interstitial insulin multiplied by sensitivity, must be kept elevated and consistent by manipulating the model parameters. Hence, in many cases, the clearance rate of 'interstitial-sensitive' insulin (p_2) reduced during identification until it became negligible resulting in high insulin sensitivity values regardless of the p_3 value.

Obviously, most Atkins cohort participants did not have the EGP response to below basal glucose concentrations that would be expected from healthy participants that is modelled by the S_G term. In fact, it may be that some of these participants had a non-basal glucose set point for endogenous glycaemic control, but lacked the endogenous capability to achieve this concentration. Thus, the exogenous insulin may have allowed a return to safe set point. However, this point is merely conjecture in the absence of more complete data, such as C-peptide assays.

The DISST sensitivity values showed that much of the important information could be obtained from the insulin sensitivity test of the Atkin's study from the initial decay of glucose with respect to interstitial insulin concentration. Changes in the DISST insulin sensitivity value obtained during this period were generally synonymous with the physiological changes observed in the participants. Note that in data not shown here, the sensitivity values identified by the Minimal Model over the same period were not stable and were worse overall than those shown in Table 7.06.)

The Minimal Model is not necessarily obsolete in light of the DISST model and these analyses. Numerous studies have found very accurate equivalence between the Minimal Model and the EIC when more suitable and more intensive protocols are used with general cohorts (Table 2.02). Furthermore, the Minimal Model has been extensively investigated since its inception over 30 years ago. Thus, researchers know precisely what might be expected from it. More recently, Bayesian techniques and the addition of a second compartment to the glucose pharmaco-kinetic model have mitigated some of the identifiability issues of the Minimal Model (Caumo *et al.* 1999; Cobelli *et al.* 1998; Denti *et al.* 2009; Pillonetto *et al.* 2003; Pillonetto *et al.* 2002). However, there seems to be very sparse additional value available from the increased intensity of the protocols required. This makes use of a 2-parameter model particularly inefficient for clinical or screening use.

The S_G term models behaviour that is variable between test participants (Del Prato *et al.* 1997) and represents a real and occasionally observable physiological phenomenon. Effectively, it defines the hepatic glucose balance as a function of blood glucose concentration. This balance seems to be impaired in diabetic individuals (Del Prato *et al.* 1997). However, the metric is not generally used to identify diabetic individuals due to it's poor resolution. Martin *et al.* showed that S_G had some limited potential for diabetes risk assessment, yet this potential was superseded by the insulin sensitivity values that were identified in tandem (Martin *et al.* 1992). Conjecture may imply that the S_G values identified in this cohort incorporated glucose dynamics that might have been attributable to *SI* given the well documented cases of parameter trade-off (Caumo *et al.* 1996; Cobelli *et al.* 1999; Pillonetto *et al.* 2002; Quon *et al.* 1994b), and fixing S_G may actually increase the resolution of *SI* for the purpose of diabetes risk assessment. If very significant glucose assay improvements occur, identification of S_G in tandem with insulin sensitivity might become sufficiently stable during identification to provide additional valuable information from moderately intense protocols. However, the current test protocols and assay techniques limit the identifiability of S_G and its utility is generally limited to the modulation of *SI*.

7.5 Conclusions

In conclusion, this chapter was not intended to argue against the use of the Minimal Model for the identification of clinically relevant parameters from dynamic tests. Rather, it was intended to show that the model has limitations amongst dynamic test protocols. This chapter strongly proved that the Minimal Model is not capable of providing two distinct sensitivity metrics from low-intensity, short duration dynamic tests. A lack of data is available for the distinction of two Minimal Model parameters that model glucose decay. Furthermore, it failed to capture the

behaviour of insulin resistant individuals of an interventional study. Although this outcome was partially due to the nature of the test, a suitable model should be capable of describing the behaviour exhibited by any cohort or protocol configuration (assuming sufficient test stimulus). In contrast, the simplicity of the DISST model parameters enabled successful replication of the EIC insulin sensitivity values, was relatively repeatable and adequately described the behaviour of the Atkins intervention cohort.

Criticism of the DISST test is often directed towards the choice of model and the low intensity of the protocol. However, the ultimate appraisal of the DISST test must be made in context of the actual performance, and pre-conceptions should not influence or override the observed outcomes that indicate a successful model and protocol.

Chapter 8. The quick DIST (DISTq)

The DISTq concept presented in this chapter was published in the open medical informatics journal (Docherty *et al*. 2009) and justification of the method assumptions was presented in computer methods and programs in biomedicine (Docherty et al. 2011c). A validation paper is forthcoming. However, a comprehensive description of the relevant clinical and parameter identification methods, study outcomes, and potential uses is presented in this chapter. The overall goal of DISTq was to provide a real-time (result at the bedside) test based on the DISST

8.1 Motivation

The quick DIST (DISTq) is a development of the fully-sampled DISST described in Chapters 4 and 6. DISTq occupies a distinct region from the DISST on the cost/accuracy spectrum with significant cost reduction, but only a slightly reduced accuracy.

The primary objective of the development of the DISTq was to eliminate the need for costly and time-consuming insulin and C-peptide assays (a 2 to 3 day turnaround is typical for these assays). Hence, it can deliver a result immediately after the test by making insulin sensitivity identifiable using only information available at the time of testing. Thus, only glucose assays and anatomical data from the fully-sampled DISST remain available for use.

With the elimination of the need for insulin and C-peptide assays, real-time insulin sensitivity identification can be done at the bedside and therapy or intervention can be tailored for the patient, but at a cost of losing participant-specific identification of endogenous insulin secretion. This approach and real-time result is unique amongst the established insulin sensitivity tests, as each of them requires assays that are generally not available in real-time and still do not deliver endogenous insulin production metrics.

8.2 DISTq process

8.2.1 Clinical protocol

The clinical protocol of the DISTq is identical to that of the fully-sampled DISST that is fully described in Section 4.2. However, no insulin or C-peptide assays are required, and thus the spinning and freezing of blood samples is not necessary. The DISTq protocol is described in brief:

- Participants attend the place of testing in the morning after an overnight fast.
- A single cannula is placed in the ante-cubital fossa (vein in inner elbow)
- Blood samples are drawn and assayed for glucose at t=0, 5, 10, 15, 20, 25, 30, 40 and 50 minutes
- A glucose bolus is administered immediately prior to the t=5 minute sample (Dosage: 5g low, 10g medium, 20g high)
- An insulin bolus is administered immediately prior to the t=15 minute sample (Dosage: 0.5U low, 1U medium, 2U high)

Hence, the DISTq is a subset of the DISST noting a missing 'S' that stood for insulin secretion.

8.2.2 *A-posteriori* parameter estimation

Accurate insulin sensitivity tests require accurate insulin concentration measurements for insulin sensitivity estimation. Furthermore, all dynamic tests, such as the IVGTT, DISST and DISTq, require accurate time-variant insulin concentration profiles to be provided. However, real-time identification prohibits insulin and C-peptide assays, as they are not typically available from most labs within less than 2 to 3 days. Thus, other methods were used to estimate this critical species profile.

Many passive physiological rates can be estimated as function of measureable anatomical data (Van Cauter *et al*. 1992). However, the estimation of the insulin concentration profile was inhibited by the lack of *a-priori* relationships between real-time measurable attributes and the kinetic parameters that define insulin clearance and the production of insulin. For example, basal insulin could not be accurately inferred from a measurement of BMI, BSA, age, sex or combination of these measurements. Neither could insulin clearance, basal, first or second phases

of insulin production. However, some relationships were evident between these kinetic or production rates and insulin sensitivity.

To generate mathematical expressions for these relationships all available fully-sampled data from the DISST pilot study, a dietary intervention study (TeMorenga *et al.* 2010), and validation study was evaluated using the iterative integral method with a slightly modified DISST model. In particular:

- All data time measurements were shifted so that all glucose boluses consistently occurred at $t=5$ minutes. This was done to co-ordinate the occurrence of the first phase of insulin production across all data sets.
- Insulin production profiles were obtained using the deconvolution process defined in Section 4.4.2.
- First pass hepatic extraction of insulin was fixed at a population average of 70% (Cobelli & Pacini 1988; Ferrannini & Cobelli 1987; Meier *et al.* 2005; Toffolo *et al.* 2006) and n_T was identified by the iterative integral method. (n_T is the combined plasma insulin clearance effect: n_K+n_L with $\alpha_I =0$)
- Insulin sensitivity and glucose distribution volume were identified using the iterative integral method described Section 3.2.

Population-level relationships between insulin sensitivity and the isolated parameters were then obtained using the identified participant-specific parameter values. Visual inspection of the relationships indicated that power relationships were appropriate. Linear regression of the log-log relationships of the population results was used to generate the coefficients of the power equation. Equation 8.01 shows how the linear regression of the $\log_e$-$\log_e$ relationships is used to define the relationships.

$$\ln(V) = A\ln(SI) + B \qquad\qquad 8.01$$

$$V = e^B(SI)^A \quad\text{or}\quad V = b(SI)^A \qquad\qquad 8.01a$$

where: V is the parameter of interest, SI is the participant insulin sensitivity [$10^{-4}\text{L}\cdot\text{mU}^{-1}\cdot\text{min}^{-1}$], A and B define the log-log slope, and $b = e^B$

8.2.2.1 Cohort details

DISST test data from three cohorts was available to develop the parameter estimation equations: the pilot investigation (18 Subjects, 46 tests), the DISST validation study (50 subjects, 50 tests), and a dietary intervention study (74 subjects, 218 tests). Participant details and study outcomes of the pilot and validation studies are presented in Section 4.4 and Chapter 6 respectively. The intervention study sought to investigate the effect of increasing the dietary composition of protein in females who are at risk of developing type 2 diabetes (TeMorenga *et al*. 2010). Inclusion criteria required either a BMI greater than 25 or a BMI greater than 23 with family history or ethnic disposition toward type 2 diabetes. Full details of the intervention study cohort and outcomes are presented in TeMorenga *et al*. The intervention cohort is summarised in Table 8.01.

Status NGT/IFG/T2D	BMI Q_1 Q_2 Q_3	Sex M/F	Age Q_1 Q_2 Q_3	HOMA-IR Q_1 Q_2 Q_3	DISST-SI† Q_1 Q_2 Q_3
63/11*/0	27.6 32.4 36.3	0/74	34.8 43 50.3	1.37 2.15 3.11	5.79 7.83 10.9

Table 8.01. Participant characteristics and insulin sensitivity results from the intervention study (TeMorenga *et al*. 2010). HOMA-IR is calculated: $IR = G_B I_B / 22.5$. DISST-SI is identified with the iterative integral method and the full data set. (* Only one participant was diagnosed with IFG in all three tests, two were diagnosed in two of the three tests and eight had IFG once. † DISST-SI units $10^{-4} L \cdot mU^{-1} \cdot min^{-1}$)

8.2.2.2 Relationship between insulin sensitivity and basal insulin

Basal insulin was related to insulin sensitivity using an exponential function. Figure 8.01 and Equation 8.02-PIVC show the relationship defined between insulin sensitivity and basal insulin for this cohort. The -C qualifier of Equation 8.02-C denotes that the equation constants were defined using the combined results of the three available cohorts. Equally, -P, -I and –V stand for: pilot study, intervention study and validation study, respectively.
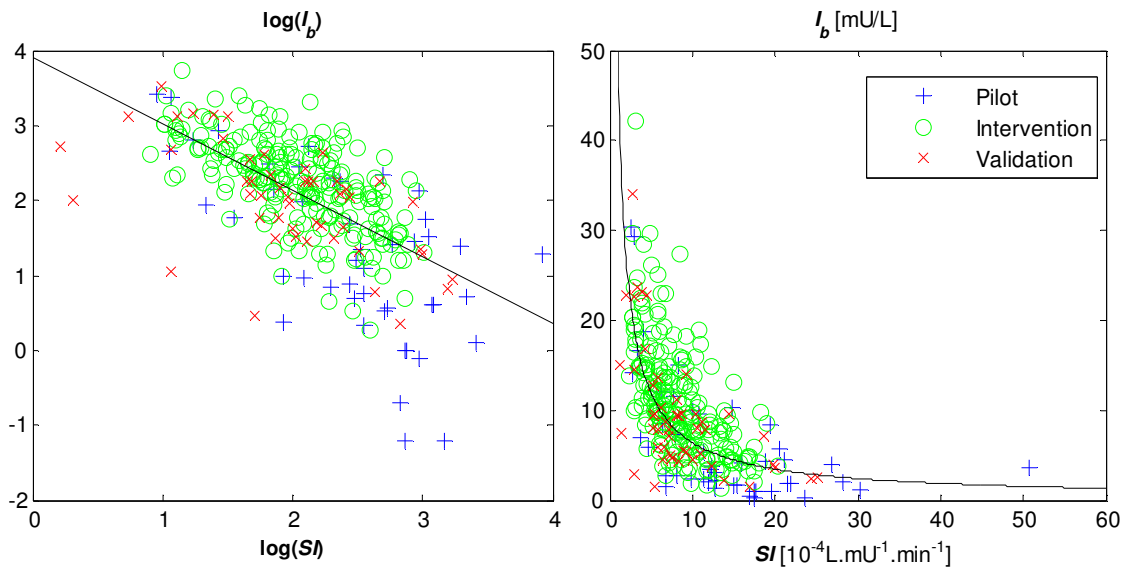
Figure 8.01. The log-log and normal plots of *SI* and basal insulin using all available data points and the derived population relationship (-).
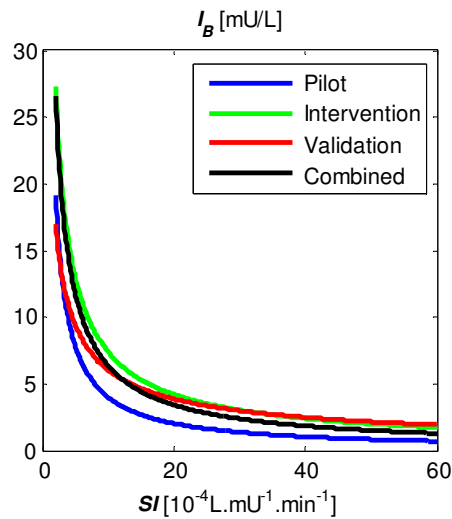
Figure 8.02. Variation in the basal insulin estimation equation between the various cohorts.

Equation 8.02 shows the mathematical distinction when the equation coefficients are evaluated using the pilot (8.02-P), intervention (8.02-I), validation (8.02-V) and the combined (8.02-C) cohorts.

$$I_b = 37.4 \cdot SI^{-0.971} \qquad\qquad\qquad 8.02\text{-P}$$

$$I_b = 47.9 \cdot SI^{-0.811} \qquad\qquad\qquad 8.02\text{-I}$$

$$I_b = 26.4 \cdot SI^{-0.639} \qquad\qquad\qquad 8.02\text{-V}$$

$$I_b = 49.4 \cdot SI^{-0.890} \qquad\qquad\qquad 8.02\text{-C}$$

This relationship is broadly justified by the acceptance of the HOMA metric as a low-cost surrogate for more accurate insulin sensitivity tests (Ferrannini & Mari 1998; McAuley *et al.* 2007; Pacini & Mari 2003). While the HOMA metric uses the multiplication of basal insulin and glucose to estimate *SI*, this relationship inverts the relationship to define basal insulin from insulin sensitivity. The relative consistency of basal glucose concentrations across a population prior to the later stages of insulin resistance in the pathogenesis of type 2 diabetes suggests that the basal insulin concentrations in a population can be inferred from insulin sensitivity measurements. Insufficient data is currently available to define the applicability of this estimation in individuals with excessively elevated basal glucose concentrations.

8.2.2.3 Relationship between insulin sensitivity and hepatic clearance of insulin

The relationships between insulin clearance and insulin sensitivity are shown in Figure 8.02 and Equation 8.03.
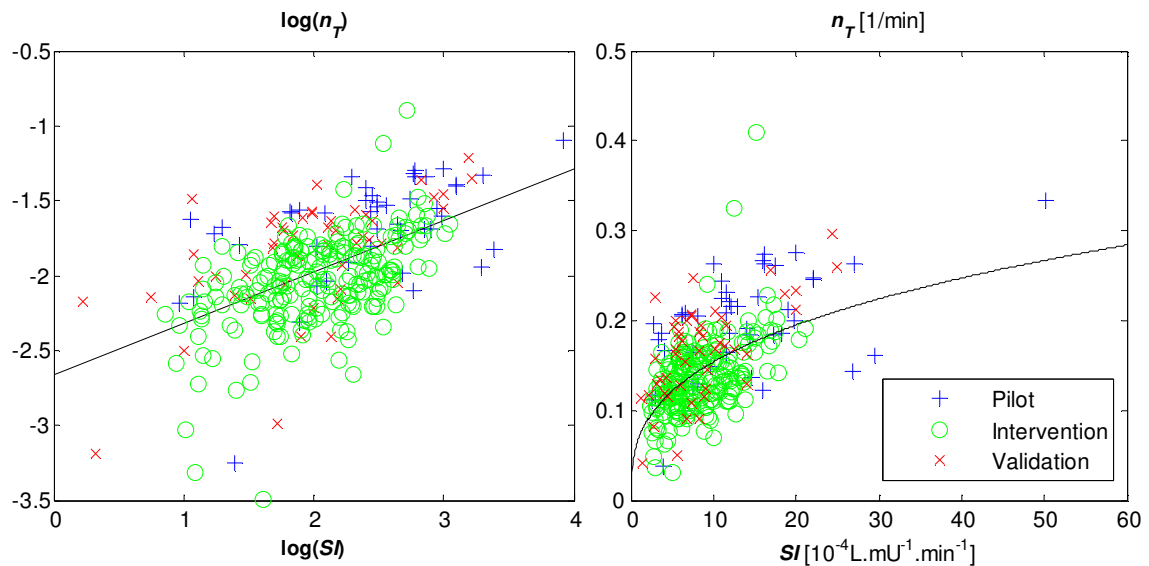
Figure 8.03. The log-log and normal plots of *SI* and hepatic clearance using all available data points and the derived population relationship (-).

It can be observed that insulin clearance had a tendency to increase with increasing insulin sensitivity. This is anecdotally reasonable, as an insulin sensitive individual may require greater clearance to avoid hypoglycaemic incidents, whereas a resistant individual would benefit from using all available insulin to clear glucose. However, there is a lack of direct observations of this behaviour in the literature. A study by Meier *et al.* (Meier *et al.* 2007) showed a greater disparity between C-peptide and insulin concentrations in insulin sensitive individuals. As the liver only clears insulin and kidney clearance of both hormones is assumed equivalent, it can be reasonable to conclude that hepatic clearance was greater in the sensitive group in the Meier *et al.* study.

The hepatic clearance metric exhibits distinct behaviour between the cohorts tested. While the pilot and validation data showed relatively similar relationships, the intervention study showed a reduced hepatic clearance at equivalent insulin sensitivity. This is likely to be an artefact of the selection criteria of the individual studies. While the pilot and validation cohorts sought diverse anatomical characteristics, the intervention study of TeMorenga *et al.* required that all participants exhibit risk factors for the development of type 2 diabetes. Thus, there may be etiological reason for the distinction. Figure 8.04 shows how the intervention cohort lies somewhat below the pilot and validation-derived lines that are relatively similar.
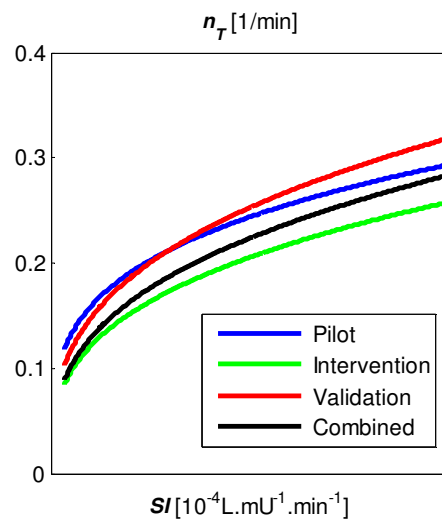
Figure 8.04. Overlaid plasma insulin clearance estimation equations from the various derivation cohorts

$$n_T = 0.0982 \cdot SI^{0.268} \hspace{5cm} \text{8.03-P}$$

$$n_T = 0.0672 \cdot SI^{0.329} \hspace{5cm} \text{8.03-I}$$

$$n_T = 0.0809 \cdot SI^{0.335} \hspace{5cm} \text{8.03-V}$$

$$n_T = 0.0697 \cdot SI^{0.343} \hspace{5cm} \text{8.03-C}$$

8.2.2.4 Relationships between insulin sensitivity and insulin production

Relationships between insulin sensitivity and insulin production at each minute were defined using the methods associated with Equations 8.01 and 8.01a. For clarity, three such production rates are isolated as indicative cases and presented in Figure 8.05 and Equations 8.04 to 8.06. These cases include the relationships between insulin sensitivity and basal (U_B), first phase (U_1) and second phase (U_2) of insulin production. Figure 8.05 shows the combined cohort relationships between insulin sensitivity and basal, first and second phases of insulin production.
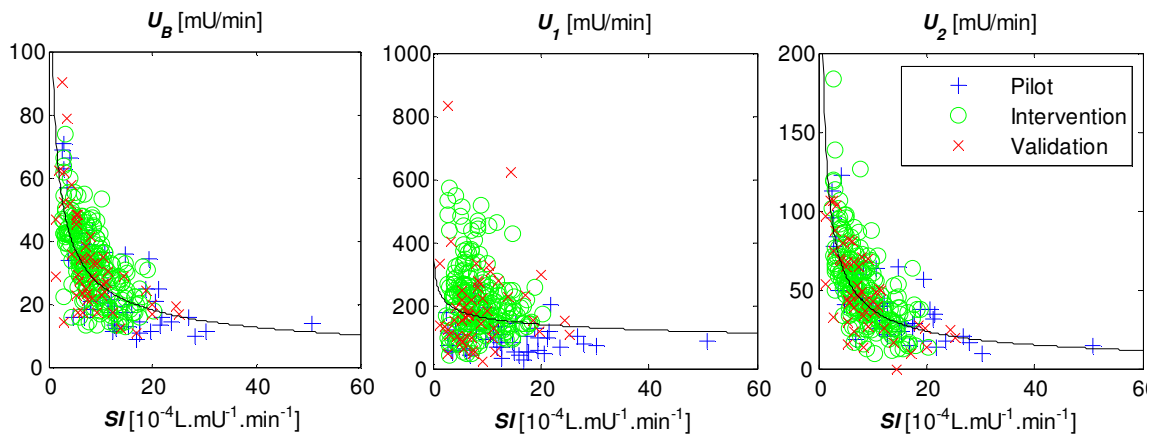
Figure 8.05. Individual plots of insulin production at basal, first phase and second phase of production as functions of insulin sensitivity.

A visual inspection of U_B and U_2 in Figure 8.05 show that power equations are appropriate for these relationships. A strong relationship between U_1 and insulin sensitivity does not exist with significant variation in U_1 across all SI values. However, as no *a-posteriori* (link to insulin sensitivity) or *a-priori* estimation method accurately predicts U_1, the power relationship to SI will be used to maintain consistency with the remainder of the insulin production profile. The relatively small exponential values in Equation 8.05 show a lack of distinction between the first phase responses of sensitive and resistant individuals.

Figures 8.06 to 8.08 show how the various cohorts affected the three indicative endogenous insulin production estimation equations. While the basal (U_B) and second phase (U_2) equations

yield remarkably similar insulin production rates for equivalent sensitivity values, the more sporadic nature of the first phase insulin production reduces the equivalence across cohorts.

Basal insulin production

$$U_B = 87.1 \cdot SI^{-0.579} \qquad\qquad 8.04\text{-P}$$

$$U_B = 84.0 \cdot SI^{-0.490} \qquad\qquad 8.04\text{-I}$$

$$U_B = 68.9 \cdot SI^{-0.435} \qquad\qquad 8.04\text{-V}$$

$$U_B = 85.7 \cdot SI^{-0.519} \qquad\qquad 8.04\text{-C}$$

First phase insulin production

$$U_1 = 140.9 \cdot SI^{-0.054} \qquad\qquad 8.05\text{-P}$$

$$U_1 = 174.3 \cdot SI^{-0.050} \qquad\qquad 8.05\text{-I}$$

$$U_1 = 220.2 \cdot SI^{-0.056} \qquad\qquad 8.05\text{-V}$$

$$U_1 = 136.7 \cdot SI^{-0.233} \qquad\qquad 8.05\text{-C}$$

Second phase insulin production

$$U_2 = 160.9 \cdot SI^{-0.624} \qquad\qquad 8.06\text{-P}$$

$$U_2 = 180.9 \cdot SI^{-0.605} \qquad\qquad 8.06\text{-I}$$

$$U_2 = 132.5 \cdot SI^{-0.487} \qquad\qquad 8.06\text{-V}$$

$$U_2 = 174.0 \cdot SI^{-0.604} \qquad\qquad 8.06\text{-C}$$
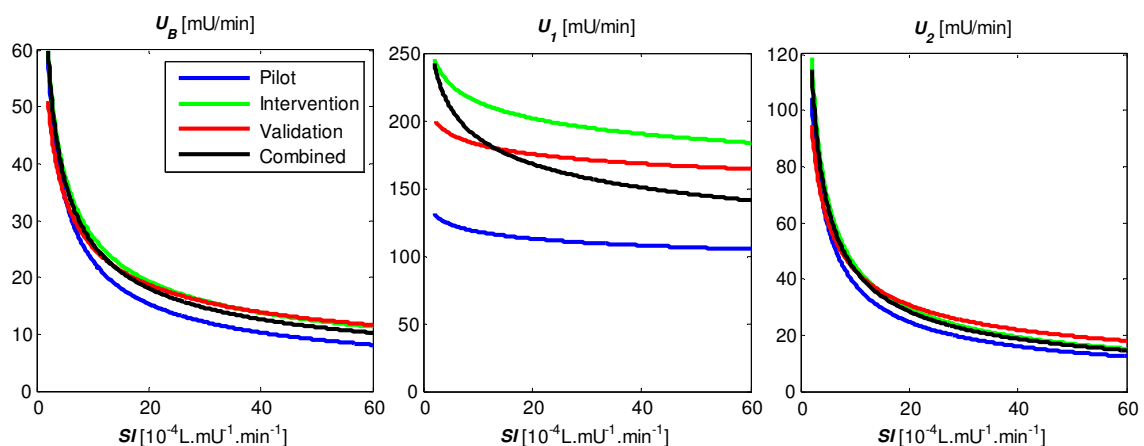
Figure 8.06. Overlaid basal insulin production estimation equations from the various derivation cohorts

The three endogenous insulin production cases presented in Figures 8.06 and Equations 8.04 to 8.06 are merely indicative of the distinct phases of insulin production. The DISTq method

estimates a participant's endogenous insulin production using a series of equations that define a profile with 1-minute resolution. Thus, 60 U_N to SI equations were derived using the available participants' 60 minute fully-sampled U_N results. Figure 8.07 shows how the 60 equations were aligned to create a surface that defines 60-minute insulin production profiles as a function of insulin sensitivity, for all participants. To predict an insulin production response to the test stimulus, the DISTq identification method takes a 'slice' of the surface map of Figure 8.07 along the relevant insulin sensitivity value.
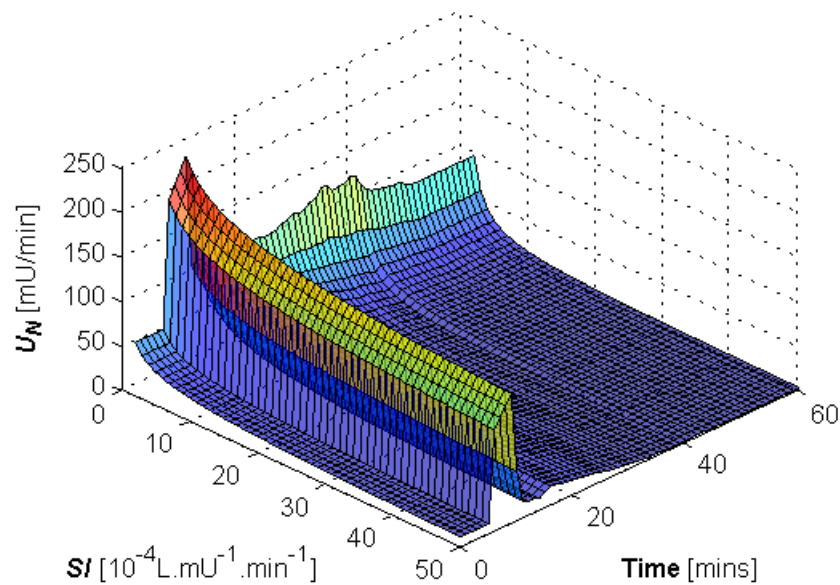
Figure 8.07. A series of insulin production profiles as a function of insulin sensitivity.

Links between insulin sensitivity and insulin production have been made in the literature (Ferrannini & Mari 2004; Mari 1998; Pacini & Mari 2003), while others have found explicitly similar relationships to those presented here (Cobelli *et al*. 2007). Furthermore, it is reasonable to assume that insulin resistant individuals who require more insulin for glucose homeostasis would attempt to produce more. However, the proposed relationship does not take into account the lack of production capability seen in those with long-term exposure to diabetes. These individuals suffer β-cell damage often because of long-term insulin resistance and become unable to produce sufficient insulin, as illustrated by some points on Figure 8.05. Thus at the lower range of insulin sensitivity, insulin production rates would be expected to 'trumpet out' covering a range of particularly high, and low rates. This feature can also be observed in Figure 8.05.

This 'trumpeting' effect may provide potential for significant improvement of the estimation processes. If duration of diabetes was known by the researcher either a third dimension could be added to the estimation graphs that could predict the extent of β-cell dysfunction, or a second

surface could be developed in which β-cell function profiles were provided as a function of the duration of diabetes rather than insulin sensitivity. However, duration of diabetes was not reported for any DISST participants and this possibility cannot be evaluated at this stage.

8.2.2.5 Alterations to published methods

To gain equivalence across all cohorts and analyses, consistent methods and processes are used for all studies. The DISTq method has been developed to maximize clinical applicability and mathematical identifiability of both insulin sensitivity and the population based equations themselves.

Preliminary presentations of the DISTq method (Docherty et al. 2009; Docherty et al. 2011c) used different expressions to link insulin sensitivity to the unknown parameters. In particular, endogenous insulin production profiles were generated from three indicative production rates (U_b, U_1, and U_2), while n_L was used as a model parameter instead of n_T. Furthermore, arduous iterative least squares methods were used to identify the constants of Equation 8.01 for the various parameters. These least squares methods exaggerated the impact of sensitive individuals, and thus, limited the applicability of the relationships to resistant individuals who arguably occupy the region of maximum desired accuracy.

Initial DISTq methods (Docherty *et al.* 2009) used three indicative insulin production rates to construct an insulin production profile. However, this method was *ad-hoc*, heuristic and required un-necessary assumptions. Using linear regression of the log-log relationships allowed equations of U_N at minute-wise resolution to be produced.

The total extraction of insulin (n_T) rate was used instead of n_L due to the number of n_L=0 cases presented by the intervention study. The occurrence of n_L=0 during the intervention study implied an overestimation of renal insulin clearance, it is almost certain that these participants had (unidentifiable) hepatic clearance of insulin. n_T is the sum of n_L and n_K assuming no saturation of hepatic clearance. The analysis of Section 5.3.2 showed that the n_L and n_K parameters have very little mathematical distinction at insulin concentrations frequently encountered during the DISST test. Thus, insulin sensitivity was linked to whole body insulin clearance from the plasma in this study. Furthermore, a power relationship was used instead of the log relationship used in (Docherty *et al.* 2009) and the linear relationship used in (Docherty et al. 2011c).

Thus, although changes have been made to the DISTq process, they are minor and their effect on clinical outcomes is negligible. Further changes could potentially be made to the parameter estimation process to maximise accuracy. In particular, there is not necessarily a necessity to use

linearisable relationships between insulin sensitivity and the metrics of interest. If sufficient fully-sampled DISST results become available, smooth, but non-linear, relationships between insulin sensitivity and the un-observable parameters could be defined.

8.2.3 DISTq insulin sensitivity identification method

The DISTq method for predicted the participant's insulinaemic response to the test stimulus *a-posteriori*. Thus, the method is dependent on *SI* and requires an iterative method to identify *SI* (Docherty *et al*. 2009). The steps were as follows:

1. A population average *SI* value was defined (10×10^{-4}L·mU^{-1}·min^{-1} was used, although convergence was confirmed using a range of possible *SI* starting values (Docherty *et al*. 2009)).

2. The *SI* value was used in Equations 8.02, 8.03 and the 60 equations that define the surface in Figure 8.07 to obtain a basal insulin value, hepatic clearance rate, and an endogenous insulin production profile with 1-minute resolution.

3. The endogenous insulin production profile was used with the basal insulin, clearance rate and known test stimulus properties to simulate the insulin concentration response of the participant using the Equations 4.15 and 4.16.

4. The interstitial insulin profile defined in Step 3 was used with the measured glucose data in the iterative integral method (Section 3.4) (Docherty et al. 2011b; Docherty et al. 2009) to identify *SI* and V_G.

5. Iterate over Steps 2 to 4 updating the insulin response estimate with the most recently evaluated *SI* from Step 4. Continue iterations until *SI* converges. Typically, eight iterations achieves convergence in the order of 0.1%.

A second DISTq protocol was investigated as part of an investigation of variations to the DISST sampling protocol, which is presented in full in Chapter 9. This second protocol required only the basal and *t*=35 minutes glucose sample to identify insulin sensitivity. This method is called DISTq30 because of the possibility of shifting the basal glucose sample to immediately before the *t*=5-minute glucose bolus, thus a 30-minute protocol would be enabled. However, this protocol removes the ability to identify V_G as a variable parameter. Hence, V_G was estimated *a-priori* using a proportion of the participants lean body mass (*LBM*), as calculated by Hume (Hume 1966). The coefficient was defined using linear regression of the relationship between the LBM and V_G and produces the relationship:

$$V_G = 0.29LBM \qquad\qquad 8.07$$

Thus, DISTq30 involves considerably less clinical intensity than the DISTq. The *SI* identification process were the same as Steps 1 to 5, with the exception that Step 4 does not include an identification of V_G.

8.3 Investigation outcomes

Primarily, the DISTq must prove an ability to replicate the identified insulin sensitivity values obtained by the fully-sampled DISST. Secondly, a demonstration of the applicability of the parameter estimation equations in an isolated cohort must be investigated. Finally, the DISTq must be compared to the gold standard of insulin sensitivity testing the euglycaemic clamp (EIC).

8.3.1 DISTq comparison to the fully-sampled DISST

To evaluate the insulin sensitivity identification capability of the DISTq and DISTq30 method, the ability of the method to replicate the values of the fully-sampled method was tested. The fully-sampled DISST sensitivity values were identified using the iterative integral method described in Section 3.4 and Docherty *et al*. (Docherty *et al*. 2009). Equivalence was assessed using Pearson's correlation in tandem with the mean gradient. While the correlation measured the linear dimensionless equivalence, the gradient measures the possible bias or proportional shift that could be introduced by un-representative population-based parameter estimation equations. The gradient (*Grad*) is defined as the ratio of 2-norms (Equation 8.08).

$$Grad = \frac{\left\|SI_{DISTq(30)}\right\|_2}{\left\|SI_{DISST}\right\|_2} \qquad\qquad 8.08$$

If *Grad*>1 DISTq has, on average, overestimated the insulin sensitivity of a cohort.

Table 8.02 shows the correlations between the DISST, DISTq and DISTq30 when the intra-cohort population-based parameter equations were used. Hence, in this case, the pilot results were derived using parameter estimation equations derived with the pilot data. Similarly, for the other cohorts, the estimation equations were derived from their own fully-sampled data.

	DISST-DISTq R (*Grad*)	DISST-DISTq30 R (*Grad*)	DISTq-DISTq30 R (*Grad*)
Pilot Cohort	0.83 *(1.00)*	0.75 *(0.96)*	0.89 *(0.97)*
Intervention Cohort	0.86 *(1.05)*	0.76 *(1.13)*	0.92 *(1.07)*
Validation Cohort	0.84 *(1.07)*	0.86 *(1.18)*	0.99 *(1.10)*
Combined Cohort	0.82 *(1.04)*	0.72 *(1.09)*	0.91 *(1.04)*

Table 8.02. Correlations and gradients of the DISST and DISTq derived insulin sensitivity values.

The correlations and gradients observed in Table 8.02 were consistent with those observed in previous analyses (Docherty et al. 2009; Docherty et al. 2011c). The DISTq method correlated relatively well with the fully-sampled DISST (R=0.82). Thus, these results validate the form of the population-based relationships and the identification method. Furthermore, the gradients are all close to one, showing the magnitude of the estimated parameters was appropriate.

The full DISTq protocol that uses all available glucose assays generally showed a superior accuracy and precision in terms of repetition of the fully-sampled DISST results than the DISTq30. This result was expected because the lack of glucose measurements taken during the DISTq30 limits the definition of the glucose concentration gradient. The LBM-based V_G estimation generated a reasonable estimate, as evidenced by the relatively high accuracy and precision between DISTq and DISTq30. However, the ability for both the fully-sampled DISST and the DISTq to measure the same glucose decay gradient maximises their equivalence.

The predominant finding of this analysis is that insulin sensitivity can be observed relatively accurately during dynamic insulin sensitivity tests using only glucose data. This outcome is a significant development in the field of insulin sensitivity testing, as the prevailing assumption is that insulin assays are critical to the identification of insulin sensitivity. Hence, a wide range of previously impossible clinical and research opportunities and applications are enabled.

In effect, DISTq succeeds where other glucose-only tests fail because the concentration of insulin can be simulated with relatively high accuracy. With the exception of very few individuals, the 1U insulin bolus overwhelms the endogenous insulin appearance (predominantly due to the ~70% first pass hepatic extraction of insulin). Thus, when the dispersion of this insulin is modelled with the pharmaco-kinetic parameters defined by Van Cauter *et al*. (Van Cauter *et al*. 1992), relatively accurate interstitial insulin concentrations can be defined.

The identified DISTq values are most sensitive to errors in the insulin clearance parameter (n_T or n_L). This value moderates the insulin bolus, which is the predominant insulin signal. Errors in

insulin production or basal insulin have an effect on the resultant insulin sensitivity values. However, this effect is mitigated by the dependence of the method on the insulin clearance term. This is why the relatively inaccurate first phase estimation equation (Figure 8.05(middle)) does not have a more deleterious effect on the resultant sensitivity values. If the protocol did not include the insulin bolus, the identification method's sensitivity to the first-phase production estimation would increase significantly. As the first phase prediction is relatively erroneous, this would inhibit the accuracy of the method, and the protocol could not yield clinically useful metrics.

8.3.2 DISTq in an isolated cohort

If DISTq were used in a clinical study, no cohort specific population-based parameter estimation equations would be available. The population derived parameter equations must be provided from a previous fully-sampled DISST investigation. Thus, the applicability of the parameter estimation equations must be evaluated in isolated cohorts. The population based equations derived using the pilot cohort were thus used to evaluate the equivalence between the fully-sampled DISST, DISTq and DISTq30 in the intervention, validation and combined cohorts. Subsequently, the intervention, validation and combined cohort derived equations were used in isolated cohorts. Tables 8.03 to 8.06 show the outcomes of these analyses

Pilot cohort derived parameter equations

	DISST-DISTq R (*Grad*)	DISST-DISTq30 R (*Grad*)	DISTq-DISTq30 R (*Grad*)
Intervention Cohort	0.86 *(1.38)*	0.77 *(1.48)*	0.92 *(1.08)*
Validation Cohort	0.84 *(1.15)*	0.86 *(1.28)*	0.99 *(1.11)*

Table 8.03. Correlations and gradients of the DISST and DISTq derived insulin sensitivity values when the pilot cohort-derived equations are used.

The pilot derived equations maintained a relatively high correlation between the fully-sampled DISST and the DISTq. The validation cohort correlation increased marginally. However, the DISTq overestimated the insulin sensitivity values in both the intervention and validation cohorts. The gradient values show that DISTq and DISTq30 both overestimated the intervention cohort's insulin sensitivity. This difference was due the disparity between the intervention behaviour and the pilot derived assumptions. Figures 8.02 and 8.06 show that the basal insulin and insulin production rates were reduced in the pilot study. Furthermore, the hepatic clearance was lowest

for the intervention study. Thus, the participant insulin concentration was frequently underestimated by the DISTq method for the intervention cohort when the pilot derived equations were used. Consequently, insulin sensitivity was overestimated. The disparity had a similar, but somewhat attenuated, effect on the outcomes of the validation cohort.

Intervention cohort derived parameter equations

	DISST-DISTq R (*Grad*)	DISST-DISTq30 R (*Grad*)	DISTq-DISTq30 R (*Grad*)
Pilot Cohort	0.82 *(0.78)*	0.74 *(0.72)*	0.88 *(0.92)*
Validation Cohort	0.84 *(0.99)*	0.86 *(0.96)*	0.99 *(1.07)*

Table 8.04. Correlations and gradients of the DISST and DISTq derived insulin sensitivity values when the intervention cohort-derived equations are used.

Given the overestimating effect that the pilot derived equations had on the intervention cohort's DISTq insulin sensitivity values, it is intuitive that the intervention derived parameter estimation equations would underestimate the pilot cohort sensitivity values. The intervention cohort-derived equation would tend to cause an overestimation of the insulin concentration for the participants of the pilot cohort. Hence, these results are expected. Note that the correlations remain high, indicating a robustness of the DISTq method.

Validation cohort derived parameter equations

	DISST-DISTq R (*Grad*)	DISST-DISTq30 R (*Grad*)	DISTq-DISTq30 R (*Grad*)
Pilot Cohort	0.83 *(0.96)*	0.74 *(0.87)*	0.88 *(0.90)*
Intervention Cohort	0.85 *(1.30)*	0.76 *(1.35)*	0.92 *(1.04)*

Table 8.05. Correlations and gradients of the DISST and DISTq derived insulin sensitivity values when the validation cohort-derived equations are used.

The insulin concentration levels in the validation cohort were between the pilot and intervention cohort levels on average. Thus, the validation cohort equations reduced the magnitude of the DISTq *SI* estimates of the pilot cohort and over-estimated those of the intervention study. Hence, this cohort represents a middle ground cohort.

Combined cohort derived parameter equations

Table 8.06 shows the effect of the combined cohort's parameter estimation equations on it's constituent cohorts DISTq insulin sensitivity values.

	DISST-DISTq R (*Grad*)	DISST-DISTq30 R (*Grad*)	DISTq-DISTq30 R (*Grad*)
Pilot Cohort	0.82 *(0.86)*	0.74 *(0.80)*	0.88 *(0.93)*
Intervention Cohort	0.86 *(1.15)*	0.76 *(1.24)*	0.92 *(1.08)*
Validation Cohort	0.84 *(0.98)*	0.86 *(1.07)*	0.99 *(1.09)*

Table 8.06. Correlations and gradients of the DISST and DISTq derived insulin sensitivity values when the combined cohort-derived equations are used.

Overall, the findings of the isolated derivation study showed that the correlation between the fully-sampled DISST and the DISTq was relatively well maintained when isolated estimation equations were used. Tables 8.03 to 8.06 show that equations from an isolated cohort had limited adverse effects on the correlation between the fully-sampled DISST and DISTq/DISTq30 insulin sensitivity values. However, the divergence from a regression gradient of 1.0 indicates that equivalence in terms of magnitude deteriorated. There was very little effect on the correlations or gradients between DISTq and DISTq30 when isolated derivation equations are used.

The effect of an isolated equation set was greatest on the intervention study. The intervention study cohort recruitment policy targeted participants with anatomical and/or inherited disposition towards diabetes (TeMorenga *et al*. 2010). Thus, the cohort exhibited systematic differing characteristics from the pilot or validation cohorts. This difference was most marked in the plasma insulin clearance rate (n_T) that was lower with respect to insulin sensitivity than the equivalent clearance values of the pilot or validation cohorts. Hence, it was a systematic bias based on the participant selection criteria for that specific investigation.

The insulin clearance term (n_T) had a significant effect on the identified insulin sensitivity value as the insulin clearance parameter has the greatest impact on the concentration of interstitial insulin. The average rate of first phase insulin production is approximately, 700 mU. Approximately 70% of this amount is cleared in the first hepatic pass extraction. Thus, approximately 200 mU of endogenous insulin enters circulation due to the first phase insulin release. This has significantly less effect than the 1000 mU of exogenous insulin that is administered IV (and thus is not affected by first pass hepatic extraction). As the insulin clearance parameter moderates the effect of the exogenous bolus, the primary contributor to the measured signal, the identification of insulin sensitivity is most sensitive to this metric.

Thus, the distinction in the behaviour of insulin clearance with respect to insulin sensitivity exhibited between the intervention cohort and the pilot and validation cohorts prompted a proportional shift in the DISTq identified insulin sensitivity values. The pilot cohort also seemed more susceptible to this insulin clearance distinction than the validation study.

The final outcome of this analysis implied that the linearity of the DISTq identified insulin sensitivity values is particularly robust when an isolated cohort is used to derive the parameter estimation equations. However, equivalence in terms of the magnitude of the identified values was reduced by the effect of the disparate insulin concentrations of the various cohorts. Thus, there is potential for the development of the parameter estimation processes by incorporating *a-priori* physiological, or epidemiological data into the parameter estimation functions that are currently functions of insulin sensitivity alone. For example, if a participant could satisfy the recruitment criteria of the intervention study, the intervention cohort-derived estimation equations should be used. Otherwise, either the pilot or the validation equations would be more appropriate.

8.3.3 DISTq verses the euglycaemic clamp

Only the validation cohort participants undertook both the DISST and euglycaemic clamp. Thus, the data obtained during the validation investigation was sufficient to obtain a comparison between the DISTq and the gold standard EIC. The study design, recruitment policies, test protocols and results were presented in Chapter 6 and will not be repeated here.

Population based parameter equations from the pilot cohort (not the validation cohort) were used in this analysis. This cohort was most representative of the anatomical range expected in the validation cohort and was also isolated and independent of the validation cohort. It is important to use an isolated cohort as the use of the DISTq in a clinical setting would not allow the patient specific identification of these parameters. Equation 6.03 was used convert the DISTq insulin sensitivity value to mimic the units of the euglycaemic clamp sensitivity index. Both DISTq and DISTq30 metrics were identified.

Figures 8.08, 8.09 and 8.10 show the correlations, Bland-Altman plots and receiver operator curves (ROC) for the EIC and DISTq/DISTq30 derived sensitivity values. The correlation between DISTq and the EIC was marginally less than that of DISTq30 (R=0.76 compared to R=0.77). The gradients between the sensitivity values of the two tests were 1.04 and 1.10 for the DISTq and DISTq30, respectively. However, a single outlier (Participant 46, discussed in Section 6.5.3), had a significant negative effect on these values and could be removed. The outlier is particularly visible in Figure 8.08 (Right) at approximately EIC=9, DISTq=22. This participant

drank copious amounts of sugary drink, and thus had an unprecedented physiological insulin secretion response relative to any assumed profile. Removal of this participant's data changed the DISTq and DISTq30 correlations to R=0.77 and R=0.80 respectively and the gradients to R=1.01, and R=1.05 respectively.
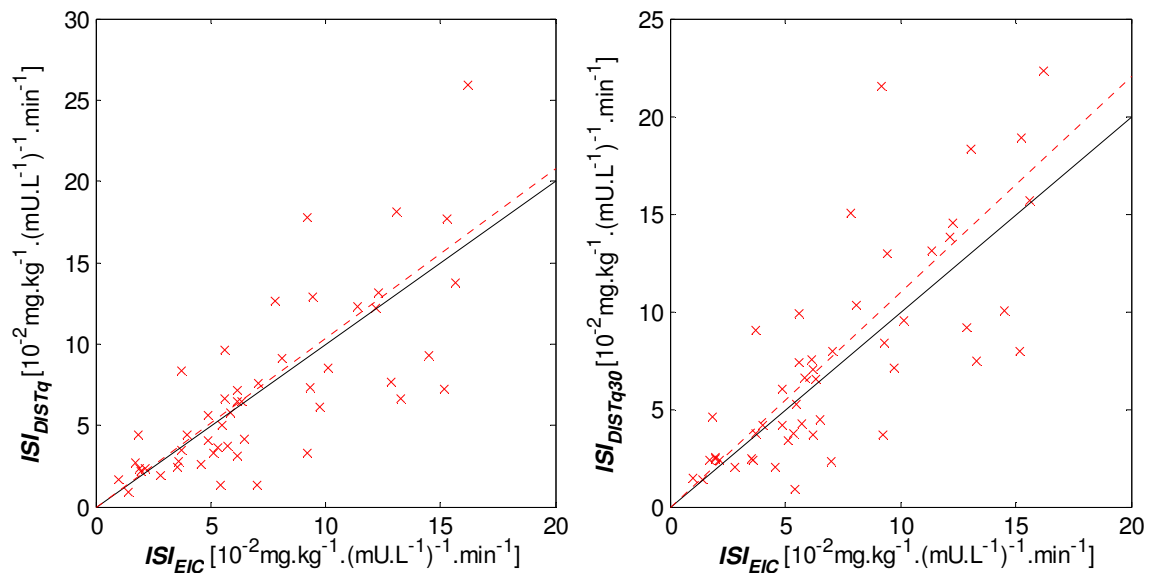
Figure 8.08. Comparison of *SI* values between the DISTq and EIC (left) and the DISTq30 and EIC(right). (1:1 line (-), mean gradient (- -)).
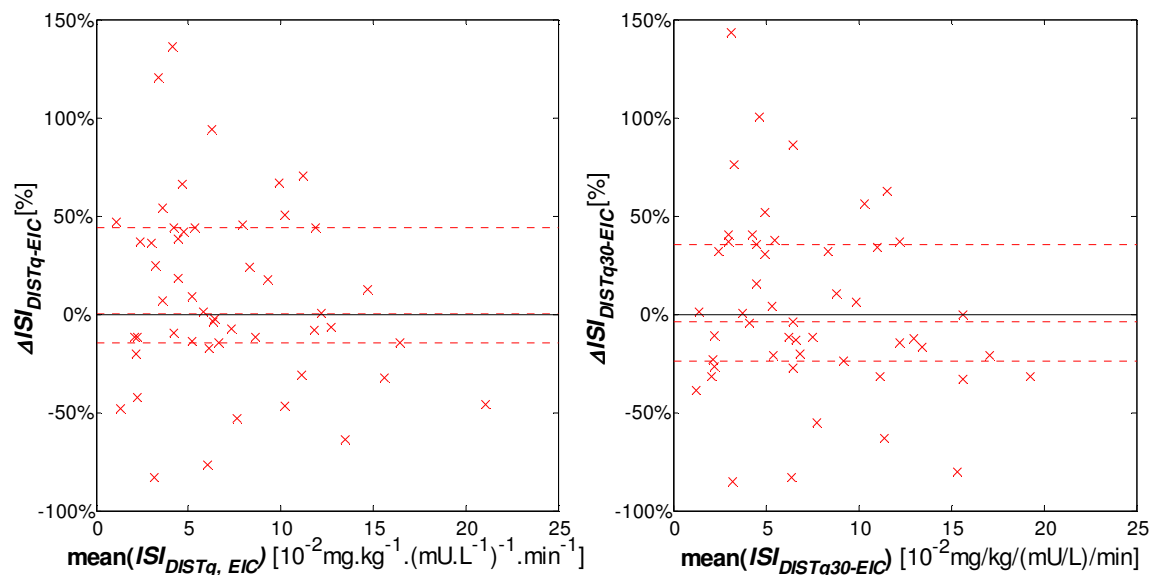
Figure 8.09. Bland-Altman plots showing the median, and inter-quartile range of the sensitivity value discrepancy between the test outcomes.

The median discrepancy for DISTq was +0.7% confirming the mean gradient shown in Figure 8.08(left). The inter-quartile range of the proportional difference was -15.9% to 43.6%. The

DISTq 30 had a similar low median error 4.1% that confirmed the mean gradient from Figure 8.08(right). The inter-quartile range was -24.1% to 35.4%. Thus, both methods performed similarly. Figure 8.10 presents the ROC curve for the DISTq and DISTq30 versus the EIC. The area under the ROC curves for were c-ROC=0.89 and 0.91 for the DISTq and DISTq30, respectively. Hence, both perform equally relative to the EIC.
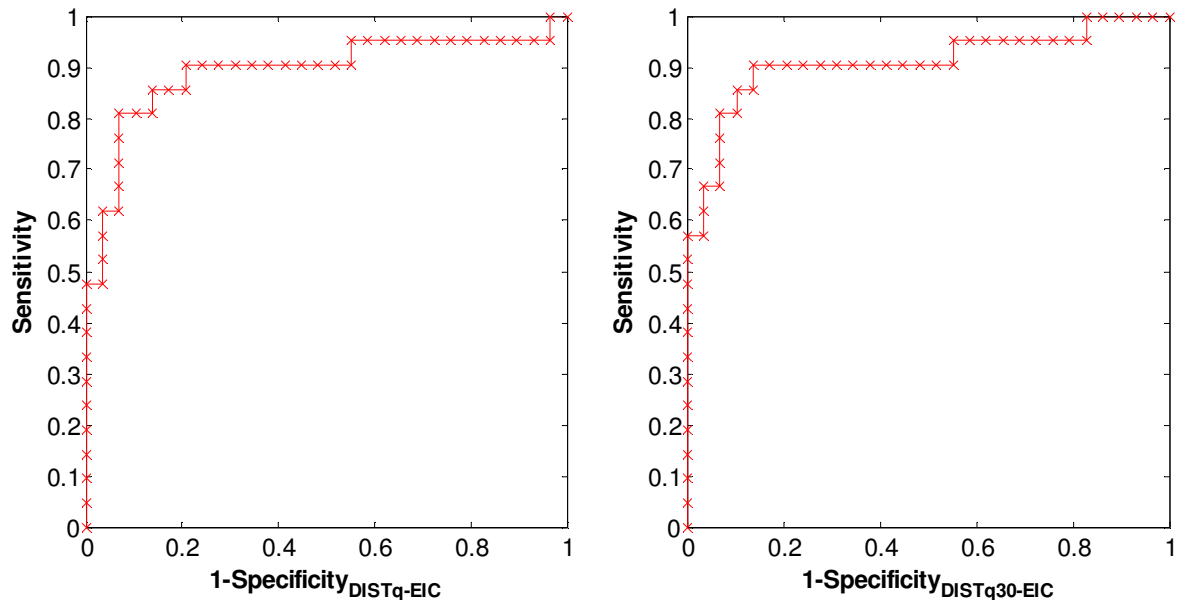
Figure 8.10. ROC curves of the DISTq metrics and the EIC using an arbitrarily selected cut-off threshold of $6.94 \times 10^{-2} mg \cdot kg^{-1} \cdot (mU \cdot L^{-1})^{-1} \cdot min^{-1}$.

8.3.4 Tracking clinical intervention

Insulin sensitivity tests are often used in clinical application to measure the effect of interventions or drugs. The effect of a high protein diet was measured using the DISST in 74 participants (TeMorenga *et al*. 2010). Thus, the data was available to investigate whether the DISTq methods can also be used to quantify the sensitivity shift.

Participants of the intervention study underwent DISST tests on weeks 0, 4 and 10. Insulin sensitivity values were identified for each test using the fully-sampled DISST and the DISTq and DISTq30 methods. Insulin sensitivity was converted to the EIC units to minimise the effect of V_G variation of the identified *SI* value. The change due to the intervention was defined as difference between the final and initial sensitivity values divided by the mean identified value (Equation 8.09).

$$\Delta = \frac{SI_3 - SI_1}{\frac{1}{3}\sum SI_{1,2,3}}\,\%$$

Figure 8.11 shows the correlation of the percentage insulin sensitivity changes as measured by the fully-sampled DISST and the DISTq and DISTq30 methods. The insulin sensitivity change observed by the DISTq correlated to the fully-sampled DISST at R=0.81, while the DISTq30 correlated to the fully-sampled DISST at R=0.72. Figure 8.12 shows the Bland-Altman plots of the fully-sampled to DISTq measured changes.
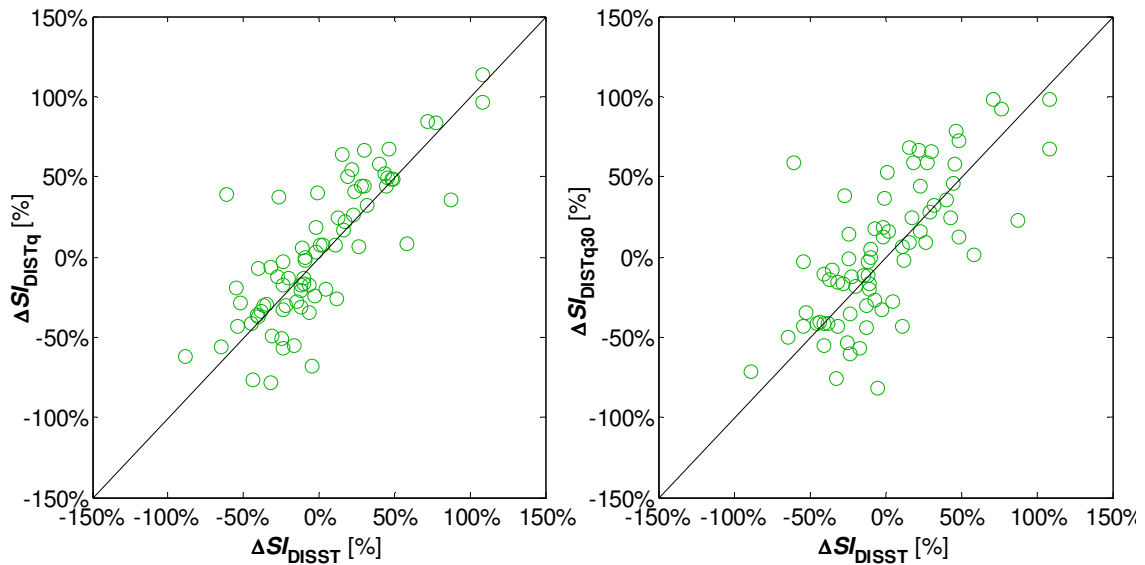
Figure 8.11. Correlations between the proportional change in insulin sensitivity measured by the fully-sampled DISST and the DISTq (left) and DISTq30 (right).
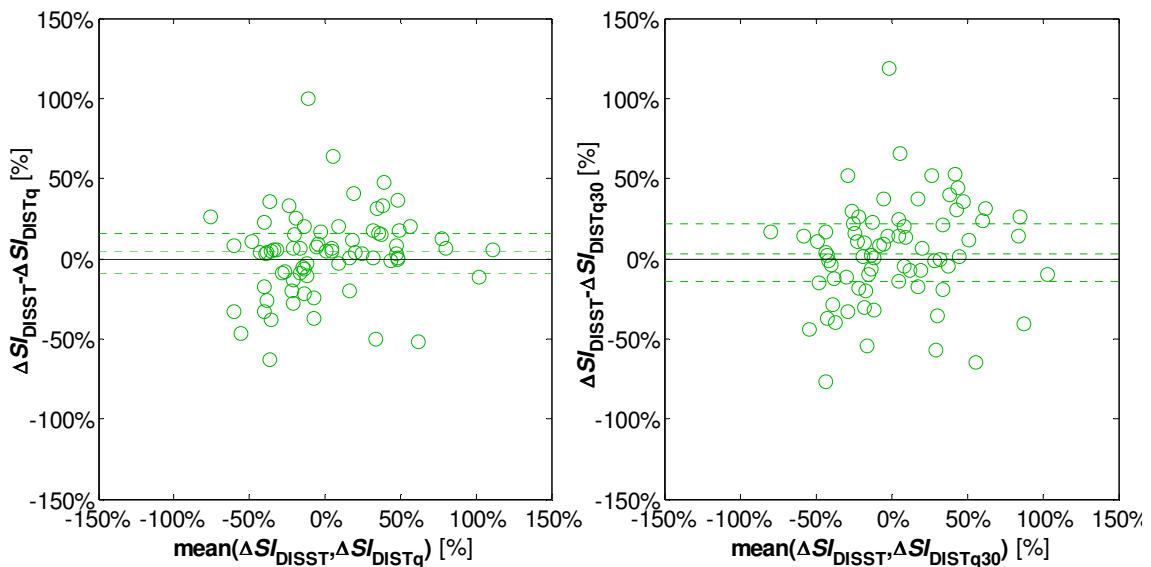
Figure 8.12. Bland-Altman plots of the proportional change measured by the DISST and the DISTq (left) and DISTq30 (right) methods.

The median discrepancy between the SI changes measured by DISTq and the fully sampled DISST was 4.4% and the interquartile range was -9.4% to 16.1%. The DISTq30 results showed a greater variation from the fully-sampled DISST measured changes (median=2.99%, IQR=-14% to 22%).
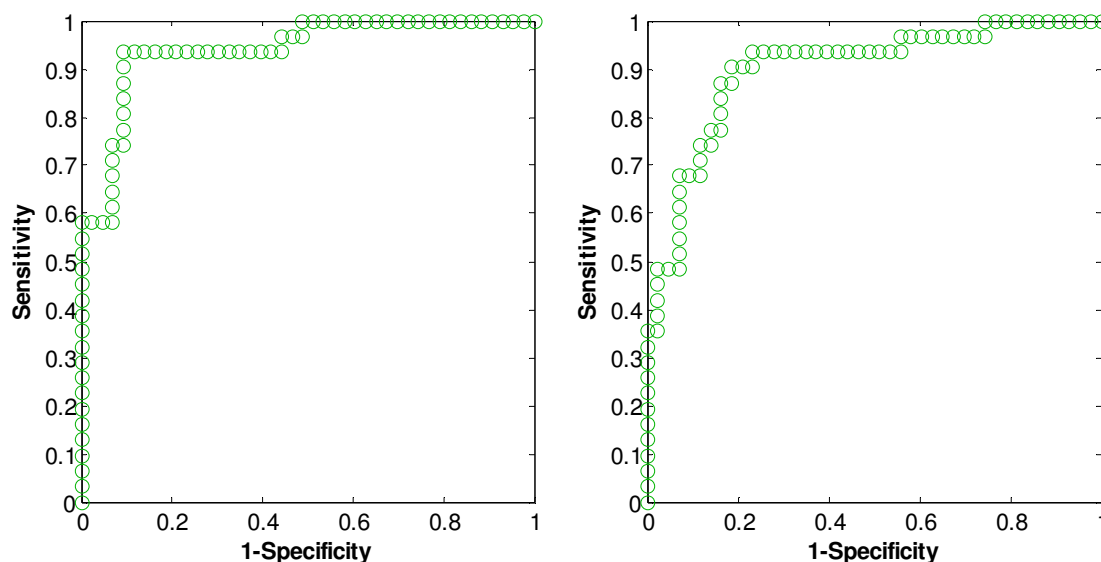
Figure 8.13. ROC curves of the fully-sampled DISST and the DISTq (left) and DISTq30 (right) using a diagnosis value of 0%.

Figure 8.13 shows the ROC curves for this analysis. The area under the ROC curves defined using a diagnostic cut off value of 0% showed a relatively high diagnostic equivalence between the insulin sensitivity identification methods. DISTq and the fully-sampled DISST provided a c-ROC=0.94 while DISTq30 and the DISST provided c-ROC=0.90.

8.4 Outcomes of the DISTq evaluation

DISTq and DISTq30 exhibited sufficient equivalence to the EIC to be considered a suitable surrogate. The ROC curves showed a strong diagnostic equivalence between DISTq and EIC. This equivalence was confirmed by the mean gradients and median differences between the two tests.

Other insulin sensitivity metrics that use only glucose assays (i.e. 2hr glucose, FPG) generally result in low-resolution metrics (Table 2.02). This poor outcome is predominantly due to the un-measured participant insulin concentration during the tests. However, DISTq is capable of higher

than expected accuracy as the protocol introduces a known amount of insulin to the body. Equally, it has the iterative ability to estimate an endogenous insulin profile.

Experience across broad cohorts has allowed the development of a method to accurately quantify how this known quantity of insulin is dispersed to the active, interstitial regions of the body and how insulin clearance and production can be roughly approximated with a function of insulin sensitivity. Furthermore, the protocol avoids issues associated with extended basal periods or hyper-physiological doses that obscure the efficiency of insulin with saturation effects. Thus, glucose and anatomical data alone *can* provide sufficient information to generate a very accurate insulin sensitivity value.

DISTq30 showed a marginally improved equivalence to the EIC than the more intense DISTq. However, the DISTq tended to correlate better to the fully-sampled DISST than DISTq30. This behaviour is a result of the greater similarity between the DISTq and DISST and the similarity between the DISTq30 and the EIC. Both the DISTq and DISST used all available glucose data, and identified V_G as a parameter. Thus, both metrics were an accurate reflection of the measured gradient of glucose disposal. DISTq30 estimated V_G and thus the glucose decay gradient was not explicitly observed. However, the V_G estimate of the DISTq30 was equivalent to the EIC methodology, which effectively estimates V_G by normalising the glucose clearance rate by the participant's body weight. Hence, the commonality of parameter identification has enhanced the equivalence of the sensitivity values across these two specific tests.

DISTq was most sensitive to errors in the insulin clearance parameter. Early attempts to mitigate this error included the application a second iterative cycle, which varied the n_T value to maximise glucose simulation fit to data. This extension achieved an increase in correlation from R=0.86 to 0.91 in the pilot cohort (Docherty *et al*. 2009). However, this method failed to improve the correlation of the intervention cohort, increased the complexity of the already complex identification method and required a frequently sampled protocol. Thus, the validation study protocol did not produce data that could use the n_T refinement cycle. Hence, the additional n_T refinement cycle was abandoned in subsequent investigations.

The tendency for the intervention study to have a lower than expected clearance rate at equivalent insulin sensitivity values indicates that slight accuracy gains may be possible by using tailored parameter estimation equations. Thus, if a participant had a genetic disposition towards type 2 diabetes, equations derived from a cohort with a similar background might improve the individual's identified insulin sensitivity result. However, an investigation of this possibility would require significant investment as sufficient participants would be required to investigate a number of targeted cohorts.

The outlier mentioned in the validation cohort (Participant 46 in Section 6.5.3) showed that the method could produce incorrect results in isolated cases of unusual physiology. The outlier had a particularly abnormal physiology that the DISTq method was unable to predict. The participant consistently drank 5+ sugary energy drinks per day and as such, had a very large first phase of insulin production. Thus, the participant's glucose decayed particularly fast, and the DISTq method predicted a smaller insulin production response (Equations 8.04-8.06). Unfortunately, the iterative nature of the DISTq identification method amplified the error. The second and subsequent iterations produced smaller insulin responses and thus greater insulin sensitivity. However, this effect could have been ameliorated by the application of the knowledge of the participant's abnormal physiology. It was known prior to testing that the participant would likely have an increased first phase of insulin production because of her abnormal daily glucose intake. This knowledge could have been incorporated into the parameter estimation equations and the participant's results would come more into line, given an appropriate, validated adjustment.

The fully-sampled DISST identification method had access to the C-peptide assays from this participant, and thus was able to accurately quantify the participant's insulin production response. This participant's sensitivity values did not produce a significant adverse affect on the correlation between the fully-sampled DISST and the EIC. The participant produced almost 3 units of insulin during the first phase of production, which was four times greater than the observed median production of 0.7 units. Although the DISST was capable of capturing this anomaly, the DISTq could not because of the fundamental assumptions it must make.

8.4.1 Comparison between the DISTq and established metrics

The DISTq performance compares favourably to many surrogate insulin sensitivity tests that are currently proposed. Table 2.02 shows that OGTT metrics have exhibited correlation values to the EIC between R=0.51 and R=0.81 (Breda *et al.* 2002; Dalla Man *et al.* 2005a; Gutt *et al.* 2000; Lorenzo *et al.* 2010; Malita *et al.* 2010; Piché *et al.* 2007; Pigeon *et al.* 2009; Soonthornpun *et al.* 2003). The DISTq30 equalled the upper limit of this range, while DISTq was very close. Furthermore, the gradient between the DISTq metrics and the EIC was very close to 1.0 implying a very strong diagnostic equivalence between the tests.

DISTq and DISTq30 are significantly less intense tests than the OGTT. The DISTq and OGTT require the same number of skin punctures. However, the oral consumption of the OGTT drink can be difficult, with incidences of vomiting. The amount lost must be estimated and made up again, thus introducing significant potential for error. Furthermore, the OGTT requires a 2 hour

protocol and requires insulin assays when identifying insulin sensitivity. Thus, it is not real-time capable. In contrast, the sampling protocol of the DISTq is more intense than the OGTT with 10 minute as compared to 30-minute sampling frequency.

Table 2.02 shows the range of correlations that the IVGGT and IM-IVGTT have achieved against the EIC (R=0.44-0.89) (Bergman *et al*. 1987; Donner *et al*. 1985; Erichsen *et al*. 2004; Ferrannini & Mari 1998; Ferrari *et al*. 1991; Foley *et al*. 1985; Galvin *et al*. 1992; Mari & Valerio 1997; Rostami-Hodjegan *et al*. 1998; Saad *et al*. 1997; Scheen *et al*. 1994). The DISTq to EIC correlations found in this investigation are comparable to the upper extent of this range and exceed most studies. This result is very significant considering the very large disparity in the clinical intensity of the IVGTT and the DISTq protocols. Many IVGTT protocols require up to 3 hours of frequent sampling. Furthermore, insulin assays are required and the results are not available in real-time. The added intensity of the IVGTT thus offers sparse, if any, benefit over the DISTq, particularly when parameter identification failure can be a concern for the IVGTT.

There are a number of potential reasons for the poorer than expected result obtained from the IVGTT protocol. The frequent sampling should lead to a rich data set from which stable and accurate parameter values are obtained. However, this result is not the case. Most notably, identification of glucose dependant uptake and insulin sensitivity often encounters parameter trade-off and identifiability issues (Caumo *et al*. 1999; Pillonetto *et al*. 2002; Quon *et al*. 1994b). The reason for this trade-off has been presented in Section 5.5.2.

Furthermore, the prolonged IVGTT protocol exaggerates the effect of basal periods on the identified parameters. In contrast, the DISST protocol incorporates a very brief basal period as the measurement of the insulin effect on glucose at physiologically relevant doses is critical to the assessment of a relevant metric. IVGTT and IM-IVGTT protocols frequently use hyper-physiological doses, and thus significant saturation effects can affect the parameter values (Docherty et al. 2010; Kolterman et al. 1980; Natali et al. 2000; Prigeon et al. 1996; Rizza et al. 1981). Thus, the IM-IVGTT performance can improve through use of Bayesian methods (Cobelli *et al*. 1999; Erichsen *et al*. 2004; Pillonetto *et al*. 2003; Pillonetto *et al*. 2002) or by fixing the glucose dependant term such as the DISST (the p_G term), or by shortening the protocol and reducing the bolus doses. However, each of these solutions has their own caveats and trade-offs.

The DISTq methods require more intense protocols than HOMA, which requires a single fasting blood sample. Table 2.02 shows that the HOMA has a wide range of reported correlations to the EIC (R=0.22 to 0.93) (Bonora *et al*. 2000; Katsuki *et al*. 2001; Katsuki *et al*. 2002; Lotz *et al*. 2008; Mari *et al*. 2001; Mather *et al*. 2001; Matthews *et al*. 1985; Pacini & Mari 2003; Piché *et al*. 2007; Wallace *et al*. 2004). Thus, the DISTq exceeds the HOMA in terms of the EIC

equivalence defined in most studies. The low intensity of HOMA in the form it is generally administered means that despite the improved performance of the DISTq metrics, HOMA is still the best choice, low cost, low accuracy metric. However, if a researcher desires a higher resolution, more dependable test the DISTq is a suitable alternative.

In summary, Figure 8.14 defines the approximate position of the DISTq amongst the established insulin sensitivity tests, including also the DISST.
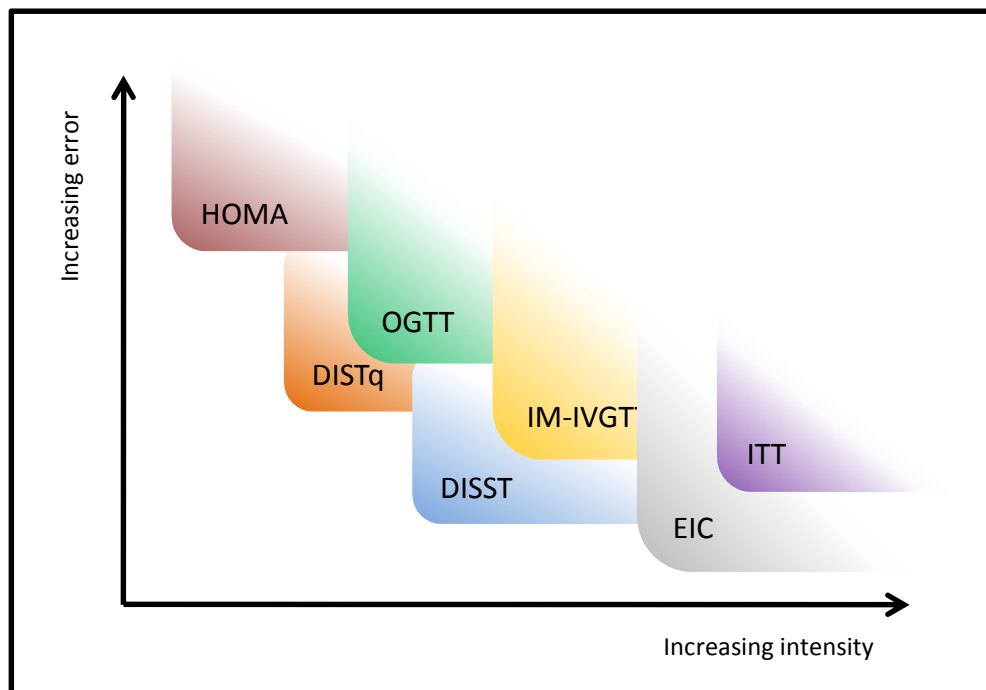
Figure 8.14. The relative position of the DISTq in comparison to other tests for the identification of insulin sensitivity.

8.4.2 Potential clinical uses for the DISTq

The comparatively strong equivalence between the DISTq and the gold standard of insulin sensitivity testing (the EIC) indicates that the DISTq may be used a suitable surrogate in a number of situations. In particular, outpatients of a general practice setting could have a test performed in the duration of a single consult. Furthermore, the result would be available within the duration of that consultation. The current method of estimating sensitivity in a general practice is a single fasting blood test for glucose. However, fasting tests are only an indicator for whether the endogenous insulin response is sufficient to maintain glucose homeostasis. Thus, it only really measures when β-cell damage has already occurred and diabetes is either a current condition or

very imminent (Figure 1.03). The DISTq offers a true insulin sensitivity based risk assessment for a cost and protocol that can be easily met by health providers.

The low cost attributes of the DISTq may enable widespread screening for type 2 diabetes. Table 2.02 shows the costs and benefits of currently available tests, these tests all have associated costs or inaccuracies that preclude the use of insulin sensitivity tests in widespread or even targeted screening. The DISTq has a unique low-cost and relatively high accuracy amongst insulin sensitivity tests and thus may be suitable. However, precisely how accurate and how low-cost a test is required for such programmes is an unknown factor.

The low cost and intensity of the DISTq protocol could also enable a greater number of participants to be recruited in clinical intervention studies with limited budgets. However, the expected shift in insulin sensitivity must be sufficient to overcome the comparatively large coefficient of variation of the DISTq sensitivity metric (Docherty *et al*. 2009). The insulin sensitivity shift measured by DISTq in the intervention study showed the expected level of equivalence with the fully-sampled DISST (Section 8.3.4) given the correlation between the sensitivity values alone (Section 8.3.1).

8.5 Future work

There are a number of potential developments of the DISTq identification method that could be investigated. These changes fall under three main categories.

1. Refining the population based parameter estimation equations.
2. Alterations to the modelling and parameter identification strategies.
3. Tailoring the clinical protocol to maximise accuracy or minimise clinical cost and intensity.

As mentioned in Section 8.3.2 the population based parameter equations could be re-evaluated at a cohort level. Thus, the some of the variation observed in the relationships between insulin sensitivity and the unknown parameters could be constrained somewhat by the application of *a-priori* epidemiological or etiological data. The observation of the reduced insulin clearance rates in the participants of the intervention study imply that alternative equation sets can be appropriate to maintain equivalence in insulin sensitivity magnitude across tests. The application of targeted cohort equations is likely to have a limited effect on the correlations of DISTq insulin sensitivity and the sensitivity derived by fully-sampled tests. However, the gradient is likely to improve. Thus, clinical diagnosis equivalence and surrogate EIC performance would improve.

Furthermore, relationships across the *a-posteriori* identified parameters could potentially be obtained. For example, basal insulin concentration is a physiological function of basal insulin production and insulin clearance. Initial ad-hoc application of the estimation of basal insulin as function of production and clearance had a mild negative effect on the correlation of the DISTq identified sensitivity values and fully-sampled values. However, it is likely that Bayesian techniques, or similar, could provide a more accurate or realistic combination of parameter estimates.

In addition, the current DISTq method assumes a first pass extraction of insulin value of 70%, which is an average of the values found in the fully-sampled tests and is justified in the literature (Cobelli & Pacini 1988; Ferrannini & Cobelli 1987; Meier *et al.* 2005; Toffolo *et al.* 2006). This effect is physiologically equivalent to the hepatic clearance of insulin and thus could be linked as a single parameter in the model. However, the current representation of first pass extraction in the DISST physiological model (Section 4.3.2) would have to be altered to become a function of the plasma insulin concentration.

Thus, Equation 4.15 would be re-defined as Equation 8.10

$$\dot{I} = -n_K I - \frac{n_I}{V_P}(I - Q) + \frac{U_X}{V_P} + U_N - x_H(U_N + I \cdot h_P) \qquad 8.10$$

where: x_H is the combined hepatic extraction rate [dimensionless], and h_P is the proportion of plasma that flows through portal vein in a minute. This latter parameter could be approximated as 1/4 of the plasma volume per minute or a population constant.

The x_L and n_L parameters are infrequently combined, as they are in Equation 8.10. It is likely that they are separated mathematically by the increased effect saturation of the first pass extraction parameter (Toffolo *et al.* 2006). Thus, a saturation parameter is appropriate in the expression and has been included in Equation 8.11.

$$\dot{I} = -n_K I - \frac{n_I}{V_P}(I - Q) + \frac{U_X}{V_P} + U_N - x_H\left(\frac{U_N + I \cdot h_P}{1 + \dfrac{U_N + I \cdot h_P}{I_{P50}}}\right) \qquad 8.11$$

where: I_{P50} is the rate that insulin passes through the portal vein at half the maximal hepatic insulin clearance and x_H is a combined hepatic clearance parameter.

The saturation parameter I_{P50} would serve to reduce the extraction of the first phase insulin in comparison to the other phases of insulin production. The term is justified by experimental observation, (Thorsteinsson 1990; Thorsteinsson *et al.* 1985), and the physical observation of a limited availability of insulin binding sites in the liver.

The model presented in Equation 8.10 contains a single parameter to define insulin pharmaco-kinetics. Thus, some of the un-modelled variation caused by the fixed x_L term in the DISTq process could potentially be mitigated. Further accuracy could perhaps be obtained by defining the population characteristics of the I_{P50} parameter.

The intensity of the clinical protocol used in the DISTq could potentially be increased to maximise resolution, or reduced to minimise cost. Increased sampling frequency would reduce the effect of assay error on the derived metric. However, this is likely to have a minimal effect on the correlations or other DISTq performance metrics. Furthermore, an extended protocol with added glucose and insulin boluses would ameliorate the effect of incomplete glucose mixing, increasing the resolution of V_G and subsequently *SI*. The period between the glucose and insulin boluses of the DISST protocol could be expanded with the second insulin bolus altered to combine glucose and insulin. This would potentially allow an observation of first phase insulin production response. This possibility is explored in Chapter 10.

DISTq30 requires a very low-intensity protocol. However, there is still potential to reduce the intensity further. The time between the glucose and insulin assays is required by the fully-sampled DISST to allow an observation of the first phase of insulin production. However, DISTq methods cannot observe this response, and as such, this period is somewhat redundant. Thus, the boluses could be combined as a single bolus, with the final sample at 20 minutes. This change would reduce the clinical intensity of the test. An *in-silico* investigation of this protocol is presented in Chapter 10.

Error in the plasma insulin clearance rate is the primary contributor to variance in the identified DISTq sensitivity value. The clearance rate is the predominant factor in the determination of the concentration of insulin in the participant during the DISST protocol. Thus, achieving greater accuracy in the estimation of this parameter during identification would yield the greatest potential for increased accuracy. If a low cost, real-time assay is available for a species that can be applied intravenously and is cleared at a rate that is indicative of the specific participant rate of insulin clearance; this species could be administered with the insulin bolus to provide a more accurate estimation of insulin sensitivity.

The basal glucose assay could potentially be removed and replaced with an estimate that is defined using the population-based functions of *SI* presented for the other unknown metrics. This would have a very minimal impact on the intensity of the test as a skin puncture is still required to administer the boluses and thus, it is very unlikely that this intensity reduction is necessary or viable.

Finally, the DISTq can be used in a hierarchy of insulin sensitivity tests, wherein increased resolution is possible through the re-assaying of frozen samples. This potential is explored thoroughly the following chapter.

Chapter 9. A hierarchy of insulin sensitivity tests

9.1 Motivation

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). Section 4.4 and Chapter 6 showed that the fully-sampled DISST is a relatively high accuracy, yet low-cost insulin sensitivity test. Chapter 8 showed that the DISTq and DISTq30 values retained much of this accuracy with a lower clinical cost than the DISST. Due to the dynamic nature of the DISST protocol, variations of the protocol could be investigated and the space between the DISST and DISTq30 on Figure 8.14 could potentially be filled with more tests that offer various different compromises of cost and accuracy.

Investigation of the test variations could measure the effect of sample and assay selection on parameter accuracy. If properly designed, such intermediate tests would offer the opportunity to store or freeze samples to be assayed later for insulin and/or C-peptide. Hence, if necessary, a low-cost, lower resolution test could be readily upgraded, for a higher resolution test. This chapter presents and evaluates a spectrum of tests between the fully-sampled DISST and the DISTq30. The overall outcome is an array of DISST-based test protocols and identification methods that could potentially provide a near continuous hierarchy of compromises between cost and accuracy.

9.2 Study design

9.2.1 Participants

The DISST protocol used in the dietary intervention study (TeMorenga *et al*. 2010) used relatively frequent sampling with consistent timing. This data was used in this investigation of the hierarchy of DISST tests, as it offered the greatest number of potential sampling variations. Eighty-two female participants from the Otago region of New Zealand took part in a longitudinal intervention investigation. All participants had characteristics associated with an increased risk of developing type 2 diabetes (T2DM) (BMI, family history and/or ethnicity). In total, 74 subjects provided data from 218 full DISST tests that were performed at 0, 4 and 10 weeks of a macronutrient intervention. Participant characteristics are summarised in Table 8.01

9.2.2 DISST test protocol

The DISST protocol is defined in Section 4.2, but is repeated in brief:

- 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.
- A cannula 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.
- Boluses of 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.

9.2.3 Design strategy of the various proposed protocols

Eight variations of the standard DISST sampling and assay protocol were evaluated by their ability to re-identify the *SI* value identified by the fully-sampled DISST. Each variation had differing advantages in terms of sample and cost reduction, with resulting different test

resolutions. These eight alternatives do not exhaust the possibilities for variation of the DISST sampling protocol. However, these eight variations were assumed distinctive enough to provide varied outcomes and illustrate the potential of test variations to provide a range of test capabilities within a DISST/DISTq framework. The 5 test protocols that utilise aspects of the DISTq identification methods (Docherty *et al*. 2009) disable the identification of patient-specific x_L, n_L and U_N values. Tables 9.01 and 9.02 summarise the proposed variations.

9.2.3.1 Fully-sampled DISST

The fully-sampled DISST protocol was designed at the University of Canterbury and is detailed in Lotz *et al*. (Lotz 2007; Lotz *et al*. 2008). The DISST identification method used C-peptide, insulin and glucose assays from every available sample time. The protocol for this test was presented in Section 4.2.

9.2.3.2 Short

The Short protocol was designed to capture all major dynamics of the three species with reduced test time and samples. The validation study of the DISST effectively used this protocol (Chapter 6).

9.2.3.3 DISST-E/SI

DISST-E/SI enabled accurate identification of insulin sensitivity and endogenous insulin production, while minimising sample assay cost. There are three significant metrics from a typical U_N profile, the basal production rate (U_B), the first-phase secretion (U_I), and the second-phase production (U_2). Only three assays are needed to uniquely identify these rates.

9.2.3.4 Sparse

The Sparse protocol significantly limits the number of assays, minimising clinical intensity. Only three samples were taken, which can be used to define the three major U_N metrics and *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 were used to define the concentration increase caused by the glucose bolus.

9.2.3.5 DIST-SI

The DIST-SI protocol identified only *SI* without any participant-specific U_N metrics. Thus, no C-peptide, measurements were taken. The DISTq population-based parameter estimations (Docherty *et al*. 2009) were used to define the U_N profile instead. Protocols that do not assay C-peptide cannot provide participant specific U_N values, and so DISST is reduced to DIST in this nomenclature.

9.2.3.6 DIST-SI-2

The DIST-SI-2 further reduced 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 two samples, taken at the end of the test, were assayed for insulin, while the full glucose response is observed with four glucose assays.

9.2.3.7 DISTq-FS

The DISTq-FS utilised all of the available glucose samples to define *SI* in an iterative process. DISTq-FS has been shown to replicate fully-sampled DISST *SI* values (Docherty et al. 2009; Docherty et al. 2011c). These outcomes, which are also presented in Chapter 8, are repeated here to allow a complete comparison.

9.2.3.8 DISTq-S

This protocol mirrors the Short DIST and used only four glucose samples to define an *SI* value. The second glucose sample (at t=15) is not used by the identification method. However, taking this sample allowed 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.

9.2.3.9 DISTq30

The DISTq30 aims to identify *SI* from very sparse data. Only two glucose samples were taken. The outcomes of this analysis were also presented in Chapter 8.

The sample times of the alternative DISST protocols are defined in Table 9.01. Table 9.02 shows the test duration, sample costs and whether the test results are available real-time.

	Sample times									Assays		
	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
Short	-	GIC	*G*IC	GIC	-	GIC	-	GIC	-	5	5	5
DISST-E/SI	-	GIC	*G*IC	GI	G	GIC	GI	GI	-	6	6	3
Sparse	-	GIC	*G*IC	-	-	-	GIC	-	-	3	3	3
DIST-SI	-	GI	I	GI	G	GI	GI	GI	-	6	6	0
DIST-SI-2	-	G	-	G	-	GI	-	GI	-	4	2	0
DISTq-FS	G	G	*G*	G	G	G	G	G	G	9	0	0
DISTq-S	-	G	*G*	G	-	G	-	G	-	5	0	0
DISTq-30	-	G	-	-	-	-	-	G	-	2	0	0
HOMA	-	GI	-	-	-	-	-	-	-	1	1	0

Table 9.01. DISST sampling schedules for the estimation of *SI*. G, I and C represent glucose, insulin and C-peptide assays. Bold-italics show a sample ignored by the specific test's parameter identification method but which may allow identification methods from other DISST protocols.

	Samples	Protocol duration	Relative sample cost	Real-time	U_N
Fully-sampled	9	50	$562	N	**Y**
Short	5	30	$312	N	**Y**
DISST-E/SI	7	30	$270	N	**Y**
Sparse	3	30	$187	N	**Y**
DIST-SI	7	30	$165	N	N
DIST-SI-2	4	30	$60	N	N
DISTq-FS	9	50	$22	**Y**	N
DISTq-S	5	30	$12	**Y**	N
DISTq-30	2	30	$5	**Y**	N
HOMA	1	2	$27	N	N

Table 9.02. Duration, relative assay cost and potential outcomes of the various 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 U_N profiles.

9.2.4 Test hierarchy

The sampling schedule of the various protocols could allow more, or less, assays from the samples taken during a particular test to enable differing analyses. For example, the sampling protocol of the DIST-SI yields seven 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 (later) for insulin and/or C-peptide, as well as glucose where not done previously, to obtain a DIST-SI or Short DISST result including U_N metrics.

This approach increases storage costs, but minimises cost for participants who can be diagnosed with a lower resolution test. Additionally, only one clinically invasive procedure is required for each participant, as the hierarchy is enabled by the storing of already taken samples. Table 9.03 shows all potential sample schedules and subsequent possible assay and identification methods for each sampling protocol defined, and is read across for left to right.

Protocol completed	Analyses possible with samples								
	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 9.03. Potential for different assay regimes to allow analyses with identification methods from other protocols.

9.2.5 Identification methods of the proposed protocols

The U_N profiles for the various protocols were 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 DISST (Lotz 2007; Lotz *et al.* 2008; McAuley *et al.* 2007) (Section 3.3.1). The DISTq methods and the population based estimates have been published previously (Docherty et al. 2009; Docherty et al. 2011c) and presented in Section 8.2.3. DISST-E/SI was an exception to the presented method. The final blood sample of the DISST-E/SI was not assayed for C-peptide, and the U_N rate was assumed constant after the final C-peptide value.

Insulin concentrations in the plasma and interstitium were either defined using the iterative integral method (IIM) (Docherty *et al.* 2009; Hann *et al.* 2005b) presented in Section 4.4 or the DISTq methods presented in Section 8.2.3. Note that the DIST-SI-2 used the DISTq parameter estimation for basal insulin (I_b), and IIM to identify n_L with a fixed x_L (70%).

Table 9.04 summarises the identification methods used by each sampling protocol.

	U_N	Insulin	Glucose
Fully-sampled	DC	IIM	IIM
Short	DC	IIM	IIM
DISST-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 9.04. Identification methods for the various protocols. (DC – deconvolution, IIM - iterative integral method, E_{DISTq} – DISTq population based estimation equations) * indicates that the identification method must be adjusted to account for sparse sampling.

DISTq parameter estimation equations for I_b, n_L, U_B, U_1 and U_2 were generated by finding smooth mathematical relationships between the parameters and *SI*. Details of this process are in (Docherty *et al.* 2009) and the parameter estimation Equations 8.02-8.06.

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

9.2.6 Analysis

The *SI*, U_B, U_1 and U_2 values from the protocols were compared to the same values obtained from the fully-sampled DISST protocol using Pearson's correlation coefficients and the gradients of the regression lines. The gradients allow a comparison of the proportional shift or bias of identified metrics. Insulin sensitivity was identified in units of L·mU^{-1}·min^{-1}. Thus, a reduction in correlation from the findings of Chapter 8 was expected. Equation 9.01 was used to force the regression line through the origin to obtain a true proportional ratio (G) between fully-sampled values (V_{FS}) and the values identified by the alternative protocols (V_{Alt}):

$$G = \frac{\|V_{Alt}\|_2}{\|V_{FS}\|_2} \qquad\qquad 9.01$$

The hepatic clearance parameters (n_L, x_L) have limited clinical diagnostic use and are thus not presented.

HOMA is also compared to the fully-sampled DISST, as it is an established, simple fasting metric that is most typically used in current screening applications.

9.3 Investigation outcomes

Table 9.05 summarises the performance of all the proposed protocols with respect to their ability to replicate the *SI* and U_N values identified using the fully-sampled DISST.

The sparser DIST-SI-2 method showed the greatest ability to replicate the *SI* metrics of the fully-sampled DISST by a small margin. It was closely followed by DIST-SI, the Short protocol, DISST-E/SI and the Sparse protocol. DISTq methods showed an expected, slightly lesser ability

to replicate *SI*. However, DISTq results were in line with previous findings (Docherty *et al.* 2009) and still represent a sound result.

DISTq-S and DISTq-30 correlated highly to DISTq-FS: R=0.94 and R=0.89 respectively. When considering the vast reduction in samples between the DISTq-FS and DISTq30, the correlation value of R=0.89 indicates strong stability and robustness.

	SI R(*G*)	U_B R(*G*)	U_1 R(*G*)	U_2 R(*G*)
Fully- sampled	**1** (1)	**1** (1)	**1** (1)	**1** (1)
Short	**0.90** (1.17)	**1** (1)	**1** (1)	**0.89** (0.99)
DISST-E/SI	**0.90** (1.10)	**1** (1)	**1** (1)	**0.72** (1.12)
Sparse	**0.89** (1.03)	**1** (1)	**1** (1)	**0.88** (0.95)
DIST-SI	**0.91** (1.10)	**0.62** (0.94)	**0.07** (0.80)	**0.75** (0.90)
DIST-SI-2	**0.92** (1.07)	**0.68** (0.97)	**0.09** (0.81)	**0.74** (0.99)
DISTq-FS	**0.83** (1.10)	**0.56** (0.94)	**-0.07** (0.80)	**0.70** (0.90)
DISTq-S	**0.77** (1.27)	**0.53** (0.92)	**-0.14** (0.80)	**0.69** (0.89)
DISTq-30	**0.71** (1.24)	**0.53** (0.98)	**-0.14** (0.80)	**0.71** (1.04)
HOMA	**-0.35** (-)	-	-	-

Table 9.05. Ability of protocols to replicate *SI* and U_N values from the fully-sampled DISST

Protocols that sampled basal and first-phase C-peptide showed absolute equivalence of U_B and U_1 to the fully-sampled DISST, as expected. Reducing the number of C-peptide samples had a greater effect on U_2. The DISTq population estimates were strongest for U_2, weaker for U_B, and poor for U_1, (although the gradient of 0.8 implies that the general magnitude of the U_1 predictions were accurate). However, it is important to recall that DISTq was not designed or intended for estimation of U_N metrics.

HOMA showed a relative inability to replicate the insulin sensitivity metrics of the fully-sampled DISST. Again, this outcome matches the great deal of literature summarised in Table 2.02.

9.4 Potential of the various individual protocols.

Relatively high correlations (R~0.9) between protocols that assayed insulin and the fully-sampled DISST test show that the limited sampling protocols could be used as surrogates for the fully-sampled test without significantly diminishing test resolution. In particular, the insulin sensitivity values identified using only three samples during the Sparse protocol correlated relatively well to the fully-sampled test (R=0.89) and the protocol also captured all major dynamics of the U_N profile. This is despite the protocol requiring half the time and one third of the assay cost. This result is not entirely surprising considering the *a-priori* analysis in Section 5.4.1 confirmed a particular robustness of the DISST glucose PD model.

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. DISTq-S and DISTq-30 also correlated relatively well to the fully-sampled DISST, 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 methods that utilised the DISTq identification equations were designed to identify *SI* alone. Thus, U_N was only adequately assessed by tests that assayed C-peptide. U_N defines β-cell function, which is of particular importance to insulin resistant individuals, as it indicates the progression rather than the risk of T2DM (Figure 1.03). U_N tends to increase during the progression toward the diabetic state and then begins to decrease as β-cell function diminishes. These changes have been referred to as Starling's curve of the pancreas (Clark *et al*. 2001; Gastaldelli *et al*. 2004). Metrics of U_N in isolation cannot define which side of the curve an individual is on and must therefore be coupled with *SI* to allow a useful observation of a participant's state on the pathogenesis of type 2 diabetes.

9.5 DISST-based tests as a hierarchy

9.5.1 Motivation

Due to the common protocol, the spectrum of tests could be used in a hierarchal approach. For example, a lower-cost DISST derivative 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 U_N, per Table 9.03. The added cost would only be needed for 'borderline' results. Hence, only a small proportion of the test cohort would potentially require higher cost assays. Importantly, no further clinical procedure must be undertaken on these borderline individuals.

9.5.2 A potential DISST-based hierarchy

To assess the validity of the DISST-based hierarchy approach, the DISST data from participants of the intervention study were used. Recruitment criteria of the intervention study was such that a relatively insulin resistant cohort was obtained (TeMorenga *et al*. 2010) (Table 8.01). Thus, the cohort is representative of the type of population that would be target by diabetes risk screening programmes.

This analysis used the short-DISST (DISST-S) protocol as the true diagnosis of insulin resistance value to be replicated by the hierarchal system. Diagnosis of insulin resistance used a cut-off SI value of 8.35×10^{-4} L·mU^{-1}·min^{-1} (50th percentile of the cohort's DISST-S SI value). This protocol produced blood samples that could be assayed differently to allow analysis with the DIST-SI2, DISTq-S or DISTq30 identification methods. Thus, a three stage hierarchy was used.

In the first stage, the glucose data was analysed using the DISTq30. In the second stage, all of the tests that produced an SI value close to the diagnosis threshold were reanalysed using the DIST-SI2 method. Clinically this required two additional glucose assays and two insulin assays. The region of re-analysis was between 6 and 12×10^{-4} L·mU^{-1}·min^{-1} and contained 78/217 (35.9%) of tests. The use of the DIST-SI-2 identification method around the diagnosis threshold allowed a targeted improvement in resolution around the diagnostic cut-off value. In a third step, tests that produced SI values between 7 and 10×10^{-4} L·mU^{-1}·min^{-1} were re-assayed to allow a DISST-S analysis. This would require an additional three insulin assays and five C-peptide assays. To enable a fair comparison, the y-axis DISST-S values incorporate 7% normally distributed noise to mimic the intra-test variation expected during the DISST test (Lotz *et al*. 2008). In this final stage, 139 (64.0%) of the cohorts tests were analysed with the low-cost DISTq30 method, 42 (19.4%) with the moderate cost DIST-SI2 and 36 (16.6%) with the comparatively higher-cost DISST-S.

Figure 9.01 shows how Stages 2 and 3 improve the resolution of the hierarchy around the diagnosis threshold. Table 9.06 summarises the assay costs, clinical burden and diagnosis accuracy of the stages of the hierarchical analysis. The final row of Table 9.06 shows the

maximum theoretical resolution of dynamic tests of this nature by comparing the diagnostic equivalence of the identified short-DISST values with the short-DISST values that had an added normally distributed noise (CV=7%). While the c-ROC values remain high and relatively similar, the sensitivity and specificity of the hierarchical system improved considerably via the addition stages.

The sensitivity and specificity of the hierarchy of DISST tests reacted very well to the re-assayed samples and began to approach the values of the short-DISST versus short-DISST analysis, the theoretical maximum for this type of test. The c-ROC value improved by a relatively small amount with the added resolution tests. The mean Stage 3 test assay cost of the DISST-based hierarchy ($71) was very low in comparison to the fully-sampled short DISST test ($312) with scarcely diminished sensitivity, specificity, or c-ROC values. Note also that this particular example had a high re-analysis rate than could be expected form a general cohort as the intervention cohort was purposefully selected for type 2 diabetes risk. This influenced the cohort's tendency toward insulin resistance. If the analysis was repeated in a more general cohort, the re-assay rate would be reduced and diagnosis accuracy would be improved.
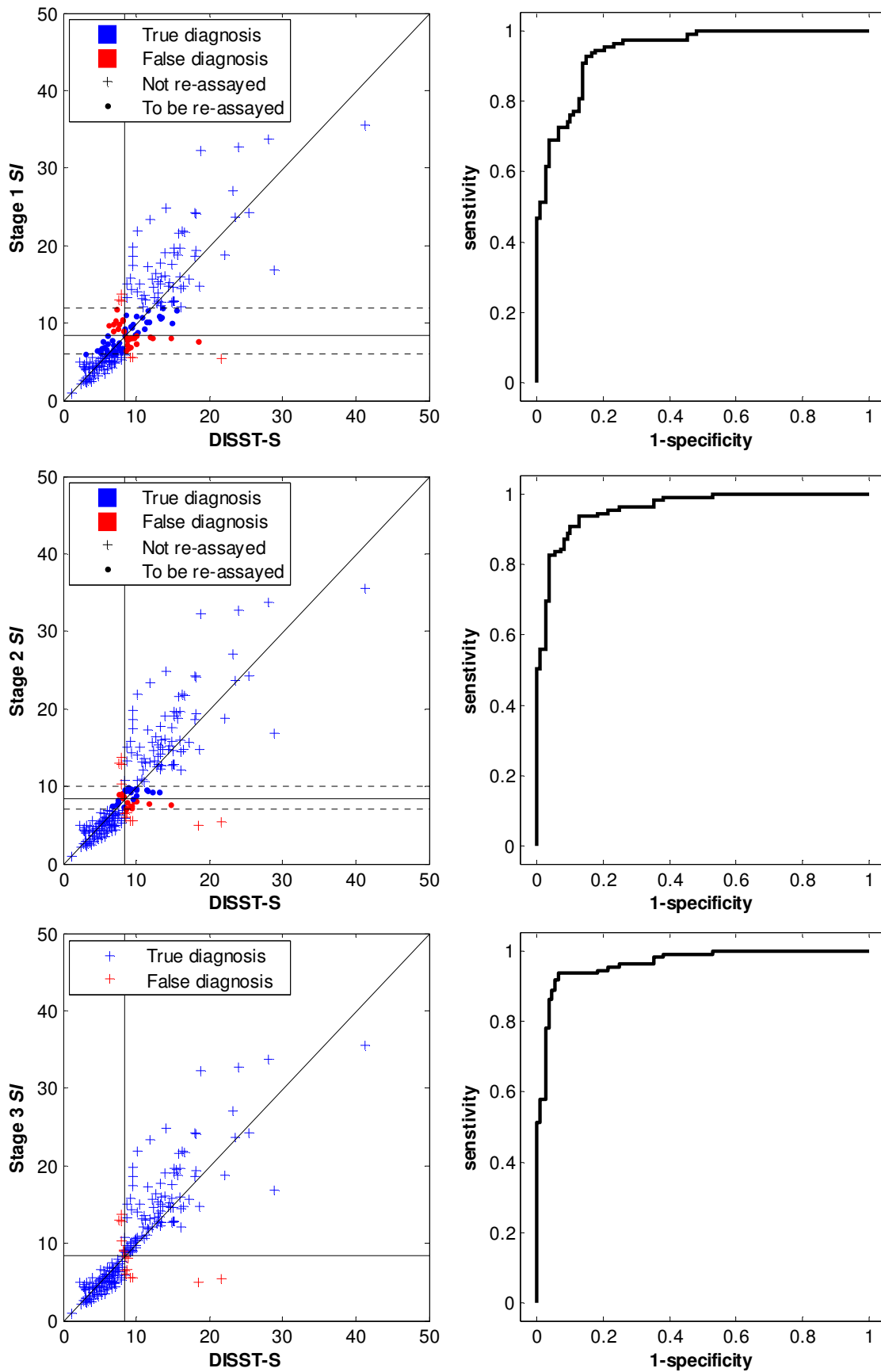
Figure 9.01. Three stages of the DISST-based hierarchal approach to diagnosing insulin resistance. (*SI* in units of $10^{-4} \text{L} \cdot \text{mU}^{-1} \cdot \text{min}^{-1}$)

	Tests			Average Clinical Time	Average Test Assay Cost	Sensitivity	Specificity	c-ROC
	DISTq	DIST-SI2	DISST-S					
DISTq vs DISST-S	217	0	0	0.6 hrs	$12	0.87	0.81	0.94
DISTq,SI2 vs DISST-S	139	78	0	0.6 hrs	$29	0.93	0.84	0.96
DISTq,SI2,-S vs DISST-S	**139**	**42**	**36**	**0.6 hrs**	**$71**	**0.95**	**0.91**	**0.97**
DISST-S vs DISST-S	0	0	217	0.6 hrs	$312	0.96	0.95	0.99

Table 9.06. Summary of the stages of the DISST-based hierarchical system of insulin sensitivity tests. Test costs obtained from Table 9.02.

9.5.3 A potential hierarchy using established tests

To use the established tests in a hierarchy would be more difficult. In particular, none of the established tests use protocols with multiple potential assay schedules and identification methods. The most likely system that could be used to diagnose high-resolution insulin resistance in clinical practice may initially use fasting glucose, then HOMA followed by the EIC.

The clinical data obtained during the EIC of the DISST validation was used to analysis these established tests in a hierarchy. Initially, participants were screened using fasting glucose. However, fasting glucose can only identify a very small proportion of a general population with very high fasting glucose due to insufficient insulin secretion or *SI*. Figure 9.02(top) shows that none of the DISST validation cohort had a fasting plasma glucose concentration above the ADA threshold for the diagnosis of diabetes or impaired fasting glucose (7.8mmol/L) (ADA 2006). If a larger cohort were used, a certain proportion would be diagnosed with the FPG assay. However, this proportion would remain very small, necessitating higher cost and effort for most participants.

In a second stage, the fasting blood sample was re-assayed for insulin. This would enable a HOMA value. Very high or low HOMA values could potentially diagnose insulin resistance or sensitivity in part of the population. However, to achieve a sensitivity and specificity in the established hierarchical system similar to the DISST hierarchy, only 22 participants of the validation study (44%) could be diagnosed with the HOMA (Ten true positive and true negative,

and one false positive and one false negative). These values were obtained when participants that produced HOMA results between 0.347 and 1.11 are re-tested with the EIC.

Thus, 28 subjects (56%) must be called back to undergo the EIC, which is a second clinical protocol. This is the third stage of the hierarchal approach to insulin resistance screening using established *SI* tests. Note that the EIC versus EIC case was completed using 5% normally distributed noise added to the y-axis EIC values in accordance with reported EIC CV values (Table 2.02).

Figure 9.02 shows the failure of FPG to diagnose insulin resistance in any of the cohort, and the improvement of diagnosis achieved when the clamp is used. Table 9.07 summarises the assay cost, clinical burden and diagnosis accuracy of the FPG/HOMA/EIC hierarchical system for insulin resistance diagnosis in a general population. The low resolution of the HOMA across most regions of insulin sensitivity meant that a significant proportion of the cohort must be reassessed using the EIC test to gain strong diagnostic performance. The EIC is a very consistent insulin sensitivity test, and thus, provides high resolution for the participants that are re-assessed. However, the clinical burden of the test increases the cost of the test considerably over the cost of the DISST-based hierarchy.

	Tests			Mean Clinician Time	Mean Test Assay Cost	Sensitivity	Specificity	c-ROC
	FPG	HOMA	EIC					
FPG vs EIC	50	0	0	0.25hrs	$2.5	-	-	0.83
HOMA vs EIC	0	50	0	0.25hrs	$27	0.84	0.84	0.89
HOMA,EIC vs EIC	**0**	**22**	**28**	**2.9hrs**	**$131***	**0.94**	**0.92**	**0.96**
EIC vs EIC	0	0	50	5hrs	$185	0.97	0.95	0.99

Table 9.07. Outcomes of the FPG/HOMA/EIC hierarchical system. (* the EIC assay cost was estimated using 24 glucose assays at $2.5 each and 5 insulin assays at 25$ each. HOMA with EIC backup requires two tests, thus there were 22 tests at $27 and 28 at $27+$185.)
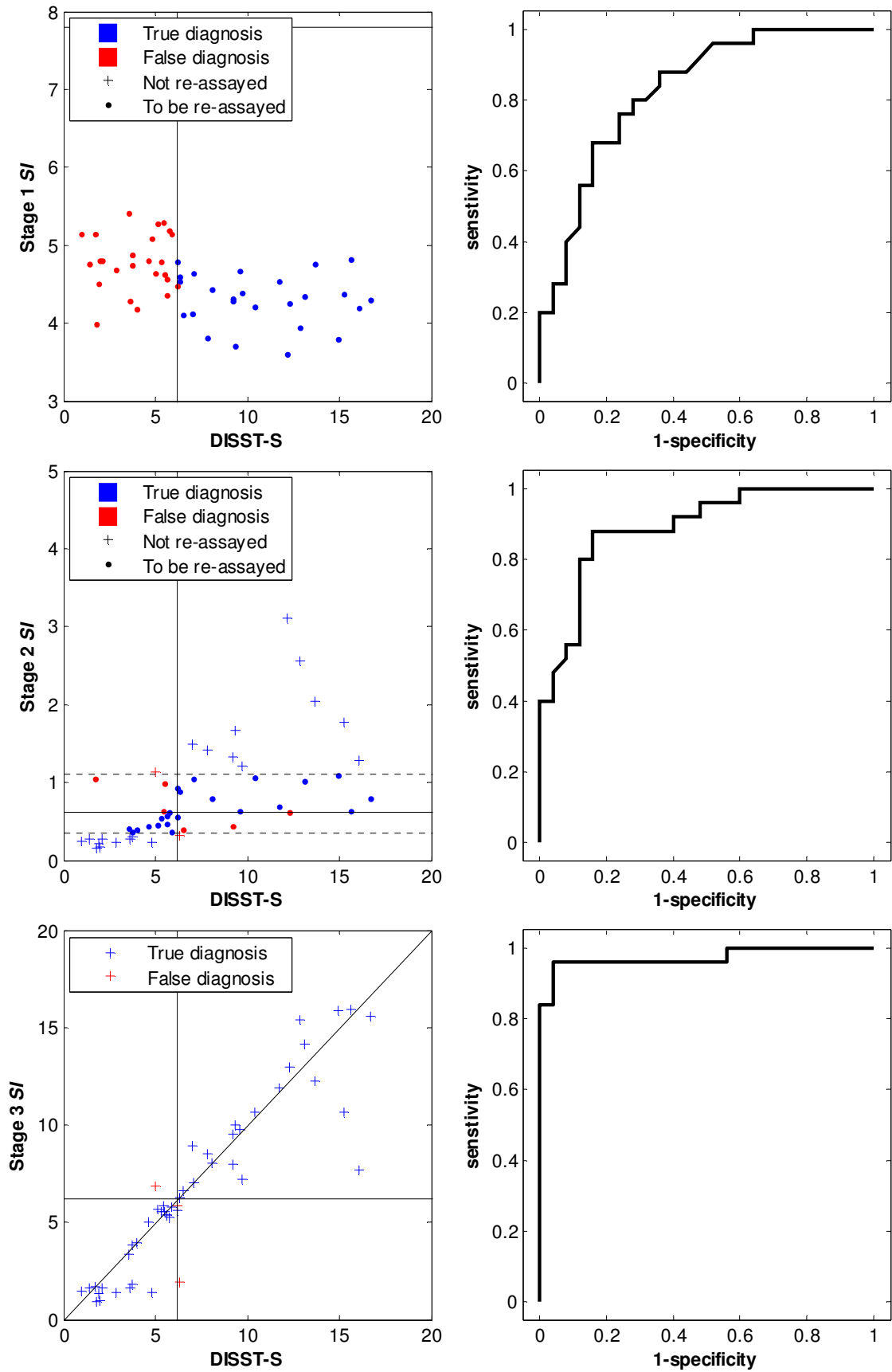
Figure 9.02. Three stages of the FPG-HOMA-EIC hierarchal approach for diagnosing insulin resistance. (HOMA scaled in Stage 3)

9.5.4 Comparison between the DISST and established test hierarchy

The thresholds used in the FPG/HOM/EIC hierarchy were defined to achieve a similar outcome in terms of sensitivity and specificity to the DISST hierarchy and allow the comparison to be in terms of clinical cost. The DISST hierarchy required a Stage 3 mean test assay cost of \$71, in comparison, the FPG/HOMA/EIC Stage 3 mean assay cost was \$131. The mean required clinician time required by the Stage 3 FPG/HOMA/EIC hierarchy was 2.9 hours in comparison to the 0.6 hours required by the DISST hierarchy. Furthermore, the DISST hierarchy did not require any call-backs. In contrast, 56% of the participants of the FPG/HOMA/EIC hierarchy must be called back after the HOMA as real-time analysis of the insulin assay is not possible. This necessitated significant added burden and effort for both the clinician and participant. There would thus be an issue of compliance for getting participants to return for the clinically intense EIC test. The DISST hierarchy does not require any call-backs, as the data obtained during the first test can be re-assayed to enable upgraded insulin sensitivity identification methods.

The DISST hierarchy was investigated using the intervention cohort that was targeted toward insulin resistant participants (TeMorenga *et al*. 2010). The FPG/HOMA/EIC hierarchy was investigated using the validation cohort that was representative of a general cohort. Thus, the spread of insulin sensitivity values in the validation study was greater than that exhibited in the intervention study. This difference meant that there was a greater concentration of values around the diagnosis region for the DISST hierarchy case, presenting a very conservative comparison and thus, slightly increasing the required proportion of higher cost tests required. If the intervention cohort was more homogenous, this would have reduced the proportion in the re-assay range and reduce the overall cost of the DISST-based hierarchy system.

The DISST hierarchy required more complex identification methods. However once these are established, there are no ongoing costs. The longitudinal outcomes of the DISST in terms of diabetes diagnosis have not been established, as they have with the EIC. Thus, the clinical outcomes provided by the FPG/HOMA/EIC hierarchy would currently have a greater value to clinicians. However, once the DISST has been validated in a longitudinal study, the value of the outcomes would be comparable, and the DISST hierarchy would be preferable by merit of its reduced clinical intensity.

9.5.5 Summary of DISST hierarchy

The utility of the DISST protocol means that a number of assay regimens and identification methods can be used on data from the same clinical trial. Thus, differing levels of accuracy can

be obtained to achieve a higher resolution about a certain diagnosis threshold without increasing clinical intensity. Furthermore, the overall clinical intensity is potentially much lower.

The DISST hierarchy described uses three tests that were defined during the initial investigation of the spectrum of DISST tests. The resolution and cost effectiveness of the hierarchy of DISST tests could perhaps be optimised using different combinations of tests or assay regimens were investigated. However, the analysis presented, while conservative, clearly show capability and potential.

PART FOUR: *In-silico* analyses of alternative DISST protocols

Chapter 10. Alternative protocols for DISTq

This chapter presents two further *in-silico* analyses of variations of the DISST protocol. The first may enable a further reduction in the DISTq30 intensity, while the second could potentially enable the concurrent identification of insulin sensitivity and first phase production.

10.1 Motivation

The DISTq method currently uses the standard DISST protocol described in Section 4.2. This approach allowed the DISTq identification method to be evaluated with the data obtained during the DISST pilot, intervention and validation studies. However, the DISST protocol was designed to work with insulin and C-peptide the assays to produce metrics for a whole body evaluation of glycaemic health including clinically important insulin secretion metrics. Hence, the clinical protocol that produced optimal results for the fully-sampled DISST may not necessarily produce the optimal results for the DISTq.

Section 10.2 presents a combined bolus protocol and its effect on the DISTq identification of insulin sensitivity. Section 10.3 presents the observability of endogenous insulin production in a more arduous protocol and the effect of combining the glucose and insulin boluses in a simple two sample tests such as DISTq30. No clinical data is available from these hypothetical protocols, and as such, the investigation of the potential of these tests will be carried out entirely *in-silico*.

10.2 Combined bolus (DISTq20C)

The combined bolus protocol recognises the lack of benefit in the period between the glucose and insulin boluses in the two-sample DISTq30 protocol. As such, the boluses are combined and the final sample can be shifted from 30 minutes to 20 minutes. Hence, it offers a further significant reduction in clinical intensity, especially if considering giving the test via two, instead of three venous punctures. This 20-minute-combined method is referred to as DISTq20C.

10.2.1 Protocol

The protocol followed the general format of the fully-sampled DISST protocol (Section 4.2). The differences are listed:

- Only two blood samples were taken at $t=0$ and $t=20$ minutes
- Samples were only assayed for glucose.
- A combined bolus is administered immediately after the $t=0$ sample is taken. The bolus consisted of 10g glucose and 1U insulin.

10.2.2 Sensitivity identification and *in-silico* study design

10.2.2.1 Relevance of DISTq population-based parameter equations

The identification method used is identical to the method of DISTq30 (Section 8.2.3). However, clinical trials of the DISTq20C protocol have not been undertaken. Thus, no C-peptide data is available to provide any endogenous insulin production profiles for this particular protocol and the response behaviour must be assumed for this *in-silico* analysis. Basal insulin concentration and production rate will not be affected by the altered behaviour, and it is very unlikely that the clearance rate of insulin will be changed. Furthermore, the glucose and insulin concentrations expected in the later stages of this test are similar to those observed in the fully-sampled DISST. Thus, the second phase of insulin production rates observed in the fully-sampled tests could be assumed relevant to this protocol. However, the combined bolus may affect the first phase of insulin production.

The first phase insulin secretion is triggered by an increase of blood glucose concentration (Del Prato *et al*. 2002). This change is sensed in the body by sensory cells typically in blood vessel walls in the periphery, and around the gastric system (Cherrington 1999). The trigger for this reaction will occur in the proposed protocol with the same effectiveness as the standard DISST protocol. However, the release of insulin is suppressed by an increased insulin concentration (Argoud *et al*. 1987). This phenomenon had only a very mild effect on the second phase insulin production of the pilot DISST participants (Lotz *et al*. 2010). In particular, only 23 of the 313 (7.3%) DISST tests undertaken during the pilot, intervention and validation studies produced second phase insulin production rates less than the respective basal rates. Thus, for the purpose of this analysis, the first phase of insulin secretion was assumed to be un-affected by the insulin

content of the bolus. Sensitivity investigations or fully-sampled clinical studies must be undertaken to test the accuracy of this assumption.

10.2.2.2 Monte-Carlo test design

The 313 data sets from the pilot, intervention and validation DISST tests are used in the iterative integral method (Section 4.4) to identify time variant U_N profiles and the x_L, n_T, S_I and V_G parameters. This data is then used with the relevant *a-priori* parameters to simulate participant-specific 'virtual' test responses to the DISTq20C and DISTq30. Both protocols yield two glucose samples: the basal and final samples. These values represent noise-less data.

The Monte-Carlo analysis identified insulin sensitivity from each dataset (DISTq20C and DISTq30) 200 times. Each simulation incorporated normally distributed noise (CV 2%) to the noiseless data. DISTq30 and DISTq20C were identified using the same magnitudes of normally distributed noise during each iteration. The insulin sensitivity values were identified using the process defined in Section 9.2.5 and the combined population based parameter equations 8.02-C to 8.06-C. The DISTq30 parameter identification method was applied with virtually no change to the DISTq20C data.

10.2.3 Study outcomes

Table 10.01 shows that DISTq30 and DISTq20C produced similar results when equivalent noise was added to the respective *in-silico* data. Interestingly, each participant had a relatively strict adherence to a particular proportional shift. The median bias was 1.02, while the 5th and 95th percentile bias' were 0.86, and 1.21, respectively. Thus, the removal of the redundant period in the test must have affected all participants differently. Figure 10.01 shows that these shifts were not a function of insulin sensitivity. Had this been the case, the shifts could be attributed to the differing relative effect of p_G and *SI* on glucose decay.

However, the lack of insulin sensitivity dependence of these shifts means that the changes must be attributable to disparity between the participant's true and simulated insulin concentrations. The median shift between tests for each participant correlated to their actual parameter values of U_B, U_1, U_2, I_B and n_T at R=-0.09, R=-0.25, R=-0.17, R=-0.17, and R=0.01 respectively. From the signs of the respective correlations, it can be concluded that an error that results in an increased estimated insulin concentration will have a slightly greater exaggerating effect on sensitivity metrics identified by the DISTq30 method.

	DISTq30 (*in-silico*) R, (Grad)	DISTq20C (*in-silico*) R, (Grad)
DISST (clinical)	0.80, (0.87)	0.82, (0.87)
DISTq30 (clinical)	0.95, (0.80)	0.94, (0.80)
DISTq30 (*in-silico*)	-	0.99, (0.99)

Table 10.01. Correlations and gradients between the insulin sensitivity values obtained by *in-silico* analyses and clinical DISST-based tests.
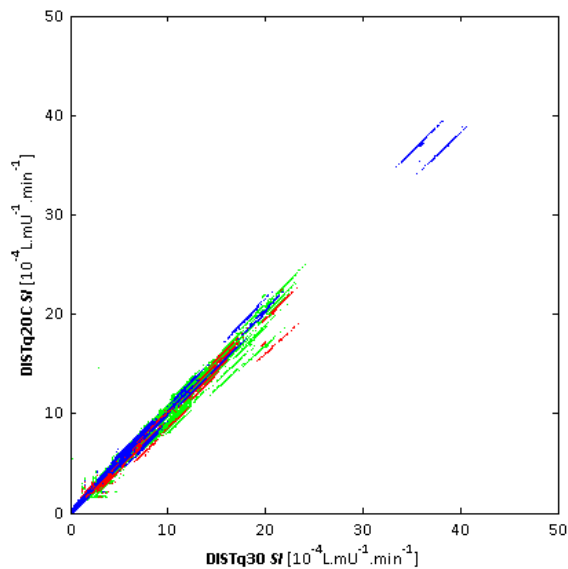
Figure 10.01. Insulin sensitivity values from the pilot, (blue) intervention (green) and validation (red) identified from the single combined bolus DISTq20C and two-bolus DISTq30 protocols.

The insulin sensitivity values obtained by the DISTq20C correlated to the fully-sampled DISST insulin sensitivity value, which was the parent value of the *in-silico* analysis, at R=0.82. In comparison, DISTq30 correlated to the parent value at R=0.80. Figure 10.02 shows the correlation between the fully-sampled DISST *SI* values and the values produced by the DISTq20C and DISTq30. The correlation figures contain vertical lines as each test produces one clinical value and 200 *in-silico* values.
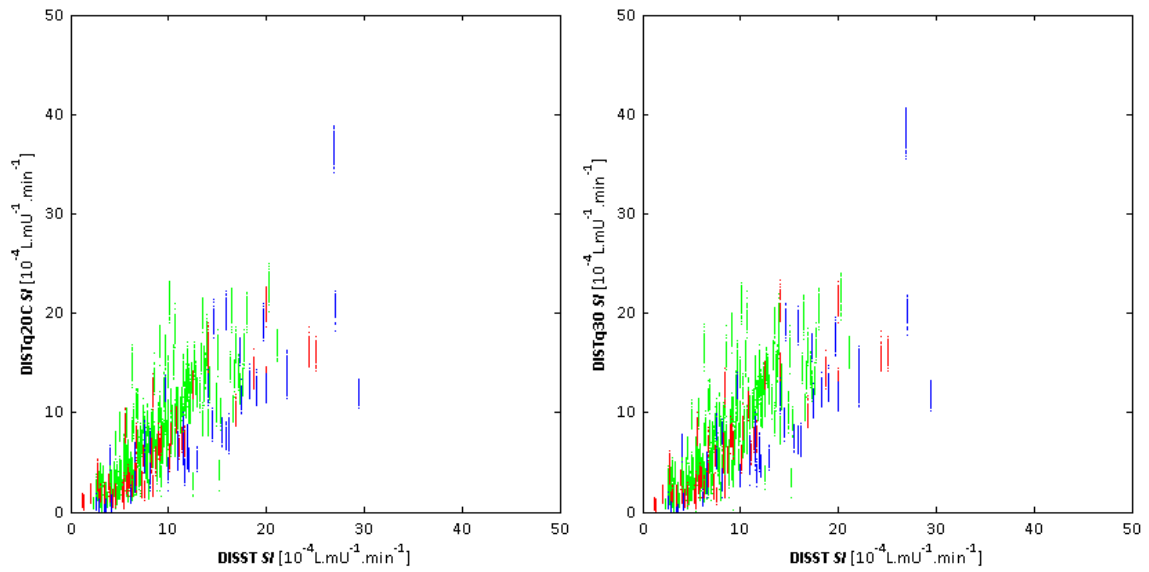
Figure 10.02. Correlation between the fully-sampled DISST and the *in-silico* derived sensitivity values of the DISTq30 and DISTq20C tests. (pilot – blue; intervention – green; and validation - red)

The DISTq20C and DISTq30 sensitivity values obtained in the *in-silico* analysis correlated relatively well to the DISTq30 values obtained clinically (R=0.94 and 0.95, respectively). Figure 10.03 shows these correlations.
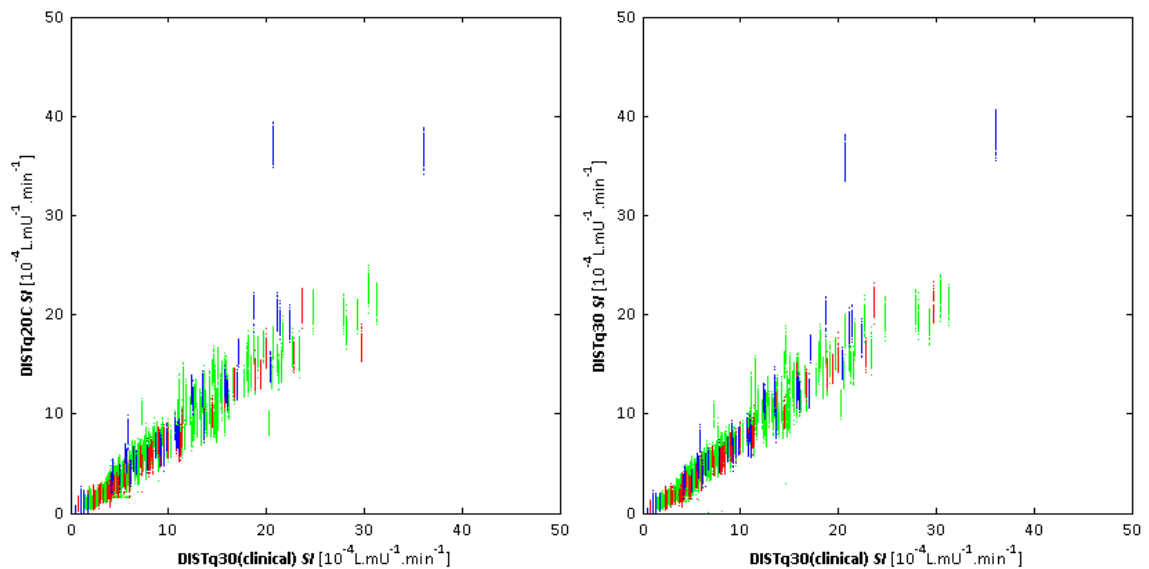
Figure 10.03. Correlations between the DISTq20C, DISTq30 and the DISTq30 values obtained clinically. (pilot – blue; intervention – green; and validation - red)

10.2.4 Clinical implications

The outcomes of this analysis indicate that the insulin sensitivity values of the DISTq20C protocol were synonymous with the values obtained by the DISTq30. Thus, by extension, it implies that the clinical observations of the DISTq30 equivalence to the fully-sampled DISST and gold standard EIC insulin sensitivity values could be assumed for the DISTq20C protocol. However, the high consistency could be a result of the *in-silico* study design, which may potentially be unrepresentative of the true disparity that can only be confirmed with a clinical study.

In particular, the study assumed that the first phase of insulin production (U_1) would remain consistent with the combined bolus. In reality, U_1 may be reduced partially by the insulin suppression of insulin production (Argoud *et al*. 1987). Whether insulin production is consistently suppressed by a certain proportion or suppressed by a random amount across or within test participants is a point of conjecture in the absence of clinical data. Thus, prior to further development or validation of DISTq20C, clinical investigations of the first phase of insulin production should be made. This testing can be as simple as obtaining C-peptide samples at *t*=0 and 5 minutes of the proposed protocol in a cohort that will enable the production of a *SI-U_1* relationship similar to those presented in Section 8.2.2.4.

Errors introduced by the first phase insulin sensitivity had a mild effect on the insulin sensitivity value. As discussed in the final paragraph of Section 8.3.1, the first phase production errors were mitigated by the comparatively large insulin bolus. Thus, even if significant intra-participant daily variation in U_1 is observed (the worst case scenario) the identified insulin sensitivity values would remain relatively unaffected.

The basal state characteristics will remain unchanged by the new protocol. Equally, the second phase of insulin production occurs under similar glucose and insulin concentrations to the DISTq30 protocol. Thus, the assumptions used in the analysis should be fundamentally valid.

If the protocol is successfully validated against the EIC, insulin sensitivity information could be obtained at a very low cost and intensity, and results would be available in real-time. In particular, it requires only one skin puncture for samples and injection. Hence, the test could potentially be given in a doctor's office or test centre. Thus, the test could be used for the similar purposes as the DISTq and DISTq30. In particular, the test could be used as a low cost alternative to HOMA in clinical studies, wherein insulin sensitivity is a secondary consideration. The test could also be used in a general practice setting for a more advanced and informative risk assessment than the fasting glucose assay that is generally used currently.

10.2.5 Future work for the DISTq20C

The next step for the DISTq20C is a clinical investigation of the effect of the combined bolus on the first phase of insulin production. This step is critical for the modification of the population-based parameter estimation equations to be determined that enable a precise first phase insulin production rate. Failing to fulfil this requirement will result in a proportional shift in sensitivity values predicted by the DISTq20C method if the DISST protocol reactions are unrepresentative. Following this step, a clinical validation study should be conducted measuring the equivalence between DISTq20C and the fully-sampled DISST or the EIC (or both).

Finally, if a clinical validation study produces favourable outcomes, the possibility of upgrading the test with additional assays could be investigated. The final outcome would be a hierarchy of tests similar to that of the standard DISST protocol presented in Chapter 9. This hierarchy could further enable a high-resolution diabetes risk screening programme comparable to that discussed in Section 9.5.2 with a slightly lower overall cost.

10.3 *SI* and U_1 from glucose data (DISTqE)

10.3.1 Motivation

Figure 1.03 shows how endogenous insulin production (U_N) can be used in conjunction with *SI* values to evaluate a participants position on pathogenesis of type 2 diabetes. U_N usually increases during the initial progression to type 2 diabetes followed by a decrease as the disease state worsens. This transition has been referred to as 'Starling's curve of the pancreas' (Clark *et al*. 2001; Gastaldelli *et al*. 2004).

This section aims to evaluate whether a surrogate of the action of the first phase of insulin production can be observed in glucose assays alone, and thus allow real-time, inexpensive identification. The DISTq parameter estimation processes outlined in Section 8.2 were used in conjunction with a modified DISST protocol and updated identification method. The protocol and method will be denoted DISTqE, representing DISTq endogenous.

10.3.2 Proposed DISTqE clinical test protocol

The DISTqE protocol aimed to allow an observation of the exogenous insulin mediated glucose disposal and compare it to an equivalent endogenous insulin mediated glucose disposal. Thus, blood samples were taken at t=0, 5, 10, 15, 25, 30, 35, 40 and 50 minutes with a 15g intravenous glucose bolus administered at t=5 minutes and an 8g/1U combined intravenous glucose and insulin bolus immediately after the t=25 minute sample. A second similar protocol was also analysed. This protocol was identical to that proposed with the exception that the 8g glucose content of the second bolus was not used and it's only distinction from the standard DISST protocol is the extended period between boluses.

Figure 10.01 shows the distinction of the integrals of the parameter coefficients for the two protocols at the proposed sample times using the identifiability analysis presented in Chapter 5. Note that the model identifiability method of Chapter 5 was not refined for the 3-parameter case and Figure 10.01 should only serve as indicative of the distinction of the model parameters, and subsequent expected parameter identifiability.
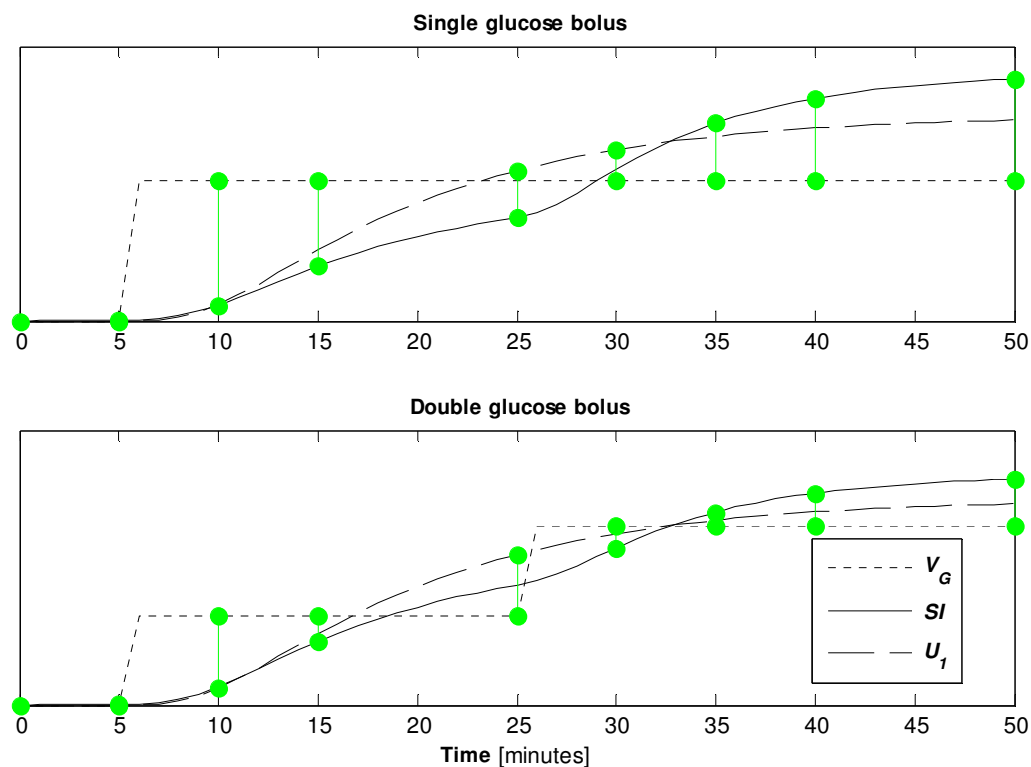
Figure 10.01. Parameter distinction of the two DISTqE protocols.

10.3.3 Sensitivity identification and *in-silico* study design

10.3.3.1 *In-silico* data generation

Data from the 313 fully-sampled DISST tests of the pilot, intervention and validation cohorts were identified using the DISST model and the iterative integral method to yield participant-specific parameters of U_N, x_L n_T, SI and V_G. These values were used with the *a-priori* identified values (n_I, V_P etc.) to simulate 313 matched responses to the protocols described in Section 10.3.2. First phase insulin production is proportional to the content of the glucose bolus. Thus, the first phase of insulin production was increased by 50%. Otherwise, U_N and the other parameters were assumed to be unchanged by the new protocol. The simulated glucose profiles were 'sampled' at the times defined in Section 10.3.2 to represent a noiseless dataset.

10.3.3.2 Identification of *SI* and U_I

An identification method similar to the method in the previously presented DISTq method was used (Docherty *et al*. 2009). However, the key difference is how the first phase of insulin was treated. Steps 1 to 4 define the modified identification method used here.

1.   A population average insulin sensitivity value was used with the DISTq population based parameter estimation equations to get values for I_B, n_T, and U_N. The first phase period was replaced in the U_N profile by zeros ($\widetilde{U_{B2}}$). A binary profile was used to define the active period of the first phase of insulin secretion ($\widetilde{U_1}$). Thus a reconstituted U_N can be defined with Equation 10.03.

$$\widetilde{U_{B2}} = \left\{ \underbrace{U_N(0 \to 5)}_{[1\times6]}, \underbrace{0}_{[1\times5]}, \underbrace{U_N(11 \to 50)}_{[1\times40]} \right\} \qquad\qquad 10.01$$

$$\widetilde{U_1} = \left\{ \underbrace{0}_{[1\times6]}, \underbrace{1}_{[1\times5]}, \underbrace{0}_{[1\times40]} \right\} \qquad\qquad 10.02$$

$$U_N = \widetilde{U_{B2}} + \frac{U_1 \cdot \widetilde{U_1}}{1 - x_L} \qquad\qquad 10.03$$

2.   The plasma and interstitial insulin concentrations were simulated using $\widetilde{U_{B2}}$ and $\widetilde{U_1}$ profiles (Equation 10.04). Figure 10.02 shows how Q_{B2} and U_I*Q_I combine to generate the total interstitial insulin concentration.

$$\dot{I}_{B2} = -n_K I_{B2} - n_L \frac{I_{B2}}{1 + \alpha_I I_{B2}} + \frac{n_I}{V_P}(Q_{B2} - I_{B2}) + \frac{U_X}{V_P} + (1 - x_L)\widetilde{U_{B2}} \qquad 10.04$$

$$\dot{Q}_{B2} = \frac{n_I}{V_Q}(I_{B2} - Q_{B2}) - n_C Q_{B2}$$

$$\dot{I}_1 = -n_K I_1 - n_L \frac{I_1}{1 + \alpha_I I_1} + \frac{n_I}{V_P}(Q_1 - I_1) + \widetilde{U_1}$$

$$\dot{Q}_1 = \frac{n_I}{V_Q}(I_1 - Q_1) - n_C Q_1$$

$$Q_T = Q_{B2} + U_1 Q_1$$
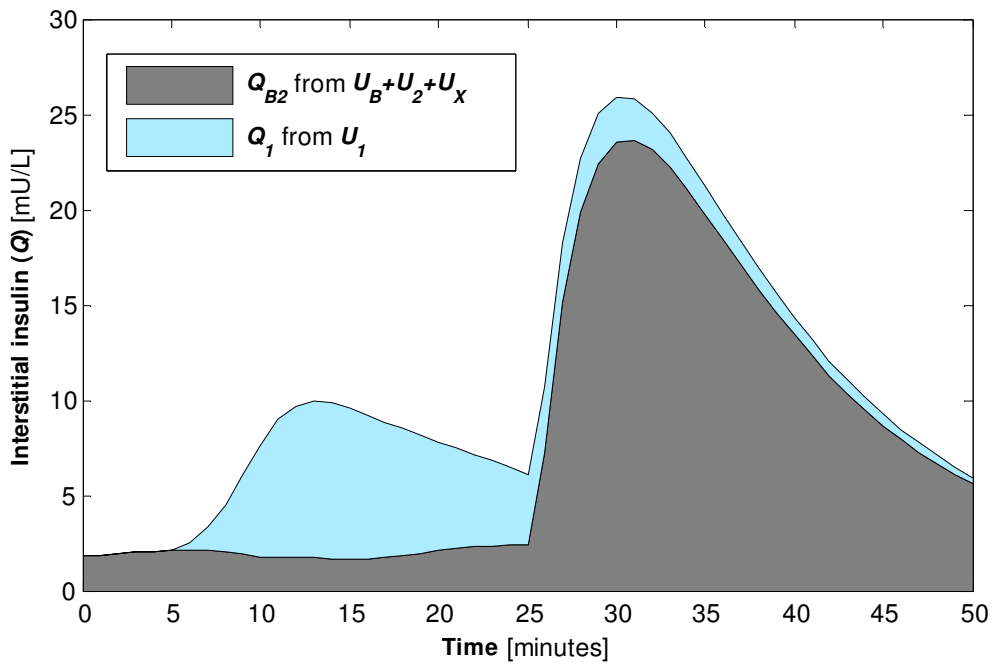
Figure 10.02. The composite $Q_T(t)$ profile from the 15g protocol contrasting the contributing profiles from a participant with a comparatively large first-phase response.

3.   The insulin-glucose dynamic represented from Equation 4.22 was re-arranged to incorporate the separated interstitial insulin concentration:

$$Q_T = Q_{B2} + U_1 Q_1 \qquad 10.05$$

$$\dot{G} = -p_G(G - G_B) - SI(G(Q_{B2} + U_1 Q_1) - G_B Q_B) + \frac{P_X}{V_G}$$

where: U_I is the post-hepatic magnitude of the first phase of insulin production and is the multiplier of the first phase insulin binary vector ($\widetilde{U_1}$) in Equation 10.03.

A non-linear least squares Levenberg-Marquardt algorithm was used to define the SI, U_I and V_G values which minimise the error between the simulated glucose concentration and the measured data.

4. Steps 1 to 3 were repeated five times using updated SI values to estimate n_T, I_B, U_B, and U_2. After five iterations, convergence of SI, Vg and U_I was generally in the order of <0.1%.

10.3.3.3 Monte Carlo analysis design

The simulated noiseless data from each participant was used 200 times, generating a total study cohort of 62600 virtual trials that were slightly inclined toward insulin resistant or at-risk females due to the cohorts of the studies used. During the Monte-Carlo simulation, 2% normally distributed noise was added to the noiseless 'sampled' data set at each iteration to model clinically obtained data. The correlation between the identified insulin sensitivity and first phase endogenous production rates were correlated to the true values and the gradients were defined using Equation 10.06.

$$Grad = \frac{\left\|SI_{DISTqE}\right\|_2}{\left\|SI_{DISST}\right\|_2}$$

10.06

Finally, the identification process was carried out on each data set once with 0% assay error to ascertain the best possible performance.

10.3.4 Results of *in-silico* investigation

Table 10.02 summarises the distribution of the *in-silico* derived parameter values for SI and U_I for the single and double dose DISTqE protocols. Note that the noiseless case does not converge to the fully-sampled DISST values, as the DISTq process is used to generate the participant's insulin concentration response, excluding the first phase insulin production. Thus, results similar to those obtained in Chapter 8 for DISTq were expected.

Protocol	Simulated assay error	*SI* variation R, (Grad)	U_1 variation R, (Grad)
Double G. Bolus	2%	0.78, (1)	0.44, (1.44)
Single G. Bolus	2%	0.78, (1.02)	0.43, (1.33)
Double G. Bolus	0%	0.81, (1.02)	0.51, (1.19)
Single G. Bolus	0%	0.75, (1.02)	0.58, (1.20)

Table 10.02. The coefficients of variation of the identified parameters of the *in-silico* analysis.

Figure 10.04 shows how the DISTqE protocols tested replicated the insulin sensitivity values of the fully-sampled DISST, while Figure 10.05 shows the similarity between the sensitivity values obtained by the two protocols when equivalent assay error was used. Each clinical trial was matched by 200 *in-silico* results, thus the lines in the figures are vertical.
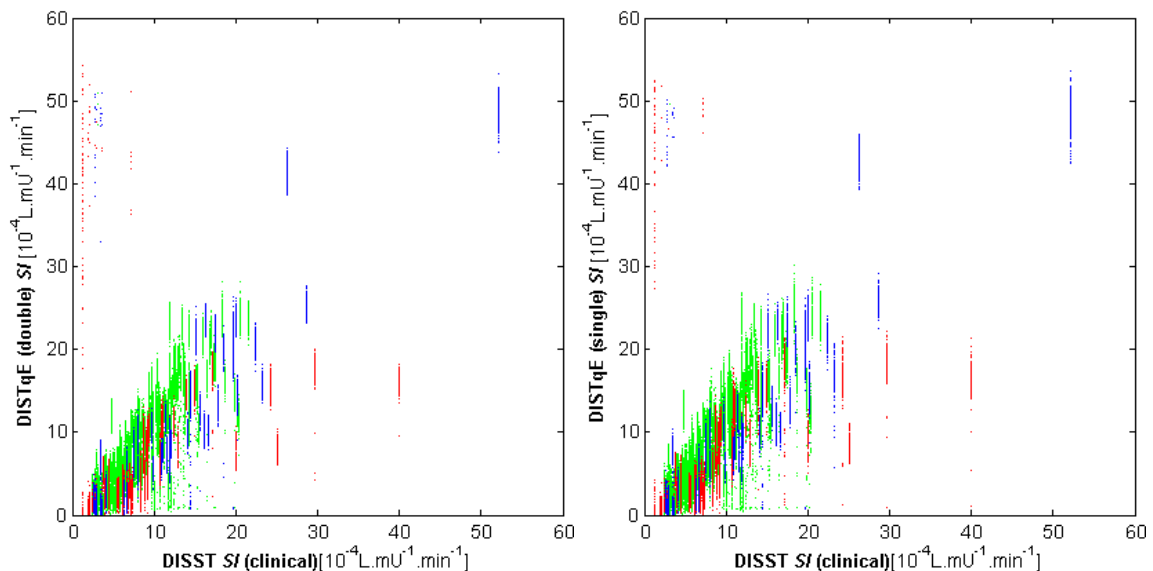
Figure 10.04. Comparison between the double (left) and single (right) bolus DISTqE insulin sensitivity and the clinically measured parent insulin sensitivity values. (blue – pilot; green – intervention; and red - validation)
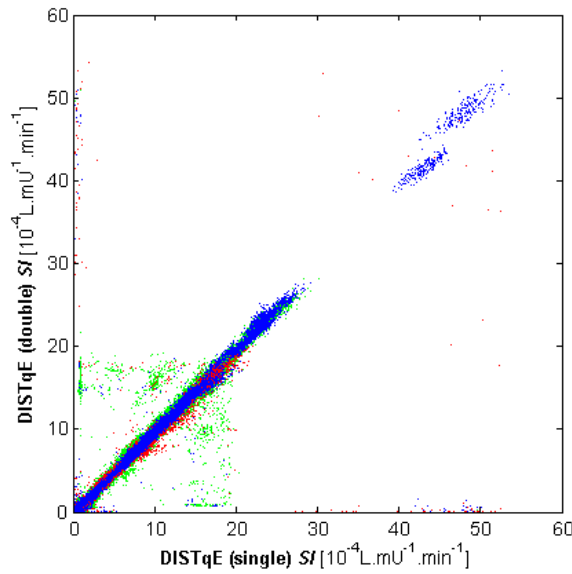
Figure 10.05. Correlation between the single and double DISTqE insulin sensitivity values. (blue - pilot, green - intervention and red - validation)

Figure 10.06 shows the similarity between the first phase insulin production rates defined by the two proposed DISTqE protocols.
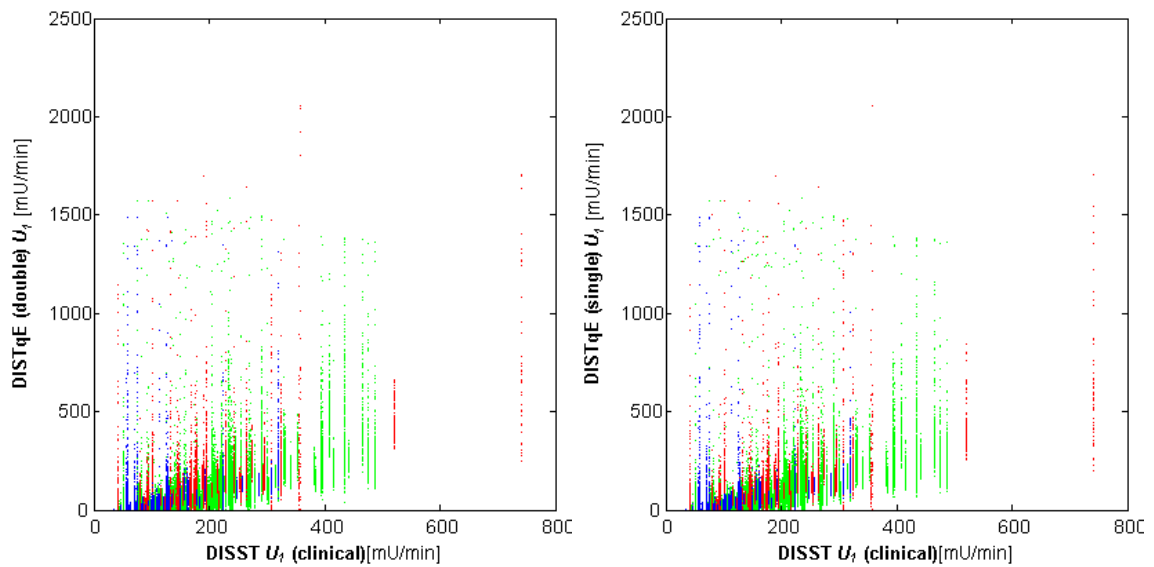
Figure 10.06. Comparison between the double (left) and single (right) bolus DISTqE first phase endogenous insulin production and the clinically measured parent first phase insulin production values. (blue – pilot; green – intervention; and red - validation)

Figure 10.07 shows the correlation between the first phase production rates identified from the two DISTqE protocols.
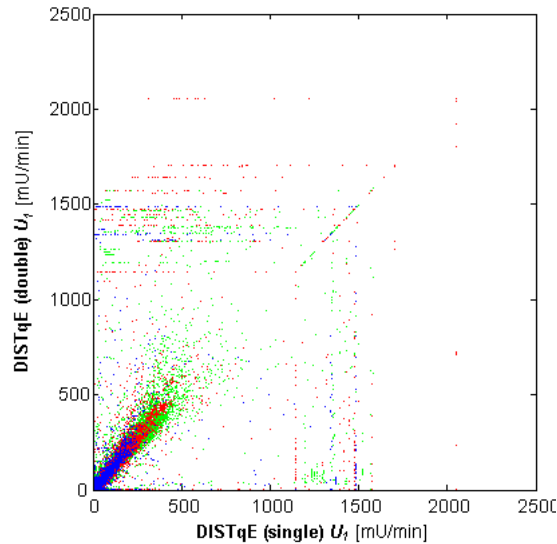
Figure 10.07. Correlation between the single and double DISTqE insulin sensitivity values. (blue – pilot; green – intervention; and red - validation)

The square shapes that occurred around 1200-1600 mU/min were due to the identified U_1 rates reaching the bounds of allowed values during identification. A consistent bound was placed at 300 $mU \cdot L^{-1} \cdot min^{-1}$ for all participants. However, when this value was normalised by the participant's plasma distribution volume, the thresholds become varied and participant-specific. Note how an identical noise can make the identified first phase rate from one protocol reach the bound, while the rate identified from the alternative protocol can be at a minimum. This behaviour shows that the first phase insulin production metric is particularly sensitive to assay error. Figure 10.08 clearly shows the parameters traded-off during identification.
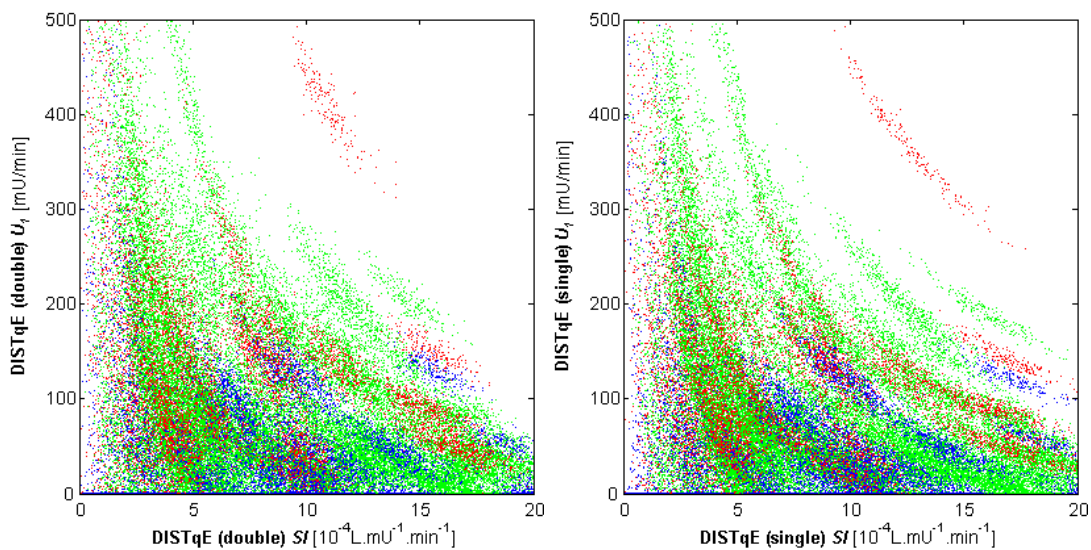
Figure 10.08. Insulin sensitivity and endogenous first phase of insulin production parameters identified from the double (left) and single (right) DISTqE protocols for the pilot (blue), intervention (green) and validation (red) cohorts. (Truncated)

10.3.5 Discussion

Unfortunately, the strength of the first-phase insulin production signal was obscured by the simulated noise. Despite the low CV of the added noise, the derived U_I values were too varied to allow promotion of the protocol for the estimation of real-time and low cost β-cell production estimates in tandem with insulin sensitivity. The appearance of the first phase response in the interstitial insulin profile that is essential to the identification of U_I was delayed and began to trade-off with SI in the same manner as S_G appeared to in Section 5.4.3 and Chapter 7. This occurrence can be confirmed with Figure 10.08, which shows that simulated assay error could shift the identified values to a significant extent by propagating the trade-off between SI and U_I.

The addition of the glucose content to the second cohort did not seem to increase identifiability, as might have been expected. The expected outcome was that the double bolus protocol would allow the measurement of the insulin sensitivity from the glucose decay of the second bolus. The decay of the initial glucose bolus could then be compared to the decay of the second bolus to allow identification of the first phase insulin production rate. Although the integral-based *a-priori* identifiability analysis has not been refined for this 3 parameter case, Figure 10.01 implies that the double glucose bolus protocol would not produce better results than the single bolus protocol. Table 10.02 and Figures 10.04 to 10.07 confirm that there is little distinction between the outcomes of the two protocols.

Furthermore, this analysis was carried out under idealised conditions. In reality, the glucose content of the second bolus may induce a second 'first' phase of endogenous insulin production. This effect could be variable across participants, and thus would be difficult to predict without direct measurement. Hence, significantly less accurate outcomes would be expected if the protocol were attempted clinically. In terms of cost effectiveness, the desired outcomes of the DISTqE protocol would be best served with the DISTq20C protocol with a C-peptide assay obtained at t=5 minutes.

10.4 Conclusions

The first phase of insulin production was barely observable in glucose data when realistic levels of assay error are simulated. Furthermore, by assuming no first phase response to the second glucose bolus, the analysis was inclined toward outcomes that are more favourable. As such, developments of the identification method or protocol must show positive outcomes *in-silico* prior to attempting clinical pilot trials of the protocol.

Potential improvements may include:

- Addition of a third passive species to the bolus that can be assayed in real-time to allow a more accurate estimation of the glucose distribution volume (V_G).
- 1:1 median ratios could be assured for this test is if the DISTq parameter estimation processes are tuned toward this protocol.
- The non-linear least-squares parameter identification method may have converged to local minima in many cases, particularly when the bounds were reached. Application of the iterative integral method could perhaps mitigate this issue.

The extent to which these developments must improve the outcomes seen here implies that the possibility of concurrently identification of insulin sensitivity and first phase production with observations of glucose responses to dynamic tests is unlikely. This analysis perhaps shows the possible limits concerning glucose-only analysis of dynamic metabolic test responses.

Chapter 11. Observing insulin sensitizer and secretagogue drug kinetics *in-silico*

The contents of this chapter describe potential protocols and identification methods based on the overall DISST concept and model to enable a relatively low intensity evaluation of the kinetic behaviour of insulin sensitizer and secretagogue drugs *in-silico*. These drugs are typically tested using 8-24 hour long tests based on the highly intensive euglycaemic clamp. Hence, a new, less intensive, but equally accurate approach would be beneficial.

11.1 Insulin sensitizer observation

Insulin sensitizer drugs are used to prevent the incidence of hyperglycaemia and maintain glucose homeostasis for individuals with type 2 diabetes mellitus. Although there are a reasonable quantity of studies investigating the long term effects of such drugs (Chan & Abrahamson 2003; D'Alessio *et al*. 1995; Juhl *et al*. 2001; Lund *et al*. 2008; Reynolds *et al*. 2002), there are a limited number of studies investigating the single dose kinetics of the drugs (Chung *et al*. 2002; Cox *et al*. 2000; Gerard *et al*. 1984; Pentikäinen *et al*. 1979). Typically, the dynamics of these drugs are measured by the companies that produce them using arduous steady-state EIC tests that last the duration of the drug's efficacy. These steady-state tests require five or ten minute sampling frequency to enable feedback control for euglycaemia. This approach is thus very costly, time consuming and clinically intensive for both the clinicians, and the test subject.

This study investigates a series of sparsely sampled dynamic tests based on the DISST model, methods and approach as a possible alternative for this clinically intense approach. It is hypothesised that a series of DISST-based tests could enable an observation of the change in effect of these drugs over time. To test this hypothesis, an *in-silico* Monte Carlo analysis is completed that simulates the expected level of clinical assay error and measures the ability of the novel identification methods to reproduce values of a theoretical sensitizer drug's pharmaco-kinetics (PK) and pharmaco-dynamics (PD).

11.1.1 Virtual participant

The parameters used to construct the virtual participant of this study were obtained from a participant of the DISST pilot study (Lotz 2007; Lotz *et al.* 2010). This particular participant was very insulin resistant with suspected undiagnosed type 2 diabetes mellitus. The participant had a significant insulin production rate, but a relative inability to effectively clear glucose. Thus, she could be the type of person who might gain an advantage from insulin sensitizer treatment, and is likely to represent the physiology of patients already on insulin sensitizer treatment.

Some key anatomical and PK/PD parameters for this participant are summarised in Table 11.01. For this analysis, saturation of hepatic insulin clearance was assumed negligible. Thus, a_I was set to zero and n_L and n_K are combined to a single parameter of insulin clearance from plasma (n_T).

Sex	Age	BMI [kg.m^{-2}]	U_N [mU/min]			Insulin clearance		V_G [L]	SI [10^{-4}L/mU/min]
			U_B	U_I	U_2	n_T [1/min]	x_L [1]		
F	57	33.9	70.9	143.6	92.5	0.064	0.822	13.4	2.24

Table 11.01. Key parameters of the participant used to generate the virtual participant used in this study.

11.1.2 Model equations

This study used the DISST physiological model defined in Equations 4.01, 4.02, 5.07, (with the n_T parameter), 4.16 and 4.22 described in Section 4.3.

If it is assumed the drug is administered orally or subcutaneously, it would be reasonable to propose a very simple two-compartment model for the PKs of the theoretical insulin sensitizer drug. It is assumed for the purpose of this preliminary, proof-of-concept investigation, that the transport between compartments will be concentration-based and the drug will not re-enter the remote compartment from the active compartment. Figure 11.01 and Equations 11.01 to 11.03 define the model used in this study and the effect of the drug on insulin sensitivity.
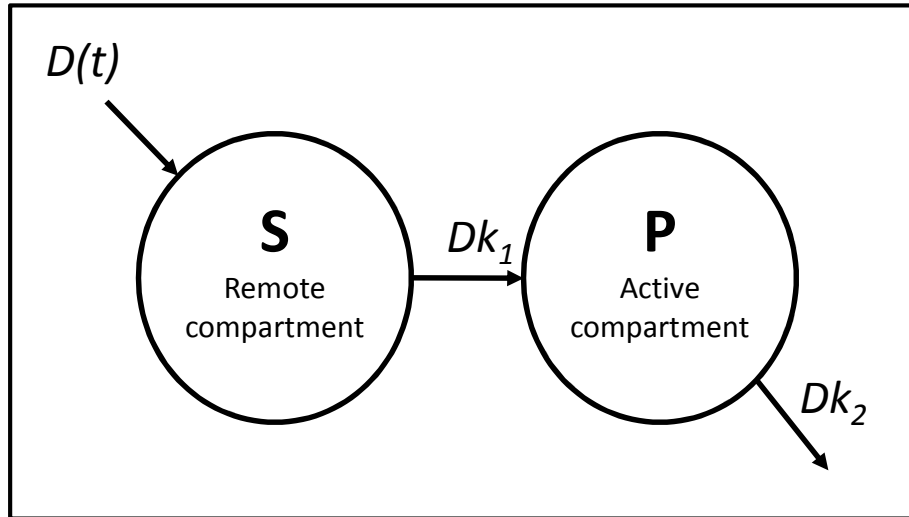
Figure 11.01. Two compartment representation of the PKs of a theoretical sensitizer drug

$$\dot{S}(t) = D(t) - Dk_1 S(t) \qquad\qquad 11.01$$

$$\dot{P}(t) = Dk_1 S(t) - Dk_2 P(t) \qquad\qquad 11.02$$

$$SI(t) = SI_B(1 + P(t)) \qquad\qquad 11.03$$

where: S Is the latent drug effect in a remote compartment [1]; P is the effect of the drug in the active compartment [1]; D is the total potential proportional effect of the drug bolus on SI [1]; Dk_1 is the passive, irreversible, transport rate from the remote to active compartments [1/min], Dk_2 is the passive, irreversible drug clearance rate from the active compartment [1/min]; SI_B is the basal (drug-free) insulin sensitivity [10^{-4}L·mU^{-1}·min^{-1}] and $SI(t)$ is the time-variant insulin sensitivity value [10^{-4}L·mU^{-1}·min^{-1}].

11.1.3 Proposed protocol

The effect of a theoretical insulin sensitizer drug during a series of DISST tests will be simulated in a virtual participant over 760 minutes. This protocol duration is typical of the longer EIC tests used for this purpose. The PKs of the theoretical drug used in this study are based on those of Metformin (Pentikäinen *et al*. 1979) a widely used insulin sensitizer drug. Seven DISST tests were begun at two hourly intervals with blood samples taken at *t*=0, 10 25 and 40 minutes in each test. Thus, the total length of the virtual trial was 760 minutes (12 hours, 40 minutes), and the total number of samples was 28. Each of the seven DISST tests requires a 10g glucose bolus and 1U insulin bolus (intravenous) immediately after the *t*=0 and 10 minute samples respectively.

The sensitizer drug is administered at t=150 minutes, toward the end of the second test. Hence, the first two tests serve as a baseline so each subject is their own control. All samples are assayed for insulin, C-peptide and glucose. The DISST-based protocol is contrasted the steady state method graphically in Figure 11.02.
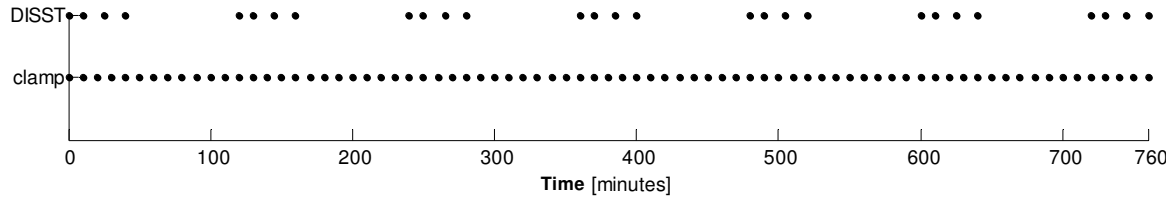
Figure 11.02. Comparison between the EIC and DISST sampling protocols for observing the PKs of insulin sensitizing and secretagogue drugs. Each mark signifies a sample to be taken. The DISST requires 28 samples, while the EIC requires 77.

11.1.4 Parameter identification

This analysis defined U_N, n_T, x_L, SI_B, V_G, D, Dk_1 and Dk_2 as variable parameters to be identified. All other parameters were assumed known as *a-priori* functions of measurable body weight, height age and sex (Lotz 2007; Van Cauter *et al*. 1992). U_N, n_T, x_L, SI_B and V_G were identified using previously presented methods: U_N is identified using a typical deconvolution approach (Van Cauter *et al*. 1992), n_T, x_L, SI_B and V_G were identified using the iterative integral method (Docherty *et al*. 2009; Hann *et al*. 2005b), SI_B and V_G were identified using only data from the first 120 minutes of the proposed protocol and were then held constant.

To identify the PK/PD of the sensitizer drugs, a comprehensive model of the glucose PDs must be generated. Combining Equations 4.22 and 11.03 provides:

$$\dot{G}(t) = -p_G(G(t) - G_B) - SI_B\big(1 + P(t)\big)G(t)Q(t) + SI_B G_B Q_B + \frac{P_X(t)}{V_G} \qquad 11.04$$

where the impact of the insulin sensitizer ($P(t)$) is defined using Equation 11.05, the analytical solution of Equations 11.01 and 11.02:

$$P(t) = Dk_1 e^{-Dk_2 t} \int_0^t (e^{Dk_2 t} e^{-Dk_1 t} \int_0^t e^{Dk_1 t} D(t)) \qquad 11.05$$

The model parameters in Equations 11.04 and 11.05 are not separable in terms of the glucose data. As such, the iterative integral method was not possible. To enable identification of SI_B, V_G, D, Dk_1 and Dk_2, Equation 11.04 must be rearranged to yield an approximation of the $P(t)$ profile from an estimation of the glucose profile from the measured data. Equation 11.04 was rearranged for $P(t)$:

$$P(t) = \frac{-(\dot{G}(t) + p_G(G(t) - G_B) - SI_B G_B Q_B - \frac{P_X(t)}{V_G})}{SI_B G(t) Q(t)} - 1 \qquad 11.06$$

With the exception of $G(t)$ all parameters of Equation 11.06 were known or had been identified. $G(t)$ was then approximated with the highest possible accuracy following the drug administration. Steps 1-4 outline this process and the various $G(t)$ profiles are shown in Figure 11.03.

1. A preliminary simulation of $G(t)$ ($G(t)_{\text{prelim}}$) was obtained for the full duration of all tests. Initially, this task was undertaken using the values for SI_B and V_G identified with the iterative integral method and the baseline data from the first 150 minutes of the test. Subsequent iterations used V_G and the time variant $SI(t)$.
2. A linear interpolation between the measured data points ($G(t)_{\text{interp}}$) was defined.
3. A linear interpolation between the values of $G(t)_{\text{prelim}}$ at the sample times ($G(t)_{\text{preint}}$) was also defined.
4. The difference between $G(t)_{\text{interp}}$ and $G(t)_{\text{preint}}$ could be attributable to the effect of the sensitizer and thus Equation 11.07 was used as an approximation of $G(t)$:

$$G(t) = G(t)_{\text{prelim}} + G(t)_{\text{interp}} - G(t)_{\text{preint}} \qquad 11.07$$
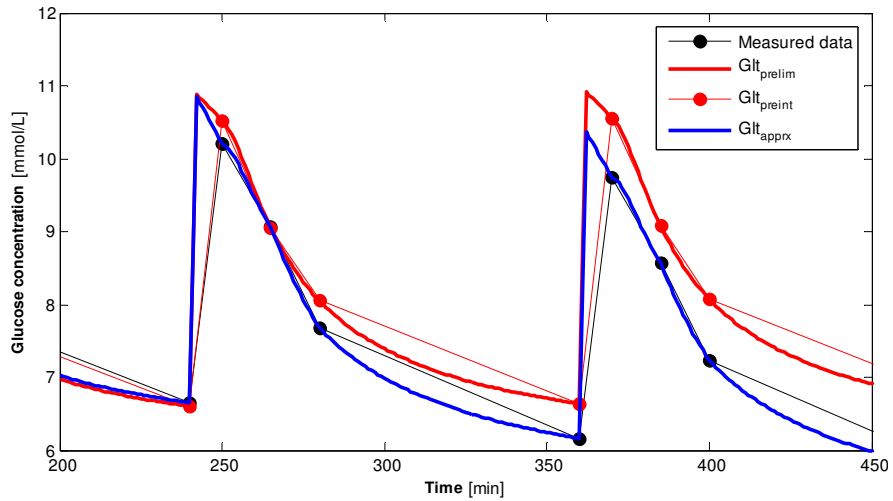
Figure 11.03. How $G(t)$ is calculated from the measured data and the estimated $G(t)_{prelim}$ profile.

With an approximation of $G(t)$, $P(t)$ can be found from Equation 11.06. This $P(t)$ profile is then used with Equation 11.05 in a non-linear least square Levenberg-Marquardt parameter identification method to find the values of Dk_1, Dk_2 and D that minimise the function in Equation 11.08.

$$error = \left\| P(t) - Dk_1 e^{-Dk_2 t} \int_0^t (e^{Dk_2 t} e^{-Dk_1 t} \int_0^t e^{Dk_1 t} D(t)) \right\|_2 \qquad 11.08$$

Once the PK/PD values of Equation 11.08 had converged sufficiently, $P(t)$ was re-evaluated using Equation 11.05. Figure 11.04 shows the form of the simulation, and the $P(t)$ profiles derived via Equation 11.06 and identified.
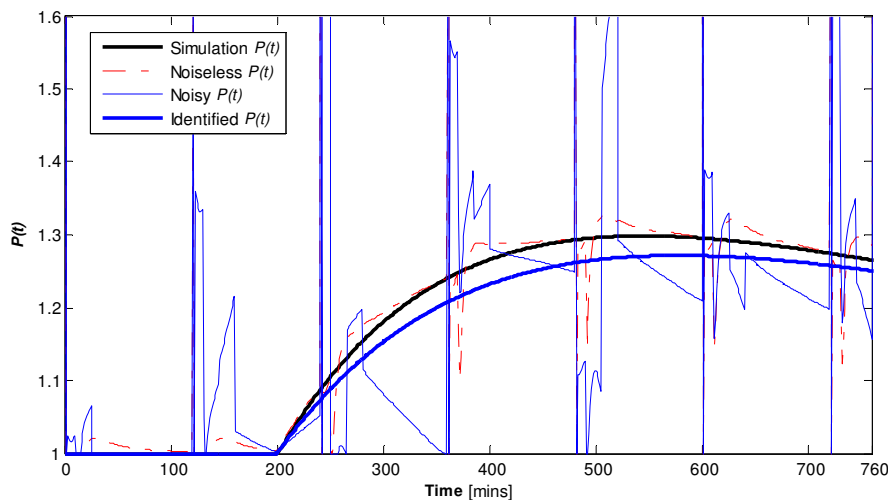
Figure 11.04. Deconvolved and simulation values of $P(t)$. The vertical lines show the discontinuities caused by the glucose boluses.

$G(t)$ was re-simulated once $P(t)$ was defined. Steps 1 to 4 and the Levenberg-Marquardt parameter identification process were iterated. In total, five iterations were used, by which time, parameter convergence for all parameters was on the order of 1%. The basal period was not re-identified.

11.1.5 Statistical evaluation

A Monte Carlo analysis was used to assess the ability of the identification method and proposed protocol to detect and quantify the PKs of the theoretical sensitizer drug. C-peptide, insulin and glucose concentration profiles were simulated using Equations 4.01, 4.02, 5.07, 4.16 and 11.04 with the protocol defined in Section 11.1.3.

The participant parameter values used in the simulation are shown in Table 11.01. The theoretical drug kinetics were based on those of Metformin (Pentikäinen *et al*. 1979): drug absorption (Dk_1) was defined as 0.005/min, representing an absorption half-life of ~140 minutes. Similarly, drug clearance (Dk_2) was defined as 0.0015/min, representing a clearance half-life of ~460 minutes. Finally, the proportional effect on *SI* (*D*) was defined as 0.5, meaning a 50% increase in insulin sensitivity could be expected if the full amount of the drug dose was in the active compartment. The three C-peptide, insulin and glucose profiles were 'sampled' at the prescribed times and these concentrations represented a noiseless data-set.

The Monte-Carlo simulation identified the eight parameters mentioned at the start of Section 11.1.4 1000 times using the identification method described. Each iteration had normally distributed random noise added to the noiseless glucose, insulin and C-peptide data sets generated using clinically measured values. The magnitude of the added noise was in accordance with realistic clinical assay error (glucose: CV=2%, insulin: CV=3%, and C-peptide: CV=4% to a maximum of three standard deviations).

The median and coefficient of variation of the identified parameters of Equations 11.01 and 11.02 are presented. Furthermore, the median and inter-quartile range (IQR) of the *SI(t)* residuals will be compared to the noiseless simulation of Equation 11.04 to assess the ability of the method to track the PDs of the drug.

11.1.6 Study Results

Table 11.02 summarises the variation in the identified parameters in Equations 11.01 and 11.02 that define the drug PK/PDs. Figure 11.05 shows the range of time-varying sensitivity profiles identified by the method described in Section 11.1.4. Table 11.03 summarises the residuals of the time varying insulin sensitivity profiles shown in Figure 11.05.

	True Value	Identified value	
		Mean (CV)	Median (IQR)
SI [10^{-4}L/mU/min]	2.24	2.25 (7.9%)	2.24 (2.11-2.36)
V_G [L]	13.4	13.4 (4.5%)	13.3 (13.0-13.7)
D [1]	0.5	0.498 (0.9%)	0.500 (0.498-0.500)
Dk_1 [10^{-3}/min]	5	10.3 (116.3%)	6.0 (4.8-8.6)
Dk_2 [10^{-3}/min]	1.5	1.3 (41.4%)	1.3 (1.0-1.7)

Table 11.02. The effect of noise on parameter identification.

It can be seen that the median profile was approximately equal to the true value for the duration of the test. The identification method generally seemed to slightly overestimate the drug absorption rate (Dk_1). However, the 100th percentile simulation (Figure 11.05) shows that some outliers drastically overestimate this absorption rate. These values must contribute to the higher than expected mean and CV for Dk_1 reported in Table 11.02. In particular, the median and IQR of Dk_1 indicates that the values typically identified were well within expected ranges. Similar results and variances occur for Dk_2 as well.

Period	Simulation residual Median (IQR)
Basal Period	0.004 (-0.122, 0.158)
`2.5-6 hours	0.091 (-0.037, 0.224)
6-12 hours	0.048 (-0.079, 0.183)
Overall	0.047 (-0.093, 0.1842)

Table 11.03. Residuals of the $SI(t)$ profiles [10^{-4}L·mU^{-1}·min^{-1}].
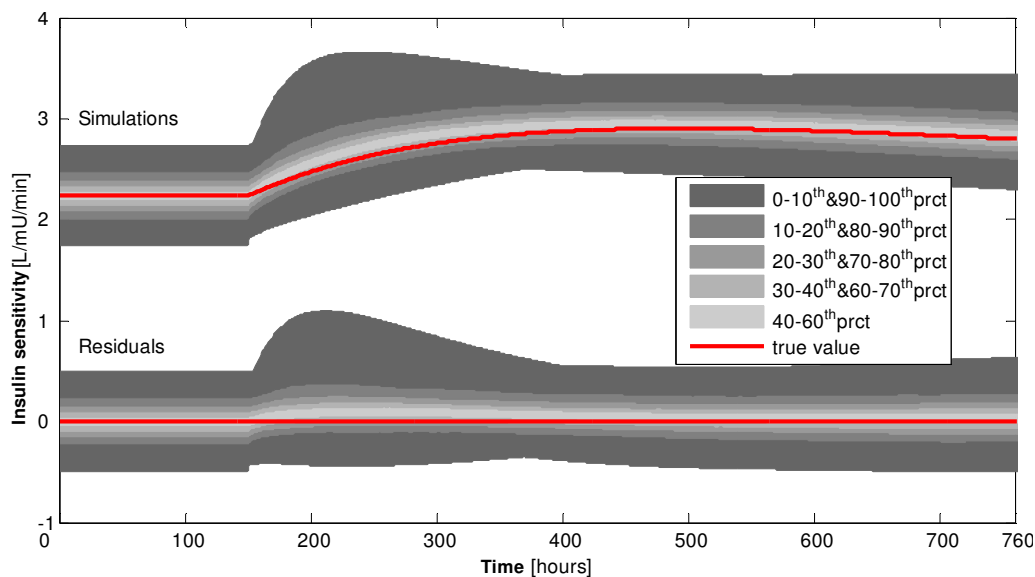
Figure 11.05. A comparison between the identified insulin sensitivity profiles and the 'true' time variant value of insulin sensitivity for this *in-silico* analysis.

11.1.7 Applicability of the DISST for tracing insulin sensitizer drugs

The simulated PKs of the composite, idealised sensitizer drug were relatively observable in the multiple DISST test data using the proposed protocol and identification methods. The variation in *SI* (7.9%) was slightly larger than previous a Monte Carlo study (Section 4.5) and (Lotz *et al*. 2008). This outcome was an artefact of the reduced sampling rate compared to the test protocol used in that study. Hence, the increased variation was somewhat expected and, to an extent, validated the other outcomes of this analysis.

The proportional drug effect (*D*) measurement was particularly stable to noise (CV of 0.9%). However, the drug absorption (Dk_1) and decay rate (Dk_2) parameters were considerably more susceptible to noise (116.3% and 41.4%, respectively). This considerable variation in the rate parameters was largely due to outliers, as the median and IQR were relatively accurate. Equally, it did not have a significant effect on the ability of the protocol and identification method to trace the kinetic and dynamic behaviours of the theoretical drug (Table 11.03).

The variation in the basal insulin sensitivity was comparable to the variation in *SI*(*t*). This result implies that parameter trade off occurs. Hence, although the methods presented might not be ideal for the identification of all parameters concurrently, they may be appropriate for predicting the overall activity of the drug over time. Importantly, this overall activity measure is the key

outcome of such tests, as the PKs can usually be directly measured with further species assays if desired.

In particular, the findings of this study imply that a clinical pilot investigation of sensitizer kinetics could be undertaken with this DISST-based approach. The existing option for the identification of sensitizer drugs PD/PKs is a continuous steady state test, such as the euglycaemic clamp. This test raises the participant's insulin concentration with a continuous insulin infusion, and euglycaemia is maintained with variable rate of glucose infusion. This variable rate is defined by using feedback control of frequently sampled glucose samples. A specific drug dose will be administered at approximately 2.5-3 hours when the glucose infusion is generally stable. This approach allows a more accurate estimation of the time-varying increase of the insulin sensitivity profile. However, it comes at the cost of significantly increased clinical burden and cost. Table 11.04 summarizes and compares the attributes of the multiple DISST approach to the existing option for tracing the kinetics and dynamics of insulin sensitizer drugs.

	DISST protocol	EIC protocol
Blood samples	28	77-154
Sample cost ($NZ)	~800-1600*	~250-300
Down-time	80 minutes every two hours	None
Validation	Sparse	Extensive
Physiological relevance	Within normal range	Hyper-physiological
Hypoglycaemic risk	Very minimal	Very minimal

Table 11.04. The costs and benefits of two possible methods for observing the kinetics and dynamics of insulin sensitizer drugs. (* the C-peptide assays could potentially be omitted to save approximately 50% of the assay cost with very limited effect on outcomes.)

The overall identification method used in this analysis was comprised of two separate types of identification method. Initially, the iterative integral method was used to identify the insulin kinetic parameters, and then the SI and V_G from the test period prior to drug administration. Following this step, non-linear least squares was used to identify the PK's of the sensitizer drug.

The iterative integral method could not identify all five parameters of Equations 11.04 and 11.05 as they are not separable in terms of measureable assays. Furthermore, when the five parameter

case was directly applied, the Levenberg-Marquardt non-linear lest squares method was either unstable, or unable to converge. As such, the iterative integral method was used to identify *SI* and V_G during the pre-drug-dose baseline period, and a change in insulin sensitivity profile is then generated by the drug that allowed a non-linear least-squares identification of the three drug PK and PD parameters.

This strategy enabled relatively fast, very stable parameter identification. The 1000 simulation Monte Carlo analysis required approximately 3 hours of simulation time. Equally importantly, the 0th and 100th percentile shown in Figure 11.05 show that none of the randomly generated data sets prompted significant failure of the identification process.

The identification process and the identified values could be further stabilised if one or more of the parameters of Equation 11.05 could be fixed. For example, the rate of drug absorption (Dk_1) may be known, but not the maximal effect (*D*) or decay rate (Dk_2). In this case, the non-linear least square step would only have two parameters and identification trade-off would become negligible. Similarly, the decay rate could be predetermined or bounded in separate prior tests.

There were some limitations in this investigation. In particular, the model was contrived for a theoretical drug based on published data and contains simplified PK's that may not fully represent the true PK/PD's of actual sensitizer drugs. Such omitted effects may include:

- Irreversible transport between the remote and active compartments may not be necessarily representative of the kinetics of drugs administered subcutaneously. If the drug is administered orally, diffusion across the gut membrane is irreversible, and the model assumption is valid in this case.
- The drug may be designed with the intension that it lie dormant, stored in fat cells for delayed dispersion. This may be modelled with an added passive third compartment with two way, condition-dependent transport.
- Glucose production suppression is not modelled as a time-variant or dependent variable parameter in this investigation
- A combined secretagogue effect will be observable with the C-peptide measurement during the trial.
- If the drug delays the absorption of food- such as GLP1 enhancers, the effect will not be captured by this type of test, nor the steady state methods. This will not affect dynamic fasting tests, but will be an important attribute of the drug that is not quantified.
- It is likely that the drug's effect on insulin sensitivity may be saturable, i.e. doubling the drug content may only increase sensitivity by 50-70%.

- The drug may require molecular changes that take time to occur. This is not modelled as such, but will be observable 'lumped-in' with the rate of absorption parameter.

All of these factors can be incorporated into the model and thus, do not invalidate the findings of this analysis. However, the model appears to be close to the limit of identification in the presence of noise. Hence, any further addition would probably require known kinetic behaviours and rates, which are likely to be available from prior tests.

The proposed multiple DISST protocol offers a comparatively low-intensity option for the identification of the kinetics and dynamics of insulin sensitizer drugs. The time-varying insulin sensitivity profiles identified in this *in-silico* analysis were quite accurate. However, the parameters that defined the profiles showed that identification trade-off was occurring. Thus, although the proposed protocol will enable the identification of the effect of the drug overall, uncertainty exists in the identified drug absorption and decay rate values.

The findings of this study indicate that a pilot trial of this protocol and the identification methods discussed would enable the observation and quantification of insulin sensitizer drugs. However, the next step for this type of test would be a clinical analysis of a particular drug, instead of the *in-silico* analysis presented here.

11.2 Observing insulin secretagogue kinetics

An *in-silico* study was also undertaken to measure the observability of insulin secretagogues. However, the observability of insulin secretagogues was very poor using dynamic tests. In particular, the first and second phase responses to the glucose bolus must be identified individually. In doing so, parameter identification interference was encountered. However, the results are presented here for contrast and completeness.

11.2.1 Study design

The series of seven DISST tests undertaken at 2-hourly intervals from Section 11.1.1.3 was repeated in the virtual participant described in Section 11.1.1.1. The sensitizer model described by Equations 11.01 and 11.02 in Section 11.1.1.2 was used to define the kinetics of the secretagogue drug. Glibenclamide kinetic parameters were used as a reference for this model.

Gerard *et al*. defined absorption and decay half-lives of 45 and 135 minutes, respectively (Gerard *et al*. 1984). This translates to kinetic parameters of Dk_1=15 and Dk_2=5 [10^{-3}/min].

It was assumed for this *in-silico* analysis that the full dose of the secretagogue would increase the first phase of production by 40% (D_1) while the second phase would be amplified by 70% (D_2). However, the time-variant increase would be dependent on the concentration of the secretagogue in the accessible compartment.

The C-peptide response of the virtual participant, defined in Table 11.01, was simulated using the endogenous insulin production responses to the DISST test. The U_N rates defined the block-wise profile shown in Figure 11.06. A five-minute first phase period was followed by a 35-minute second phase period. This period was followed by a linear reduction back to the basal rate over 40 minutes, where it remained until the next DISST test. The secretagogue concentration was assumed to have a proportional effect on the first and second production rates above basal (Equation 11.09). Thus, the U_B rate remained constant throughout the protocol, but the first and second phases are amplified by the insulin secretagogues.

$$U_{(1|2)\text{S`gogue}}(t) = U_B + \left(1 + P(t) * D_{(1|2)}\right)\left(U_{(1|2)} - U_B\right) \qquad 11.09$$

where $D_{(1|2)}$ is the maximal effect that the drug dose could have on the first or second insulin production period.
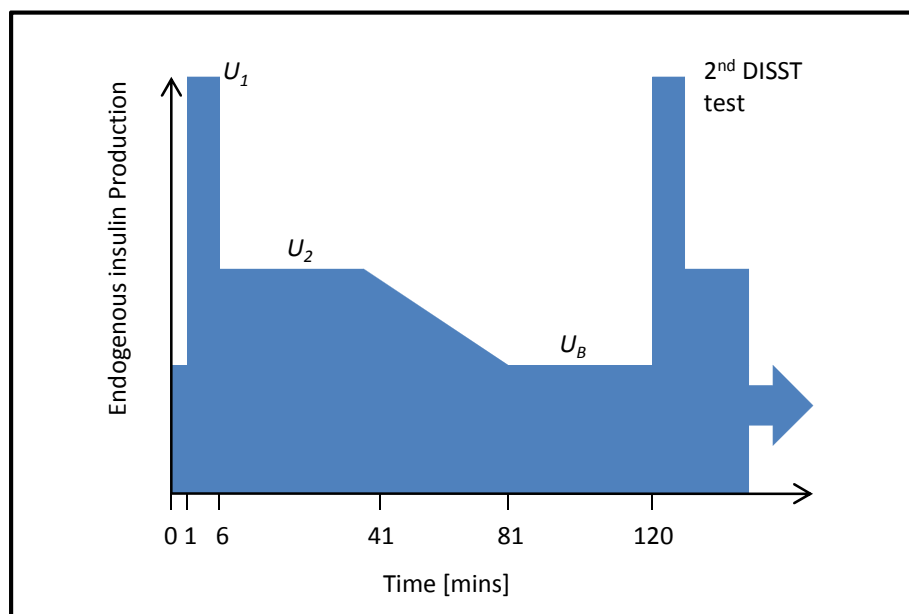
Figure 11.06. Indicative shape of the simulated insulin production profile.

The insulin production profile was then used to simulate the plasma and interstitial C-peptide concentrations (*C(t)* and *Y(t)*). A noiseless data set was obtained by sampling the synthetic data at the times defined in Section 11.1.3 for use as the input in a Monte Carlo simulation. During the Monte Carlo simulation, normally distributed noise was added to the noiseless set in accordance with reported C-peptide assay error (4%) at each iteration. In total, 100 noisy data sets were generated from which the model parameters were identified.

11.2.2 Parameter identification

Steps 1-3 were repeated for each run of the Monte-Carlo analysis to obtain values for the model parameters.

1. The data between *t*=0 and *t*=120 was used with the iterative integral method to identify the first and second phase insulin production responses of the first DISST protocol. Thus the pre-bolus sample of the 2nd DISST test was also used.

2. The subsequent production responses to the following DISST protocols were also identified in isolation. (The first sample of the following test is used as a data point.) Thus, seven first phase and seven second phase values were obtained.

3. These values were used in a Levenberg-Marquardt non-linear least squares identification method to obtain values for Dk_1, Dk_2, D_1 and D_2 that minimise the error in Equation 11.09.

The outcomes of each Monte Carlo run were recorded and the parameter accuracy and variability is reported.

11.2.3 Investigation results

The 4% noise simulation produced consistently failed results. Thus, 0.4% noise was used to observe the level of assay accuracy required to achieve clinically useful results and test the identifiability of the parameters. The analysis defined in Section 11.2.1 was repeated for the reduced assay error. Table 11.05 shows the spread of the values obtained by the identification method using both the clinically realistic 4% noise and the hypothetical 0.4% noise case.

Although the parameter identification was relatively inaccurate, even for the 0.4% noise case, the re-simulations of the time-variant first and second phase production rates adhered to the initial simulation. Figure 11.06 shows the deciles of the re-simulated profiles in comparison to the initial simulated drug effect profile, while Table 11.06 quantifies the residuals of the re-simulated profiles.

Parameter	True Value	0%	0.4% noise		4% noise	
			Mean (CV)	Median (IQR)	Mean (CV)	Median (IQR)
$Dk1$ [10^{-3}/min]	15	14.9	15.6 (28.0%)	15.1 (12.0, 18.0)	71.2 (169.6%)	14.6 (7.3, 68.0)
$Dk2$ [10^{-3}/min]	5	5.2	5.3 (22.9%)	5.1 (4.4, 6.2)	13.9 (462.4%)	4.8 (2.0, 8.5)
D_1 [1]	0.4	0.41	0.41 (21.1%)	0.42 (0.36, 0.48)	0.61 (94.9%)	0.49 (0.01, 1.01)
D_2 [1]	0.7	0.69	0.70 (16.7%)	0.69 (0.61, 0.79)	0.87 (68.8%)	0.82 (0.40, 1.28)
U_1 [mU/min]	233.6	227.9	227.9 (0.991%)	227.8 (226.5, 229.2)	227.5 (9.0%)	227.2 (213.6, 241.2)
U_2 [mU/min]	150.7	152.2	152.3 (0.940%)	152.2 (151.9, 152. 7)	152.1 (4.0%)	152.3 (148.0, 156.2)

Table 11.05. Identified kinetic and effect parameters of the secretagogue model.
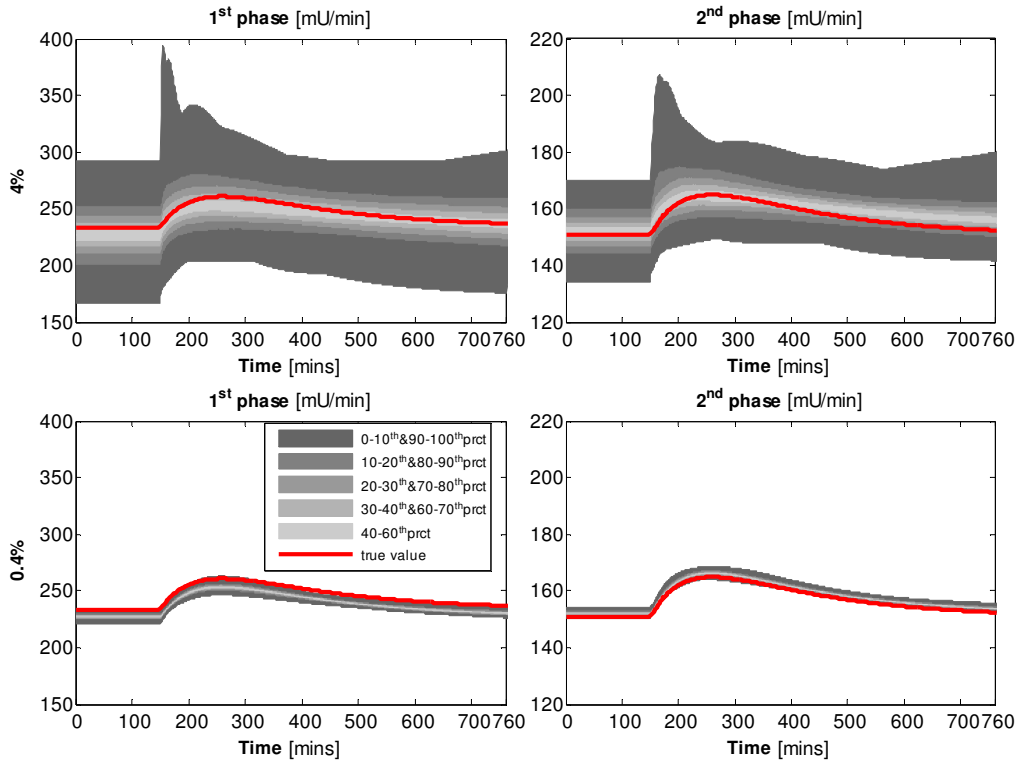
Figure 11.07. Re-simulations of the time variant secretagogue amplification of the first (left) and second phases (right) of insulin production while 4% (top) and 0.4% (bottom) assay error is applied.

| Period | Phase | Simulation residual | |
| | | 0.4% noise | 4% noise |
		Median (IQR)*	Median (IQR)*
Basal Period	U_1	-5.86 (-7.11, -4.41)	-6.40 (-20.00, 7.62)
	U_2	1.55 (1.16, 1.97)	1.59 (-2.67, 5.55)
2.5-6 hours	U_1	-6.66 (-8.13, -5.18)	-5.52 (-17.09, 7.75)
	U_2	1.60 (1.09, 2.10)	0.97 (-3.16, 5.54)
6-12 hours	U_1	-6.41 (-7.45, -5.31)	-1.25 (-11.79, 9.49)
	U_2	1.48 (1.12, 1.87)	2.89 (-0.08, 5.97)
Overall	U_1	-6.32 (-7.55, -5.08)	-2.90 (-14.67, 8.99)
	U_2	1.52 (1.12, 1.94)	2.33 (-1.33, 5.91)

Table 11.06. Residuals between the simulation and identified *P(t)* values.

11.2.4 Implication of the results

Concurrent identification of time variant profiles for first and second phase insulin production rates seemed to be limited by parameter trade off. When 4% assay error was simulated in the virtual data, the coefficients of variation in the kinetic parameters was between 65%-500%. Thus, useful parameter values could not be obtained using the protocol and identification method tested here.

Furthermore, when the 0.4% noise was added to the idealised data, which represents a 90% reduction in the assay error that would otherwise be expected in a clinical setting, the parameter coefficients of variation were still about 20%. This smaller parameter variation is perhaps the lower limit of resolution that may be clinically usable. However, the assay error required to obtain this result is unrealistic. Thus, for the method to be usable to trace both first and second phase insulin production, improvements in assay technique must enable a 10x increase in accuracy, which is highly unlikely in the foreseeable future.

The median re-simulations were relatively well in accordance with the true simulation values. Thus, the median simulations converged to relatively accurate values and thus the method was validated to a small degree. However, the wide inter-quartile range and the apparent trade-off between first and second phases of insulin production imply that the model and DISST approach may not be particularly suited to this particular application.

To improve resolution, the number of parameters should be reduced. Either some known drug kinetic information must be provided, or either first or second phase secretion must be set as a constant or dependent parameter. However, in making this change, much of the information that is being sought, must be provided, seriously limiting the potential utility of method presented. The only realistic choice of reducing unknowns would typically be more knowledge of the drug kinetics, which can be readily tested independently prior to such PK/PD tests.

The hyperglycaemic EIC is unable to distinguish the first and second phases of insulin production. The results obtained are most representative of the second phase of insulin secretion. However, the EIC protocol is quite stable with relatively high resolution outcomes, from a high intensity test. The performance of the presented dynamic test protocol is not sufficient to displace the hyperglycaemic EIC for tracing secretagogue drugs.

PART FIVE: Conclusions and future work

Chapter 12. Conclusions

12.1 Clinical applications

This thesis expanded upon the DISST test that was created during the doctoral investigation of Dr Thomas Lotz (Lotz 2007). The DISST test was developed to provide diabetes risk assessments through the quantification of insulin sensitivity and insulin production metrics. Although both of these metrics are required to observe an individual's position on the pathogenesis of type 2 diabetes, they are not provided in tandem by the established insulin sensitivity tests. The validation investigation presented in Chapter 6 confirmed that the DISST occupies a favourable position on axes of accuracy and economy in comparison to established insulin sensitivity tests. Accordingly, the DISST has sufficient merit to be considered as a suitable method of insulin sensitivity testing, and makes the more much arduous, yet less accurate, IVGTT obsolete.

The Minimal Model of the insulin/glucose PDs is generally regarded as intrinsic to the identification of any dynamic insulin sensitivity test data. However, the investigation presented in Chapter 7 showed that the Minimal Model fails to accurately quantify insulin sensitivity using DISST data. In contrast, the DISST model captured the behaviour measured by other tests and means. This outcome did not necessarily invalidate the Minimal Model, which has been extensively validated. However, it does unequivocally show that the DISST model is more suited to the identification of parameters for the low intensity and duration DISST protocol and can produce more reasonable results in insulin resistant cohorts. Equally, it provides further clinically important metrics for metabolic health appraisal and management, with greater test robustness.

Chapter 8 contained the most significant, novel discovery presented in this thesis: the quick DIST (DISTq). DISTq showed that contrary to established paradigms in the field, accurate insulin sensitivity identification can be possible in real-time. The real-time aspect means that insulin and C-peptide assays cannot be used during parameter identification. Thus, the participant's insulin concentration must be estimated. Previous attempts to do this with OGTT data failed and thus, it is generally assumed that the participant's insulin concentration must be accurately measured in order for accurate quantification of insulin sensitivity. The DISTq identification method overcomes this limitation with the application of a novel, purpose-designed, *a-posteriori* iterative process that exploited the relationships between insulin parameters and insulin sensitivity.

The outcomes of the DISTq investigation show that it can replicate the fully-sampled DISST with a relatively high accuracy (R=0.82 to 0.86) and, perhaps more importantly, that it can replicate the gold standard EIC insulin sensitivity values (R=0.77). In correlating to the EIC at R=0.77 the DISTq achieves a greater performance than most established insulin sensitivity tests, even those that assay insulin. It trails only the fully-sampled DISST and some isolated IM-IVGTT outcomes, while exceeding the correlation obtained by the majority of investigations. The ITT outcomes also exceeded the DISTq. However, the ITT protocol has been abandoned due to excessive participant risk. Furthermore, the DISTq30 was proposed, which correlated to the EIC at R=0.80, and thus offers a slight improvement upon the DISTq. Hence, it further exceeds the performance of most established tests.

These outcomes are significant and should prompt an evaluation of whether much more widespread diabetes risk screening programmes can be undertaken. Such programmes have not been pursued in the past, in part, due to the inhibitive economic and clinical burden of protocols that produce values that are sufficiently accurate. With the DISTq, insulin sensitivity values can be obtained for $10 and 30 minutes with a resolution that would previously cost more than $750 and 3 hours. It remains to be investigated whether this development is sufficient to instigate risk screening programmes.

The DISST, DISTq and DISTq30 do not necessarily represent the optimum tests possible from the DISST format. Rather, they exist upon a spectrum of possible tests that use the basic DISST protocol and specific assays to obtain differing compromises of accuracy, information and economy. This spectrum was investigated in Chapter 9. A hierarchical test design was developed using the tests defined in the spectrum analysis. In essence, the hierarchy system showed that very high resolution could be obtained for a very low cost by exploiting the equivalent protocol of the tests on the DISST spectrum. If a participant's low-cost test value was within a range close to the diagnosis threshold, a higher resolution value could be obtained by re-assaying some, or all, of the samples obtained during the original test for insulin or C-peptide. Assuming sufficient numbers can be identified using the original low-cost test, a very high resolution can be obtained for a very minimal average per-participant cost.

Chapter 10 explored two variants of the DISST protocol that were tailored for the DISTq identification method. The DISST protocol was designed to obtain insulin sensitivity in tandem with insulin production though assaying of C-peptide, insulin and glucose. Thus, the period between the glucose and insulin boluses could be assumed to provide no advantage to the DISTq identification. An *in-silico* investigation showed that this assumption is valid and that the DISTq30 protocol could perhaps be reduced to 20 minutes with a combined bolus. This approach

would enable extremely low-cost insulin sensitivity values that could perhaps be obtained during a consultation in a general practice setting.

The second alternative DISTq protocol investigated *in-silico* aimed to show whether the first phase of insulin production (U_1) was observable as a surrogate of its action in glucose data when the glucose bolus was increased and the following bolus was delayed. Unfortunately, the first phase manifestation on the interstitial insulin profile that is required for identification of U_1 interfered with the glucose bolus coefficient, and the observability was severely limited. Thus, pilot investigations of this protocol are not recommended without further advances. This study may define some limits as to how widely such glucose-only tests might be effective.

Chapter 11 showed how a DISST framework or approach could replace the existing EIC protocols for the tracking of insulin sensitizer and secretagogue drugs. Parameter trade-off during identification of the secretagogue drug effects limited the applicability of dynamic tests for this purpose. However, the proposed multiple DISST protocol and identification method was able to quantify and track the effect of insulin sensitizers relatively well.

12.2 Mathematical developments

The iterative integral method was developed and applied for the first time to identify parameters of the DISST model. Chapter 4 presented this method, as well as a method that uses positive derivative feedback control on the iterative process to accelerate the convergence to the least-squares parameter values. The method is particularly fast for non-linear cases and is robust to initial estimates and assay error.

An integral-based *a-priori* model identifiability analysis method was developed and presented in Chapter 5. This method was developed after the frustrating false positive outcomes from the existing algebraic derivative-based model identifiability analyses that do not recognise or account for assay placement or error. The method successfully captured the true nature of identifiability, which is analogous in the presence of assay error. Furthermore, it predicted parameter trade-off during identification in a number of cases including the famous Minimal Model trade-off that is exacerbated in insulin resistant participants. This trade-off had been extensively reported without explanation until this development.

Chapter 13. Future work

To achieve full and un-equivocal acceptance of the DISST methodology, investigations similar to the DISST pilot and validation studies must be undertaken by an isolated research group. Furthermore, the test or model must be investigated directly for the primary purpose it was designed: type 2 diabetes risk assessment. As such, participants of the DISST must be recalled 10 to 15 years after their initial test and undergo a 2hr-OGTT for a diagnosis of diabetes. This will directly and explicitly validate the DISST for type 2 diabetes risk assessment as opposed to the implied validation of EIC equivalence.

Identifiability of the Minimal Model in insulin resistant cohorts has been an on-going issue for the Minimal Model. A protocol was proposed in Section 5.4.3 that increased the distinction in interfering parameters' coefficients and thus identifiability. However, the protocol was limited to allow a fair comparison to the DISST protocol parameter identifiability and was thus not the best possible protocol for this purpose. A protocol could be developed and tested *in-silico* to show precisely what is required for the Minimal Model to become identifiable in an insulin resistant cohort.

DISTq was validated in tandem with the fully-sampled DISST and exhibited very positive outcomes. However, further refinement of the population-based parameter estimation equations or model may increase the accuracy or precision of the derived metrics. In particular, the estimated values could be modified by a participant's family history of diabetes/metabolic disease, anatomical makeup, abnormal physiology or exposure to diabetes. An immediately recognisable example is type 1 diabetic individuals for whom the parameter estimation equations for U_N are not representative.

In terms of application, the real-time and low-cost attributes of DISTq, DISTq30 and perhaps DISTq20C, if clinically validated, enable a myriad of previously impossible applications of insulin sensitivity. The efficacy of the test for the following uses must be investigated:

- Diabetes risk assessment during a general practice consult
- Tailored insulin therapy for newly diagnosed IDDM individuals
- Daily testing for insulin sensitivity high dependency units (HDU) to track recovery or identify sepsis.
- Low-cost surrogate for insulin sensitivity in clinical intervention or metabolic tracking trials
- Screening for diabetes risk (possibly with the hierarchical system)

The *in-silico* analysis of DISTq20C used some assumptions that must be clinically validated. The outcomes of the *in-silico* analysis were very positive. However, if the analysis assumptions prove to be incorrect, the actual clinical correlation to the EIC may be marginally less than the correlation implied by the *in-silico* analysis.

The DISTqE analysis showed that the parameter values identified are not sufficiently accurate to pursue clinical pilots at this stage. However, the identifiability of the DISTqE protocol could perhaps be improved if a third species could be added to the initial bolus that could be assayed in real time to enable the identification of V_G. This would mitigate the identifiability issues and greatly increase the observability of U_1. However, this remains to be investigated *in-silico*.

The integral-based *a-priori* identifiability analysis method was able to describe, predict and finally quantify the parameter trade-off exhibited in a number of cases related to insulin sensitivity. However, the method currently cannot predict the coefficient of variation prior to analysis, merely the shift in variation due to changes in the protocol or participant. It is very likely that with further investigation of the matrix algebra that drives the method, the coefficient of variation could be identified prior to any simulation. Furthermore, the method could be adapted to appraise models with three or more parameters, in contrast to the current method that can only appraise two-parameter models. The method could perhaps be used in a wide variety of models not limited to physiology.

References

ADA 2005, 'Diagnosis and Classification of Diabetes Mellitus', *Diabetes Care,* vol. 28, no. suppl 1, pp. S37-S42.

ADA 2006, 'Diagnosis and Classification of Diabetes Mellitus', *Diabetes Care,* vol. 29, no. suppl 1, pp. S43-S8.

ADA 2007, 'Standards of Medical Care in Diabetes 2007', *Diabetes Care,* vol. 30, no. suppl 1, pp. S4-S41.

ADA 2010, 'Summary of Revisions for the 2010 Clinical Practice Recommendations', *Diabetes Care,* vol. 33, no. Suppl 1, p. S3.

Ader, M & Bergman, RN 1987, 'Insulin sensitivity in the intact organism', *Baillieres Clin Endocrinol Metab,* vol. 1, no. 4, pp. 879-910.

Ahrén, B & Pacini, G 2005, 'Islet adaptation to insulin resistance: mechanisms and implications for intervention', *Diabetes, Obesity and Metabolism,* vol. 7, no. 1, pp. 2-8.

Amato, MC, Giordano, C, Galia, M, Criscimanna, A, Vitabile, S, Midiri, M, Galluzzo, A & for the AlkaMeSy Study, G 2010, 'Visceral Adiposity Index', *Diabetes Care,* vol. 33, no. 4, pp. 920-2.

Antuna-Puente, B, Disse, E, Faraj, M, Lavoie, M-E, Laville, M, Rabasa-Lhoret, R & Bastard, J-P 2009, 'Evaluation of insulin sensitivity with a new lipid-based index in non-diabetic postmenopausal overweight and obese women before and after a weight loss intervention', *Eur J Endocrinol,* vol. 161, no. 1, pp. 51-6.

Argoud, GM, Schade, DS & Eaton, RP 1987, 'Insulin suppresses its own secretion in vivo', *Diabetes,* vol. 36, no. 8, pp. 959-62.

Arleth, T, Andreassen, S, Federici, MO & Benedetti, MM 2000, 'A model of the endogenous glucose balance incorporating the characteristics of glucose transporters', *Comput Methods Programs Biomed,* vol. 62, no. 3, pp. 219-34.

Assmann, G, Schriewer, H, Schmitz, G & Hagele, EO 1983, 'Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2', *Clin Chem,* vol. 29, no. 12, pp. 2026-30.

Audoly, S, Bellu, G, D'Angio, L, Saccomani, MP & Cobelli, C 2001, 'Global identifiability of nonlinear models of biological systems', *IEEE Trans Biomed Eng,* vol. 48, no. 1, pp. 55-65.

Audoly, S, D'Angio, L, Saccomani, MP & Cobelli, C 1998, 'Global identifiability of linear compartmental models: a computer algebra algorithm', *IEEE Trans Biomed Eng,* vol. 45, no. 1, pp. 36-47.

Avogaro, A, Bristow, JD, Bier, DM, Cobelli, C & Toffolo, G 1989, 'Stable-label intravenous glucose tolerance test minimal model', *Diabetes,* vol. 38, no. 8, pp. 1048-55.

Barr, RG, Nathan, DM, Meigs, JB & Singer, DE 2002, 'Tests of Glycemia for the Diagnosis of Type 2 Diabetes Mellitus', *Annals of Internal Medicine,* vol. 137, no. 4, pp. 263-72.

Barrett, EJ, Eggleston, EM, Inyard, AC, Wang, H, Li, G, Chai, W & Liu, Z 2009, 'The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action', *Diabetologia,* vol. 52, no. 5, pp. 752-64.

Bellman, R & Åström, KJ 1970, 'On structural identifiability', *Math Biosci,* vol. 7, no. 3-4, pp. 329-39.

Bellu, G, Saccomani, MP, Audoly, S & D'Angio, L 2007, 'DAISY: A new software tool to test global identifiability of biological and physiological systems', *Comput Methods Programs Biomed,* vol. 88, no. 1, pp. 52-61.

Benedict, C, Hallschmid, M, Hatke, A, Schultes, B, Fehm, HL, Born, J & Kern, W 2004, 'Intranasal insulin improves memory in humans', *Psychoneuroendocrinology,* vol. 29, no. 10, pp. 1326-34.

Bergman, RN, Ider, YZ, Bowden, CR & Cobelli, C 1979, 'Quantitative estimation of insulin sensitivity', *Am J Physiol,* vol. 236, no. 6, pp. E667-77.

Bergman, RN, Phillips, LS & Cobelli, C 1981, 'Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose', *J Clin Invest,* vol. 68, no. 6, pp. 1456-67.

Bergman, RN, Prager, R, Volund, A & Olefsky, JM 1987, 'Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp', *J Clin Invest,* vol. 79, no. 3, pp. 790-800.

Bluestone, JA, Herold, K & Eisenbarth, G 2010, 'Genetics, pathogenesis and clinical interventions in type 1 diabetes', *Nature,* vol. 464, no. 7293, pp. 1293-300.

Bonora, E, Moghetti, P, Zancanaro, C, Cigolini, M, Querena, M, Cacciatori, V, Corgnati, A & Muggeo, M 1989, 'Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies', *J Clin Endocrinol Metab,* vol. 68, no. 2, pp. 374-8.

Bonora, E, Targher, G, Alberiche, M, Bonadonna, RC, Saggiani, F, Zenere, MB, Monauni, T & Muggeo, M 2000, 'Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity', *Diabetes Care,* vol. 23, no. 1, pp. 57–63.

Borghouts, LB & Keizer, HA 2000, 'Exercise and Insulin Sensitivity: a Review.', *Int J Sports Med,* vol. 21, no. 1, pp. 1-12.

Boston, RC, Stefanovski, D, Moate, PJ, Sumner, AE, Watanabe, RM & Bergman, RN 2003, 'MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test', *Diabetes Technol Ther,* vol. 5, no. 6, pp. 1003-15.

Breda, E, Toffolo, G, Polonsky, KS & Cobelli, C 2002, 'Insulin release in impaired glucose tolerance: oral minimal model predicts normal sensitivity to glucose but defective response times', *Diabetes,* vol. 51 Suppl 1, pp. S227-33.

Carson, ER & Cobelli, C 2001, *Modelling methodology for physiology and medicine*, Academic Press, San Diego.

Caumo, A, Bergman, RN & Cobelli, C 2000, 'Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index', *J Clin Endocrinol Metab,* vol. 85, no. 11, pp. 4396-402.

Caumo, A & Cobelli, C 1993, 'Hepatic glucose production during the labeled IVGTT: estimation by deconvolution with a new minimal model', *Am J Physiol,* vol. 264, no. 5 Pt 1, pp. E829-41.

Caumo, A, Vicini, P & Cobelli, C 1996, 'Is the minimal model too minimal?', *Diabetologia,* vol. 38, no. 8, pp. 997-1000.

Caumo, A, Vicini, P, Zachwieja, JJ, Avogaro, A, Yarasheski, K, Bier, DM & Cobelli, C 1999, 'Undermodeling affects minimal model indexes: insights from a two-compartment model', *Am J Physiol,* vol. 276, no. 6 Pt 1, pp. E1171-93.

Cederholm, J & Wibell, L 1990, 'Insulin release and peripheral sensitivity at the oral glucose tolerance test', *Diabetes Res Clin Pract,* vol. 10, no. 2, pp. 167-75.

Chan, JL & Abrahamson, MJ 2003, 'Pharmacological Management of Type 2 Diabetes Mellitus: Rationale for Rational Use of Insulin', *Mayo Clinic Proceedings,* vol. 78, no. 4, pp. 459-67.

Chase, JG, Mayntzhusen, K, Docherty, PD, Andreassen, S, McAuley, KA, Lotz, TF & Hann, CE 2010, 'A three-compartment model of the C-peptide-insulin dynamic during the DIST test', *Math Biosci,* vol. 228, no. 2, pp. 136-46.

Chen, H, Sullivan, G & Quon, MJ 2005, 'Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model', *Diabetes,* vol. 54, no. 7, pp. 1914-25.

Cherrington, AD 1999, 'Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo', *Diabetes,* vol. 48, no. 5, pp. 1198-214.

Chin, SV & Chappell, MJ 2010, 'Structural identifiability and indistinguishability analyses of the Minimal Model and a Euglycemic Hyperinsulinemic Clamp model for glucose-insulin dynamics', *Comput Methods Programs Biomed,* vol. In Press, Corrected Proof.

Chung, M, Kourides, I, Canovatchel, W, Sutfin, T, Messig, M & Chaiken, RL 2002, 'Pharmacokinetics and pharmacodynamics of extended-release glipizide GITS compared with immediate-release glipizide in patients with type II diabetes mellitus', *The Journal of Clinical Pharmacology,* vol. 42, no. 6, pp. 651-7.

Clark, A, Jones, LC, de Koning, E, Hansen, BC & Matthews, DR 2001, 'Decreased insulin secretion in type 2 diabetes: a problem of cellular mass or function?', *Diabetes,* vol. 50, no. Suppl 1, pp. S169-71.

Clark, M, Hampson, SE, Avery, L & Simpson, R 2004, 'Effects of a tailored lifestyle self-management intervention in patients with type 2 diabetes.', *Br J Health Psychol,* vol. 9, no. 3, pp. 365-79.

Cobelli, C, Bettini, F, Caumo, A & Quon, MJ 1998, 'Overestimation of minimal model glucose effectiveness in presence of insulin response is due to undermodeling', *Am J Physiol,* vol. 275, no. 6 Pt 1, pp. E1031-6.

Cobelli, C, Caumo, A & Omenetto, M 1999, 'Minimal model SG overestimation and SI underestimation: improved accuracy by a Bayesian two-compartment model', *Am J Physiol,* vol. 277, no. 3 Pt 1, pp. E481-8.

Cobelli, C & Pacini, G 1988, 'Insulin secretion and hepatic extraction in humans by minimal modeling of C-peptide and insulin kinetics', *Diabetes,* vol. 37, no. 2, pp. 223-31.

Cobelli, C, Pacini, G, Toffolo, G & Sacca, L 1986, 'Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT', *Am J Physiol,* vol. 250, no. 5 Pt 1, pp. E591-8.

Cobelli, C, Toffolo, G, Dalla Man, C, Campioni, M, Denti, P, Caumo, A, Butler, P & Rizza, R 2007, 'Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests', *Am J Physiol Endocrinol Metab,* vol. 293, no. 1, pp. E1-E15.

Consoli, A 1992, 'Role of Liver in Pathophysiology of NIDDM', *Diabetes Care,* vol. 15, no. 3, pp. 430-41.

Cox, PJ, Ryan, DA, Hollis, FJ, Harris, A-M, Miller, AK, Vousden, M & Cowley, H 2000, 'Absorption, Disposition, and Metabolism of Rosiglitazone, a Potent Thiazolidinedione Insulin Sensitizer, in Humans', *Drug Metabolism and Disposition,* vol. 28, no. 7, pp. 772-80.

Craft, S, Asthana, S, Newcomer, JW, Wilkinson, CW, Matos, IT, Baker, LD, Cherrier, M, Lofgreen, C, Latendresse, S, Petrova, A, Plymate, S, Raskind, M, Grimwood, K & Veith, RC 1999, 'Enhancement of Memory in Alzheimer Disease With Insulin and Somatostatin, but Not Glucose', *Arch Gen Psychiatry,* vol. 56, no. 12, pp. 1135-40.

D'Alessio, DA, Prigeon, RL & Ensinck, JW 1995, 'Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes. A physiological role of glucagon-like peptide I', *Diabetes,* vol. 44, no. 12, pp. 1433-7.

Dalla Man, C, Caumo, A, Basu, R, Rizza, R, Toffolo, G & Cobelli, C 2005a, 'Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model', *Am J Physiol Endocrinol Metab,* vol. 289, no. 5, pp. E909-14.

Dalla Man, C, Yarasheski, KE, Caumo, A, Robertson, H, Toffolo, G, Polonsky, KS & Cobelli, C 2005b, 'Insulin sensitivity by oral glucose minimal models: validation against clamp', *Am J Physiol Endocrinol Metab,* vol. 289, no. 6, pp. E954-9.

Davidson, MB, Harris, MD, Ziel, FH & Rosenberg, CS 1987, 'Suppression of Sleep-Induced Growth-Hormone Secretion Abolishes the Dawn Phenomenon in Type-I Diabetic-Patients', *Clinical Research,* vol. 35, no. 1, pp. A148-A.

De Gaetano, A & Arino, O 2000, 'Mathematical modelling of the intravenous glucose tolerance test', *J Math Biol,* vol. 40, no. 2, pp. 136-68.

DeFronzo, RA & Ferrannini, E 1991, 'Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease', *Diabetes Care,* vol. 14, no. 3, pp. 173-94.

DeFronzo, RA, Tobin, JD & Andres, R 1979, 'Glucose clamp technique: a method for quantifying insulin secretion and resistance', *Am J Physiol,* vol. 237, no. 3, pp. E214-23.

Del Prato, S, Marchetti, P & Bonadonna, RC 2002, 'Phasic insulin release and metabolic regulation in type 2 diabetes', *Diabetes,* vol. 51 Suppl 1, pp. S109-16.

Del Prato, S, Matsuda, M, Simonson, DC, Groop, L, Sheehan, P, Leonetti, F, Bonadonna, RC & DeFronzo, RA 1997, 'Studies in the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance.', *Diabetologia,* vol. 40, no. 6, pp. 687-97.

Denti, P, Bertoldo, A, Vicini, P & Cobelli, C 2009, 'Nonlinear mixed effects to improve glucose minimal model parameter estimation: A simulation study in intensive and sparse sampling', *IEEE Trans Biomed Eng,* vol. 56, no. 9, pp. 2156-66.

Devreese, K & Leroux-Roels, G 1993, 'Laboratory assessment of five glucose meters designed for self-monitoring of blood glucose concentration', *Eur J Clin Chem Clin Biochem,* vol. 31, no. 12, pp. 829-37.

Docherty, P, Chase, JG, Lotz, T & Desaive, T 2011a, 'A graphical method for practical and informative identifiability analyses of physiological models: A case study of insulin kinetics and sensitivity', *Biomedical Engineering Online,* vol. 10, no. 1, p. 39.

Docherty, PD, Chase, JG & David, T 2011b, 'Characterisation of the iterative integral parameter identification method', *Med. Biol. Eng. Comput. ,* vol. (In Review).

Docherty, PD, Chase, JG, Hann, CE, Lotz, TF, Lin, J, McAuley, KA & Shaw, GM 2010, 'The identification of insulin saturation effects during the dynamic insulin sensitivity test', *Open Med. Inform. J.,* vol. 4, pp. 141-8.

Docherty, PD, Chase, JG, Lotz, T, Hann, CE, Shaw, GM, Berkeley, JE, Mann, JI & McAuley, KA 2009, 'DISTq: An iterative analysis of glucose data for low-cost real-time and accurate estimation of insulin sensitivity', *Open Med. Inform. J.,* vol. 3, pp. 65-76.

Docherty, PD, Chase, JG, Lotz, TF, Hann, CE, Shaw, GM, Berkeley, JE, TeMorenga, L, Mann, JI & McAuley, K 2011c, 'Independent cohort cross-validation of the real-time DISTq estimation of insulin sensitivity', *Comput Methods Programs Biomed,* vol. 102, no. 2, pp. 94-104.

Docherty, PD, Chase, JG, Lotz, TF & Krebs, JD 2011d, 'Evaluation of a Glomerular Filtration Term in the DISST Model to Capture the Glucose Pharmacodynamics of an Insulin-Resistant Cohort', in *18th IFAC World congress*, ed. S Andreassen, Milan, Italy

Donner, CC, Fraze, E, Chen, YD, Hollenbeck, CB, Foley, JE & Reaven, GM 1985, 'Presentation of a new method for specific measurement of in vivo insulin-stimulated glucose disposal in humans: comparison of this approach with the insulin clamp and minimal model techniques', *J Clin Endocrinol Metab,* vol. 60, no. 4, pp. 723-6.

Eaton, RP, Allen, RC, Schade, DS, Erickson, KM & Standefer, J 1980, 'Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior', *J Clin Endocrinol Metab,* vol. 51, no. 3, pp. 520-8.

Edsberg, B, Herly, D, Hildebrandt, P & Kuhl, C 1987, 'Insulin bolus given by a sprinkler needle: effect on absorption and glycaemic response to a meal', *Br Med J (Clin Res Ed),* vol. 294, no. 6584, pp. 1373-6.

Erichsen, L, Agbaje, OF, Luzio, SD, Owens, DR & Hovorka, R 2004, 'Population and individual minimal modeling of the frequently sampled insulin-modified intravenous glucose tolerance test', *Metabolism,* vol. 53, no. 10, pp. 1349-54.

Ferrannini, E 1997, 'Insulin resistance is central to the burden of diabetes', *Diabetes Metab Rev,* vol. 13, no. 2, pp. 81-6.

Ferrannini, E & Cobelli, C 1987, 'The kinetics of insulin in man. II. Role of the liver', *Diabetes Metab Rev,* vol. 3, no. 2, pp. 365-97.

Ferrannini, E, Gastaldelli, A, Miyazaki, Y, Matsuda, M, Mari, A & DeFronzo, RA 2005, 'beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis', *J Clin Endocrinol Metab,* vol. 90, no. 1, pp. 493-500.

Ferrannini, E & Mari, A 1998, 'How to measure insulin sensitivity', *J Hypertens,* vol. 16, no. 7, pp. 895-906.

Ferrannini, E & Mari, A 2004, 'Beta cell function and its relation to insulin action in humans: a critical appraisal', *Diabetologia,* vol. 47, no. 5, pp. 943-56.

Ferrari, P, Alleman, Y, Shaw, S, Riesen, W & Weidmann, P 1991, 'Reproducibility of insulin sensitivity measured by the minimal model method', *Diabetologia,* vol. 34, no. 7, pp. 527-30.

Finegood, DT, Pacini, G & Bergman, RN 1984, 'The insulin sensitivity index. Correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp', *Diabetes,* vol. 33, no. 4, pp. 362-8.

Foley, JE, Chen, YD, Lardinois, CK, Hollenbeck, CB, Liu, GC & Reaven, GM 1985, 'Estimates of in vivo insulin action in humans: comparison of the insulin clamp and the minimal model techniques', *Horm Metab Res,* vol. 17, no. 8, pp. 406-9.

Gakidou, E, Mallinger, L, Abbot-Klafter, J, Guerrero, R, Villalpando, S, Ridaura, RL, Aekplakorn, W, Naghavi, M, Lim, SK, Lozano, R & Murray, CJL 2010, 'Management of diabetes and associated cardiovascular risk factors in seven countries: a comparison of data from national health examination surveys', *Bull World Health Organ,* vol. 89, no. 3, pp. 161-240.

Galvin, P, Ward, GM, Walters, JM, Pestell, R, Koschmann, M, Vaag, A, Martin, I & Best, JD 1992, 'A simple method for quantitation of insulin sensitivity and insulin release from an intravenous glucose tolerance test', *Diabet Med,* vol. 9, no. 10, pp. 921-8.

Garcia-Estevez, DA, Araujo-Vilar, D, Fiestras-Janeiro, G, Saavedra-Gonzalez, A & Cabezas-Cerrato, J 2003, 'Comparison of several insulin sensitivity indices derived from basal plasma insulin and glucose levels with minimal model indices', *Horm Metab Res,* vol. 35, no. 1, pp. 13-7.

Gastaldelli, A, Ferrannini, E, Miyazaki, Y, Matsuda, M & DeFronzo, RA 2004, 'Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study', *Diabetologia,* vol. 47, no. 1, pp. 31-9.

Gelding, SV, Robinson, S, Lowe, S, Niththyananthan, R & Johnston, DG 1994, 'Validation of the low dose short insulin tolerance test for evaluation of insulin sensitivity', *Clinical Endocrinology,* vol. 40, no. 5, pp. 611-5.

Gerard, J, Lefebvre, PJ & Luyckx, AS 1984, 'Glibenclamide pharmacokinetics in acarbose-treated type 2 diabetics', *European Journal of Clinical Pharmacology,* vol. 27, no. 2, pp. 233-6.

Greisen, J, Juhl, CB, Grofte, T, Vilstrup, H, Jensen, TS & Schmitz, O 2001, 'Acute pain induces insulin resistance in humans', *Anesthesiology,* vol. 95, no. 3, pp. 578-84.

Groop, LC, Widen, E & Ferrannini, E 1993, 'Insulin resistance and insulin deficiency in the pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus: errors of metabolism or of methods?', *Diabetologia,* vol. 36, no. 12, pp. 1326-31.

Grulet, H, Durlach, V, Hecart, AC, Gross, A & Leutenegger, M 1993, 'Study of the rate of early glucose disappearance following insulin injection: insulin sensitivity index', *Diabetes Res Clin Pract,* vol. 20, no. 3, pp. 201-7.

Guerrero-Romero, F, Simental-Mendia, LE, Gonzalez-Ortiz, M, Martinez-Abundis, E, Ramos-Zavala, MG, Hernandez-Gonzalez, SO, Jacques-Camarena, O & Rodriguez-Moran, M 2010, 'The Product of Triglycerides and Glucose, a Simple Measure of Insulin Sensitivity. Comparison with the Euglycemic-Hyperinsulinemic Clamp', *J Clin Endocrinol Metab,* vol. 95, no. 7, pp. 3347-51.

Gutt, M, Davis, CL, Spitzer, SB, Llabre, MM, Kumar, M, Czarnecki, EM, Schneiderman, N, Skyler, JS & Marks, JB 2000, 'Validation of the insulin sensitivity index ($ISI_{0,120}$): comparison with other measures', *Diabetes Res Clin Pract,* vol. 47, no. 3, pp. 177-84.

Hallschmid, M & Schultes, B 2008, 'The potential of intranasal insulin in the management in metabolic disorders', *US Endocrinology,* vol. 4, no. 2, pp. 55-8.

Hanley, AJ, Williams, K, Festa, A, Wagenknecht, LE, D'Agostino, RB, Jr. & Haffner, S 2005, 'Liver markers and development of the metabolic syndrome: The insulin resistance atherosclerosis study', *Diabetes,* vol. 54, no. 11, pp. 3140-7.

Hanley, AJ, Williams, K, Gonzalez, C, D'Agostino, RB, Jr., Wagenknecht, LE, Stern, MP & Haffner, SM 2003, 'Prediction of type 2 diabetes using simple measures of insulin resistance: combined results from the San Antonio Heart Study, the Mexico City Diabetes Study, and the Insulin Resistance Atherosclerosis Study', *Diabetes,* vol. 52, no. 2, pp. 463-9.

Hann, C, Chase, J, Andreassen, S, Smith, B & Shaw, G 2005a, 'Diagnosis Using a Minimal Cardiac Model Including Reflex Actions', *Intensive Care Med,* vol. 31, no. S1, p. S18.

Hann, C, Chase, J & Shaw, G 2006, 'Integral-based Identification of Patient Specific Parameters for a Minimal Cardiac Model', *Comput Methods Programs Biomed,* vol. 81, no. 2, pp. 181-92.

Hann, CE, Chase, JG, Lin, J, Lotz, T, Doran, CV & Shaw, GM 2005b, 'Integral-based parameter identification for long-term dynamic verification of a glucose-insulin system model', *Comput Methods Programs Biomed,* vol. 77, no. 3, pp. 259-70.

Harris, RH, Donahue, K, Rathore, SS, Frame, P, Woolf, SH & Lohr, KL 2003, 'Screening Adults for Type 2 Diabetes: A Review of the Evidence for the U.S. Preventative Services Task Force', *Ann. Intern. Med,* vol. 138, no. 3, pp. 215-29.

Hauschke, D, Kieser, M, Diletti, E & Burke, M 1999, 'Sample size determination for proving equivalence based on the ratio of two means for normally distributed data', *Statistics in Medicine,* vol. 18, pp. 93-105.

Hollenbeck, CB & Reaven, G 1987, 'Treatment of patients with non-insulin-dependent diabetes mellitus: diabetic control and insulin secretion and action after different treatment modalities.', *Diabet Med,* vol. 4, no. 4, pp. 311-6.

Hovorka, R, Chassin, L, Luzio, SD, Playle, R & Owens, DR 1998, 'Pancreatic beta-cell responsiveness during meal tolerance test: model assessment in normal subjects and subjects with newly diagnosed noninsulin-dependent diabetes mellitus', *J Clin Endocrinol Metab,* vol. 83, no. 3, pp. 744-50.

Hrebicek, J, Janout, V, Malincikova, J, Horakova, D & Cizek, L 2002, 'Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention', *J Clin Endocrinol Metab,* vol. 87, no. 1, pp. 144-7.

Hume, R 1966, 'Prediction of lean body mass from height and weight', *J Clin Pathol.,* vol. 19, no. 4, pp. 389-91.

Ingelsson, E, Sundstrom, J, Arnlov, J, Zethelius, B & Lind, L 2005, 'Insulin Resistance and Risk of Congestive Heart Failure', *Jama,* vol. 294, no. 3, pp. 334-41.

Juhl, CB, Parksen, N, Pincus, SM, Hansen, ÃP, Veldhuis, JD & Schmitz, O 2001, 'Acute and Short-Term Administration of a Sulfonylurea (Gliclazide) Increases Pulsatile Insulin Secretion in Type 2 Diabetes', *Diabetes,* vol. 50, no. 8, pp. 1778-84.

Kahn, SE, Hull, RL & Utzschneider, KM 2006, 'Mechanisms linking obesity to insulin resistance and type 2 diabetes', *Nature,* vol. 444, no. 7121, pp. 840-6.

Katsuki, A, Sumida, Y, Gabazza, EC, Murashima, S, Furuta, M, Araki-Sasaki, R, Hori, Y, Yano, Y & Adachi, Y 2001, 'Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes', *Diabetes Care,* vol. 24, no. 2, pp. 362-5.

Katsuki, A, Sumida, Y, Urakawa, H, Gabazza, EC, Murashima, S, Morioka, K, Kitagawa, N, Tanaka, T, Araki-Sasaki, R, Hori, Y, Nakatani, K, Yano, Y & Adachi, Y 2002, 'Neither Homeostasis Model Assessment nor Quantitative Insulin Sensitivity Check Index Can Predict Insulin Resistance in Elderly Patients with Poorly Controlled Type 2 Diabetes Mellitus', *J Clin Endocrinol Metab,* vol. 87, no. 11, pp. 5332-5.

Katz, A, Nambi, SS, Mather, K, Baron, AD, Follmann, DA, Sullivan, G & Quon, MJ 2000, 'Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans', *J Clin Endocrinol Metab,* vol. 85, no. 7, pp. 2402-010.

Kim, C & Kim, SB 2007, 'Modelling contaminant transport in a three-phase groundwater system with the Freundlich-type retardation factor', *Environmental Technology,* vol. 28, no. 2, pp. 205-16.

Kolterman, OG, Insel, I & Saekow, M 1980, 'Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects.', *J. Clin. Invest.,* vol. 65, no. 6, pp. 1272-84.

Krebs, JD, Bell, D, Hall, R, Parry-Strong, A, Docherty, PD, Clarke, K & Chase, JG 2011, 'Improvements in glucose metabolism and insulin sensitivity with a low-carbohydrate diet in obese patients with type 2 diabetes: A pilot study', *Journal of the American College of Nutrition,* vol. (in review).

Laakso, M, Edelman, SV, Brechel, G & Baron, AD 1990, 'Decreased effect of insulin to stimulate skeletal muscle blood flow in Obese man: A novel mechanism for insulin resistance.', *J. Clin. Invest.,* vol. 85, no. 6, pp. 1844-52.

Levenberg, K 1944, 'A method for the solution of certain non-linear problems in least squares', *Quarterly of Applied mathematics,* vol. 2, pp. 164-8.

Levy, JC, Morris, RJ, Hammersley, M & Turner, RC 1999, 'Discrimination, adjusted correlation, and equivalence of imprecise tests: application to glucose tolerance', *Am J Physiol,* vol. 276, no. 2 pt 1, pp. E365-75.

Liljenquist, JE, Horwitz, DL, Jennings, AS, Chiasson, JL, Keller, U & Rubenstein, AH 1978, 'Inhibition of insulin secretion by exogenous insulin in normal man as demonstrated by C-peptide assay', *Diabetes,* vol. 27, no. 5, pp. 563-70.

Lin, J, Razak, NN, Pretty, CG, Le Compte, A, Docherty, PD, Parente, JD, Shaw, GM, Hann, CE & Geoffrey Chase, J 2011, 'A physiological Intensive Control Insulin-Nutrition-Glucose (ICING) model validated in critically ill patients', *Comput Methods Programs Biomed,* vol. 102, no. 2, pp. 192-205.

Lorenzo, C, Wagenknecht, LE, D'Agostino, RB, Jr., Rewers, MJ, Karter, AJ & Haffner, SM 2010, 'Insulin resistance, beta-cell dysfunction, and conversion to type 2 diabetes in a multiethnic population: the Insulin Resistance Atherosclerosis Study.', *Diabetes Care,* vol. 33, no. 1, pp. 67-72.

Lotz, T 2007, *High resolution clinical model-based assessment of insulin sensitivity*, PhD Thesis Thesis, University of Canterbury, Christchurch, New Zealand. http://hdl.handle.net/10092/1571

Lotz, T, Chase, JG, McAuley, KA, Shaw, GM, Wong, J, Lin, J, Le Compte, AJ, Hann, CE & Mann, JI 2008, 'Monte Carlo analysis of a new model-based method for insulin sensitivity testing', *Comput Methods Programs Biomed,* vol. 89, pp. 215-25.

Lotz, TF, Chase, JG, McAuley, KA, Shaw, GM, Docherty, PD, Berkeley, JE, Williams, SM, Hann, CE & Mann, JI 2010, 'Design and clinical pilot testing of the model based Dynamic insulin sensitivity and secretion test (DISST)', *J Diabetes Sci Technol,* vol. 4, no. 6, pp. 1195-201.

Lotz, TF, Goltenbolt, U, Chase, JG, Docherty, PD & Hann, CE 2009, 'A minimal C-peptide sampling method to capture peak and total prehepatic insulin secretion in model-based experimental insulin sensitivity studies', *J Diabetes Sci Technol,* vol. 3, no. 4, pp. 875-86.

Lund, SS, Tarnow, L, Frandsen, M, Smidt, UM, Pedersen, O, Parving, H-H & Vaag, AA 2008, 'Impact of metformin versus the prandial insulin secretagogue, repaglinide, on fasting and postprandial glucose and lipid responses in non-obese patients with type 2 diabetes', *Eur J Endocrinol,* vol. 158, no. 1, pp. 35-46.

Malita, FM, Messier, V, Lavoie, JM, Bastard, JP, Rabasa-Lhoret, R & Karelis, AD 2010, 'Comparison between several insulin sensitivity indices and metabolic risk factors in overweight and obese postmenopausal women: A MONET study', *Nutrition, Metabolism and Cardiovascular Diseases,* vol. 20, no. 3, pp. 173-9.

Mari, A 1998, 'Assessment of insulin sensitivity and secretion with the labelled intravenous glucose tolerance test: improved modelling analysis', *Diabetologia,* vol. 41, no. 9, pp. 1029-39.

Mari, A, Pacini, G, Brazzale, AR & Ahren, B 2005, 'Comparative evaluation of simple insulin sensitivity methods based on the oral glucose tolerance test', *Diabetologia,* vol. 48, no. 4, pp. 748-51.

Mari, A, Pacini, G, Murphy, E, Ludvik, B & Nolan, JJ 2001, 'A model-based method for assessing insulin sensitivity from the oral glucose tolerance test', *Diabetes Care,* vol. 24, no. 3, pp. 539-48.

Mari, A, Tura, A, Gastaldelli, A & Ferrannini, E 2002, 'Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation', *Diabetes,* vol. 51 Suppl 1, pp. S221-6.

Mari, A & Valerio, A 1997, 'A Circulatory Model for the Estimation of Insulin Sensitivity', *Control Eng Practice,* vol. 5, no. 12, pp. 1747-52.

Marquardt, DW 1963, 'An Algorithm for Least-Squares Estimation of Nonlinear Parameters', *SIAM Journal on Applied Mathematics,* vol. 11, no. 2, pp. 431-41.

Martin, BC, Warram, JH, Krolewski, AS, Bergman, R, Soeldner, JS & Kahn, CR 1992, 'Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study', *Lancet,* vol. 340, no. 8825, pp. 925-9.

Mather, KJ, Hunt, AE, Steinberg, HO, Paradisi, G, Hook, G, Katz, A, Quon, MJ & Baron, AD 2001, 'Repeatability characteristics of simple indices of insulin resistance: implications for research applications', *J Clin Endocrinol Metab,* vol. 86, no. 11, pp. 5457-64.

Matsuda, M & DeFronzo, RA 1999, 'Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp', *Diabetes Care,* vol. 22, no. 9, pp. 1462-70.

Matthews, DR, Hosker, JP, Rudenski, AS, Naylor, BA, Treacher, DF & Turner, RC 1985, 'Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man', *Diabetologia,* vol. 28, no. 7, pp. 412-9.

McAuley, KA, Berkeley, JE, Docherty, PD, Lotz, TF, Te Morenga, LA, Shaw, GM, Williams, SM, Chase, JG & Mann, JI 2011, 'The dynamic insulin sensitivity and secretion test--a novel measure of insulin sensitivity', *Metabolism,* vol. In Press, Corrected Proof.

McAuley, KA, Mann, JI, Chase, JG, Lotz, TF & Shaw, GM 2007, 'Point: HOMA - Satisfactory for the Time Being: HOMA: the best bet for the simple determination of insulin sensitivity, until something better comes along', *Diabetes Care,* vol. 30, no. 9, pp. 2411-3.

McAuley, KA, Williams, SM, Mann, JI, Goulding, A, Chisholm, A, Wilson, N, Story, G, McLay, RT, Harper, MJ & Jones, IE 2002, 'Intensive lifestyle changes are necessary to improve insulin sensitivity: a randomized controlled trial', *Diabetes Care,* vol. 25, no. 3, pp. 445-52.

McAuley, KA, Williams, SM, Mann, JI, Walker, RJ, Lewis-Barned, NJ, Temple, LA & Duncan, AW 2001, 'Diagnosing insulin resistance in the general population', *Diabetes Care,* vol. 24, no. 3, pp. 460-4.

McDonald, C, Dunaif, A & Finegood, DT 2000, 'Minimal-model estimates of insulin sensitivity are insensitive to errors in glucose effectiveness', *J Clin Endocrinol Metab,* vol. 85, no. 7, pp. 2504-8.

McLaughlin, T, Abbasi, F, Lamendola, C & Reaven, G 2007, 'Heterogeneity in prevalence of risk factors for cardiovascular disease and type 2 diabetes in obese individuals: impact of differences in insulin sensitivity', *Archives Internationales De Physiologie De Biochimie Et De Biophysique,* vol. 167, pp. 642-8.

Meier, JJ, Holst, JJ, Schmidt, WE & Nauck, MA 2007, 'Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans', *Am J Physiol Endocrinol Metab,* vol. 293, pp. E849-56.

Meier, JJ, Veldhuis, JD & Butler, PC 2005, 'Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans', *Diabetes,* vol. 54, no. 6, pp. 1649-56.

Monzillo, LU & Hamdy, O 2003, 'Evaluation of insulin sensitivity in clinical practice and in research settings', *Nutr Rev,* vol. 61, no. 12, pp. 397-412.

Natali, A, Gastaldelli, A, Camastra, S, Sironi, AM, Toschi, E, Masoni, A, Ferrannini, E & Mari, A 2000, 'Dose-response characteristics of insulin action on glucose metabolism: a non-steady-state approach', *Am J Physiol Endocrinol Metab,* vol. 278, no. 5, pp. E794-801.

Nathan, DM, Kuenen, J, Borg, R, Zheng, H, Schoenfeld, D & Heine, RJ 2008, 'Translating the A1C Assay Into Estimated Average Glucose Values', *Diabetes Care,* vol. 31, no. 8, pp. 1473-8.

Nishida, Y, Tokuyama, K, Nagasaka, S, Higaki, Y, Shirai, Y, Kiyonaga, A, Shindo, M, Kusaka, I, Nakamura, T, Ishibashi, S & Tanaka, H 2004, 'Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartment-labeled minimal model', *Diabetes,* vol. 53, no. 2, pp. 315-20.

NZMoH 2007, *Diabetes Surveillance: Population-based estimates and projections for New Zealand, 2001-2011:* , Ministry of Health, Wellington.

O'Gorman, DJ, Karlsson, HKR, McQuiad, S, Yousif, O, Rahman, Y, Gasparro, D, Glund, S, Chibalin, AV, Zierath, JR & Nolan, JJ 2006, 'Exercise training increases insulin-simulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes.', *Diabetologia,* vol. 49, no. 12, pp. 2983-92.

Oterdoom, LH, de Vries, APJ, van Son, WJ, van der Heide, JJH, Ploeg, RJ, Gansevoort, RT, de Jong, PE, Gans, ROB & Bakker, SJL 2005, 'Validation of Insulin Resistance Indexes in a Stable Renal Transplant Population', *Diabetes Care,* vol. 28, no. 10, pp. 2424-9.

Pacini, G & Mari, A 2003, 'Methods for clinical assessment of insulin sensitivity and beta-cell function', *Best Pract Res Clin Endocrinol Metab,* vol. 17, no. 3, pp. 305-22.

Pentikäinen, P, Neuvonen, P & Penttilä, A 1979, 'Pharmacokinetics of metformin after intravenous and oral administration to man', *European Journal of Clinical Pharmacology,* vol. 16, no. 3, pp. 195-202.

Piché, M-È, Lemieux, S, Corneau, L, Nadeau, A, Bergeron, J & Weisnagel, SJ 2007, 'Measuring insulin sensitivity in postmenopausal women covering a range of glucose tolerance: comparison of indices derived from the oral glucose tolerance test with the euglycemic-hyperinsulinemic clamp', *Metabolism,* vol. 56, no. 9, pp. 1159-66.

Pigeon, E, Riou, ME, St-Onge, J, Couillard, E, Tremblay, A, Marette, A, Weisnagel, SJ & Joanisse, DR 2009, 'Validation of a simple index (SIis OGTT) of insulin sensitivity in a population of sedentary men', *Diabetes & Metabolism,* vol. 35, no. 5, pp. 398-403.

Pillonetto, G, Sparacino, G & Cobelli, C 2003, 'Numerical non-identifiability regions of the minimal model of glucose kinetics: superiority of Bayesian estimation', *Math Biosci,* vol. 184, no. 1, pp. 53-67.

Pillonetto, G, Sparacino, G, Magni, P, Bellazzi, R & Cobelli, C 2002, 'Minimal model S(I)=0 problem in NIDDM subjects: nonzero Bayesian estimates with credible confidence intervals', *Am J Physiol Endocrinol Metab,* vol. 282, no. 3, pp. E564-73.

Pohjanpalo, H 1978, 'System identifiability based on the power series expansion of the solution', *Math Biosci,* vol. 41, no. 1-2, pp. 21-33.

PriceWaterhouseCoopers 2007, *Type 2 Diabetes - Outcomes Model Update*, PricewaterhouseCoopers, Wellington.

Prigeon, RL, Roder, ME, Porte, D, Jr. & Kahn, SE 1996, 'The effect of insulin dose on the measurement of insulin sensitivity by the minimal model technique. Evidence for saturable insulin transport in humans', *J Clin Invest,* vol. 97, no. 2, pp. 501-7.

Quon, MJ, Cochran, C, Taylor, SI & Eastman, RC 1994a, 'Direct comparison of standard and insulin modified protocols for minimal model estimation of insulin sensitivity in normal subjects', *Diabetes Res,* vol. 25, no. 4, pp. 139-49.

Quon, MJ, Cochran, C, Taylor, SI & Eastman, RC 1994b, 'Non-insulin-mediated glucose disappearance in subjects with IDDM. Discordance between experimental results and minimal model analysis', *Diabetes,* vol. 43, no. 7, pp. 890-6.

Reynolds, LR, Konz, EC, Frederich, RC & Anderson, JW 2002, 'Rosiglitazone amplifies the benefits of lifestyle intervention measures in long-standing type 2 diabetes mellitus', *Diabetes, Obesity and Metabolism,* vol. 4, no. 4, pp. 270-5.

Ritt, JF 1950, *Differential Algebra*, Amer. Math. Soc., Providence, RI.

Rizza, RA, Mandarino, LJ & Gerich, JE 1981, 'Dose-response characteristics for effects of insulin on production and utilization of glucose in man', *Am J Physiol,* vol. 240, no. 6, pp. E630-9.

Roche 2004, *Data Sheet - Insulin Immunoassay, Elecsys 1010/2010/Modular Analytics E170*, Roche Diagnostics, Mannheim, Germany, 12017547 122.

Roche 2005, *Data Sheet - C-Peptide Immunoassay, Elecsys 1010/2010/Modular Analytics E170*, Roche Diagnostics, Mannheim, Germany, 03184897 190.

Rostami-Hodjegan, A, Peacey, SR, George, E, Heller, SR & Tucker, GT 1998, 'Population-based modeling to demonstrate extrapancreatic effects of tolbutamide', *Am J Physiol Endocrinol Metab,* vol. 274, no. 4pt1, pp. E758-71.

Ruige, JB, Mertens, IL, Bartholomeeusen, E, Dirinck, E, Ferrannini, E & Van Gaal, LF 2006, 'Fasting-based Estimates of Insulin Sensitivity in Overweight and Obesity: A Critical Appraisal[ast]', *Obesity,* vol. 14, no. 7, pp. 1250-6.

Saad, MF, Anderson, RL, Laws, A, Watanabe, RM, Kades, WW, Ida Chen, YD, Sands, RE, Pei, D, Savage, PJ & Bergman, RN 1994, 'A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance', *Diabetes,* vol. 43, pp. 1114-21.

Saad, MF, Steil, GM, Riad-Gabriel, M, Khan, A, Sharma, A, Boyadjian, R, Jinagouda, SD & Bergman, RN 1997, 'Method of insulin administration has no effect on insulin sensitivity estimates from the insulin-modified minimal model protocol.', *Diabetes,* vol. 46, no. 12, pp. 2044-8.

Santaguida, PL, Balion, C, Hunt, D, Morrison, K, Gerstein, HC, Raina, P, Booker, L & Yazdi, H 2005, 'Diagnosis, prognosis, and treatment of impared glucose tolerance and impared fasting glucose', *Evid Rep Technol Assess,* vol. 128, pp. 1-11.

Scheen, AJ, Paquot, N, Castillo, MJ & Lefebvre, PJ 1994, 'How to measure insulin action in vivo', *Diabetes Metab Rev,* vol. 10, no. 2, pp. 151-88.

Schwartz, B, Jacobs, DR, Moran, A, Steinberger, J, Hong, C-P & Sinaiko, AR 2008, 'Measurement of Insulin Sensitivity in Children', *Diabetes Care,* vol. 31, no. 4, pp. 783-8.

Sherwin, RS, Kramer, KJ, Tobin, JD, Insel, PA, Liljenquist, JE, Berman, M & Andres, R 1974, 'A model of the kinetics of insulin in man', *J Clin Invest,* vol. 53, no. 5, pp. 1481-92.

Sjostrand, M, Holmang, A & Lonnroth, P 1999, 'Measurement of interstitial insulin in human muscle', *Am J Physiol,* vol. 276, no. 1 Pt 1, pp. E151-4.

Soonthornpun, S, Setasuban, W, Thamprasit, A, Chayanunnukul, W, Rattarasarn, C & Geater, A 2003, 'Novel insulin sensitivity index derived from oral glucose tolerance test', *J Clin Endocrinol Metab,* vol. 88, no. 3, pp. 1019-23.

Steil, GM, Hwu, C, Janowski, R, Hariri, F, Jinagonda, S, Darwin, C, Tadros, S, Rebrin, K & Saad, MF 2004, 'Evaluation of Insulin Sensitivity and beta-cell Function Indexes Obtained from Minimal Model Analysis of a Meal Tolerance Test', *Diabetes,* vol. 53, no. 5, pp. 1201-7.

Stumvoll, M, Mitrakou, A, Pimenta, W, Jenssen, T, Yki-Jarvinen, H, Van Haeften, T, Renn, W & Gerich, J 2000, 'Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity', *Diabetes Care,* vol. 23, no. 3, pp. 295-301.

Swinnen, S, Holleman, F & DeVries, J 2008, 'The interpretation of glucose clamp studies of long-acting insulin analogues: from physiology to marketing and back', *Diabetologia,* vol. 51, no. 10, pp. 1790-5.

TeMorenga, L, Williams, SM, Brown, R & Mann, JI 2010, 'Effect of a relatively high protein, high fiber diet on body composition and metabolic risk factors in overweight women', *Eur J Clin Nutr,* vol. 64, no. 11, pp. 1323-31.

Thorsteinsson, B 1990, 'Kinetic models for insulin disappearance from plasma in man', *Dan Med Bull,* vol. 37, no. 2, pp. 143-53.

Thorsteinsson, B, Fugleberg, S & Binder, C 1986, 'Non-linearity of insulin kinetics', *Diabetologia,* vol. 29, no. 12, p. 898.

Thorsteinsson, B, Fugleberg, S, Feldt-Rasmussen, B & Binder, C 1985, 'Kinetic models for plasma disappearance of insulin in normal subjects', *Acta Pharmacol Toxicol (Copenh),* vol. 57, no. 5, pp. 309-16.

Toffolo, G, Campioni, M, Basu, R, Rizza, RA & Cobelli, C 2006, 'A minimal model of insulin secretion and kinetics to assess hepatic insulin extraction', *Am J Physiol Endocrinol Metab,* vol. 290, no. 1, pp. E169-E76.

Trout, KK, Homko, C & Tkacs, NC 2007, 'Methods of measuring insulin sensitivity', *Biological Research for Nursing,* vol. 8, no. 4, pp. 305-18.

Van Cauter, E, Mestrez, F, Sturis, J & Polonsky, KS 1992, 'Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance', *Diabetes,* vol. 41, no. 3, pp. 368-77.

Van Cauter, E, Polonsky, KS & Scheen, AJ 1997, 'Roles of circadian rhythmicity and sleep in human glucose regulation', *Endocr Rev,* vol. 18, no. 5, pp. 716-38.

Van den Berghe, G, Wilmer, A, Hermans, G, Meersseman, W, Wouters, PJ, Milants, I, Van Wijngaerden, E, Bobbaers, H & Bouillon, R 2006, 'Intensive Insulin Therapy in the Medical ICU', *N Engl J Med,* vol. 354, no. 5, pp. 449-61.

Vozarova, B, Weyer, C, Lindsay, RS, Pratley, RE, Bogardus, C & Tataranni, PA 2002, 'High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes', *Diabetes,* vol. 51, no. 2, pp. 455-61.

Wallace, TM, Levy, JC & Matthews, DR 2004, 'Use and abuse of HOMA modeling', *Diabetes Care,* vol. 27, no. 6, pp. 1487-95.

Ward, GM, Walters, JM, Barton, J, Alford, FP & Boston, RC 2001, 'Physiologic modeling of the intravenous glucose tolerance test in type 2 diabetes: a new approach to the insulin compartment', *Metabolism,* vol. 50, no. 5, pp. 512-9.

Widjaja, A, Morris, RJ, Levy, JC, Frayn, KN, Manley, SE & Turner, RC 1999, 'Within- and Between-Subject Variation in Commonly Measured Anthropometric and Biochemical Variables', *Clin Chem,* vol. 45, no. 4, pp. 561-6.

Youssef, IK & El-Arabawy, HA 2007, 'Picard iteration algorithm combined with Gauss-Seidel technique for initial value problems', *Appl. Math. Comp.,* vol. 190, no. 1, pp. 345-55.

Zethelius, B, Hales, CN, Lithell, HO & Berne, C 2004, 'Insulin resistance, impaired early insulin response, and insulin propeptides as predictors of the development of type 2 diabetes: a population-based, 7-year follow-up study in 70-year-old men.', *Diabetes Care,* vol. 27, no. 6, pp. 1433-8.

Zierler, K 1999, 'Whole body glucose metabolism', *Am J Physiol,* vol. 276, no. 3 Pt 1, pp. E409-26.

Zimmet, P, Boyko, EJ, Collier, GR & deCourten, M 1999, 'Etiology of the metabolic syndrome: Potential role of insulin resistance, leptin resistance, and other players', *Ann N Y Acad Sci,* vol. 892, pp. 25-44.