Analysis and Optimisation of Model-Based Insulin Sensitivity and Secretion Tests

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Nomenclature

Acronyms and abbreviation

Physiological conditions

T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
NGT	Normal Glucose Tolerance
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IR	Insulin Resistance

Insulin sensitivity tests

EIC	Euglycemic Hyperinsulinaemic Clamp
IVGTT	Intravenous Glucose Tolerance Test
IM-IVGT	Insulin Modified IVGTT
OGTT	Oral Glucose Tolerance Test
HOMA	Homeostasis Model Assessment
DISST	Dynamic Insulin Sensitivity and Secretion Test
DISTq	Quick Dynamic Insulin Sensitivity test

Institutions

ADA	American Diabetes Association
IDF	International Diabetes Federation
WHO	World Health Organization

Model parameters

C	Plasma C-peptide concentration
Y	Interstitial C-peptide concentration
I	Plasma insulin concentration
Q	Interstitial insulin concentration

G	Basal plasma glucose concentration
G_0	Fasting plasma glucose concentration
G_B	Basal plasma glucose concentration
V_Q	Interstitial insulin distribution volume
n_I	Plasma-interstitial diffusion rate
n_C	Interstitial insulin degradation rate
P_t	Exogenous glucose input rate
p_{gu}	Non-insulin mediated glucose disposal rate
V_G	Glucose distribution volume
SI	Insulin sensitivity
U_N	Endogenous insulin secretion
U_B	Basal insulin secretion
U_1	First phase secretion
U_2	Second phase secretion
ϕ_D	Derivative control gain
ϕ_P	Proportional control gain

Abstract

Type 2 diabetes (T2D) is a chronic disease characterised by a range of dysfunctions in glycaemic regulation. These dysfunctions are known to include insulin resistance (*IR*), hyper-insulin secretion, hypo-insulin secretion and altered hepatic glucose balance, all in the course of developing the disease. *IR*, in particular, is a condition in which the circulating insulin is less effective in lowering the glucose levels in blood. Insulin hypersecretion is most associated with pre-diabetes, but can sometimes occur in early diabetes in concert with sufficient *IR*. Insulin hyposecretion, on the other hand, is most often present in longer-term diabetes and is a result of reduction in β-cell mass. Hence, the ability to accurately monitor and diagnose these stages of progression would offer unique insight and clinical opportunity.

As an individual progresses towards T2D, the amount of insulin required to deal with the glucose loads increases. This outcome is ultimately driven by low insulin sensitivity ($SI = IR^{-1}$). Additionally, T2D is said to have a lower insulin secretion capability, and thus, resulted in consistently increase glucose levels in the blood. Thus, more specifically, precisely observing and understanding the metabolic disorder as changes in both *SI* and endogenous insulin secretion (U_N) may provide further insight into the heterogeneous etiology of type 2 diabetes, and clinical intervention opportunities.

Although, several test protocols and mathematical modelling strategies have been developed to quantify these key aspects of T2D, particularly in *SI* and U_N, the goal of this

thesis is to find how to effectively improve the precision and clinical utility of these model-based assessments when assessing the *SI* and U_N. This thesis focuses on minimising the identification error or accurately identify the *SI* value particularly for individual with established T2D. In addition, this thesis also develops and analyses a proportional-derivative (PD) control model that may potentially be able to replace the conventional and accepted methods for estimating the participant-specific U_N profile, which are not precise and thus introduce error.

In particular, many modelling strategies use fasting glucose (G_0) as basal glucose concentration (G_B) when assessing the insulin sensitivity. With the assumption of $G_B = G_0$, most of the model-based *SI* assessment able to produce a highly correlated of an *SI* value to gold standard euglycaemic hyperinsulinaemic clamp (EIC). However, some of the model-based like dynamic insulin sensitivity and secretion test (DISST), was developed in a relatively healthy, normoglycaemic cohort. Thus, the assumption of $G_B = G_0$ might be untrue as prior studies have suggested that G_B and G_0 should be treated differently particularly for T2D individuals. Hence, the outcomes of identifying G_B potentially provide accurate assessment of *SI* value, in particular, for pre-diabetes individual, are investigated and quantified for the first time.

It is understandable that U_N plays a leading role in glucose homeostasis. Pathological changes in U_N can enable early diagnosis of metabolic dysfunction before the emergence of type 2 diabetes. A PD control model that defines U_N as a function of glucose concentration is proposed and analysed to provide further insight and modelling capability for this

prediabetic state. In addition, it offers the ability to add precision to estimating *SI* and additional diagnostics around U_N.

Thus, finally, the proposed PD U_N model is further analysed to provide more information in determining each participant's glycemic condition. The characterised gains of derivative control, ϕ_D and proportional control, ϕ_P are used in identifying and discriminating the U_N profile for each metabolic state. Hence, the outcome will potentially improve the overall identification of U_N profile.

Chapter 1. Introduction

Diabetes has gone from rarity to global epidemic. Global estimations of diabetes prevalence by King *et. al.* estimate that there will be a total of 300 million adults with diabetes in 2025 (King *et al*. 1998). The World Health Organization (WHO) estimates an increase of more than 110%, from 171 million in 2000 to 366 million in 2030, of diabetes case worldwide (Wild *et al*. 2004). The International Diabetes Federation (IDF) estimates that the total number of people living with diabetes will rise about 55% from 382 million in 2013 to 592 million in 2035 (IDF 2013). While these estimates vary wildly, they share common themes of exponential growth and large number of people with diabetes.

Diabetes continues to increase in prevalence and significance due to developments in economics and urbanization that lead to sedentary lifestyles changes leading to reduced physical activity and increasing obesity (Whiting *et al*. 2011). Diabetes is characterised by high levels of insulin resistance and insulin secretory dysfunction that affect glucose uptake and utilization by most cells of the human body. Thus, a combination of resistance to insulin action and an increasingly inadequate compensatory insulin secretory response results in changing the metabolic state of an individual a healthy state, through pre-diabetes and early onset type 2 diabetes to manifest diabetes (Ferrannini 1997; WHO 2006). In particular, an absolute deficiency of insulin secretion occurs in manifest diabetes (ADA 2014). Hence, understanding the underlying metabolic disorder in the pathogenesis of diabetes could provide valuable information to instigate therapies to mitigate or delay the onset of the disease.

This chapter reviews and discusses the physiological characteristics of glucose and insulin that are associated with insulin resistance and the pathogenesis of type 2 diabetes.

1.1 Glucose

Glucose is a simple sugar or monosaccharide that consists of three elements: carbon, hydrogen and oxygen. Glucose is the most important carbohydrate and is used by the body as the main source of energy. During the conversion of glucose to pyruvate via glycolisis, energy is released for use in the cells (Guyton & Hall 2006). Hence, adequate glucose is essential in providing energy to maintain cellular function and thus, the body.

Figure 1.1 illustrates the level of glucose in the blood stream in a simulated healthy individual. Immediately after a meal, the digestive system breaks down the carbohydrate in food into glucose. The glucose produced is absorbed into the blood stream and results in sharp increase in blood glucose level. A healthy individual has a blood glucose level of 72 - 90 $mg \cdot dL^{-1}$ (4 - 5 $mmol \cdot L^{-1}$) before a meal. However, the blood glucose level of most healthy individuals will increase above 140 $mg \cdot dL^{-1}$ (7.78 $mmol \cdot L^{-1}$) after a meal containing large amounts of carbohydrates (Guyton & Hall 2006). In response to the increased blood glucose level, pancreatic β-cells secrete a significant amount of insulin to lower the blood glucose level back to a safe level.

Glucose is transported around the body passively via the blood stream. Insulin is required to mediate glucose uptake into cells, thus removing it from the blood, particularly in the liver,

muscles and adipose tissue. Glucose can also be directly taken up by cells itself in the brain and central nervous system without the need of insulin.

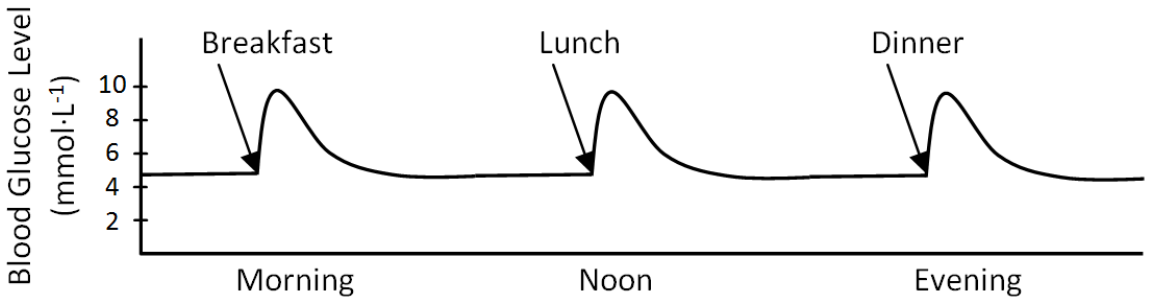

Figure 1.1: Schematic illustration of the effect of blood glucose level after meal.

Although, glucose is used by the cells to produce energy, excess glucose can be stored in the liver and muscles, primarily as glycogen, for future use. However, the body's ability to store glycogen is limited. Hence, further excess glucose is then stored in adipose tissue as fat.

Naturally, the body constantly regulates the blood glucose level as a part of metabolic homeostasis. A healthy fasting blood glucose level is in the range of 4 – 5 $mmol \cdot L^{-1}$. However, if this glucose balancing system is disrupted, it can lead to hyperglycemia, where the blood glucose level is elevated above safe levels (≥ 200 $mg \cdot dL^{-1}$, 11.1 $mmol \cdot L^{-1}$) (ADA 2014; WHO 2006). If left untreated, hyperglycemia can lead to the emergence of T2D and significant complications (Duckworth 2001; Laakso 1999a,b). On the other hand, if blood glucose levels fall too low, into the range of 20 – 50 $mg \cdot dL^{-1}$ (1.11 – 2.78 $mmol \cdot L^{-1}$), hypoglycaemia occurs (Guyton & Hall 2006), which carries its own significant risk (Cryer 1997; Cryer & Polonsky 2008; McCrimmon & Frier 1994; Wilson 1983) and thus adds risk to treating hyperglycemia with insulin.

An individual is defined as having impaired fasting glucose (IFG) when the value of fasting plasma glucose is in the range of 100 $mg \cdot dL^{-1}$ (5.6 $mmol \cdot L^{-1}$) to 125 $mg \cdot dL^{-1}$ (6.9 $mmol \cdot L^{-1}$), or impaired glucose tolerance (IGT) when the plasma glucose value in the range of 140 $mg \cdot dL^{-1}$ (7.8 $mmol \cdot L^{-1}$) to 199 $mg \cdot dL^{-1}$ (11 $mmol \cdot L^{-1}$) at the 2-hour mark of an oral glucose tolerance test (OGTT) (ADA 2014). These IFG and IGT individuals have been referred to as having a pre-diabetes state, indicating a high risk of further development of diabetes (ADA 2014; Shaw *et al*. 1999; Tuomilehto et al. 2001; Vendrame & Gottlieb 2004). Maintaining a balanced glucose level in the blood is crucial for health. Failing to continually control blood glucose levels leads to the development of metabolic disorders, particularly diabetes, all of which have significant complications that can reduce quality and length of life.

1.2 Insulin

One of the primary hormones produced by pancreatic β-cells of the islets of Langerhans is insulin (Guyton & Hall 2006). The insulin hormone is composed of two polypeptide chains, A (21 amino acids) and B (30 amino acids) chains. These two amino acids are connected by disulfide bonds (Chevenne *et al*. 1999).

Insulin plays the primary significant role in maintaining glucose homeostasis. The β-cells, representing 60% of all the cells of the islets of Langerhans, secrete insulin in response to elevated glucose levels in the blood stream. These insulin secretions enable glucose to be absorbed by muscle and adipose tissue cells, regulate storage and release of glucose in the liver, and promote fat synthesis and storage (Guyton & Hall 2006; Jefferson *et al*. 2001). Hence, it dominates the glucose removal portion of human metabolic regulation.

Figure 1.2 illustrates the important role played by insulin in glucose uptake. Once released into the blood stream, insulin is then distributed to interstitial fluid. There, it binds to its receptor of muscle and adipose tissue cell outer membranes. This action, in turn, activates an intercellular reaction that include translocation of glucose transporter proteins from intracellular vesicles to the plasma membrane (Holman & Kasuga 1997; Myers & White 1996), which then results in influx of glucose (Jefferson *et al*. 2001).

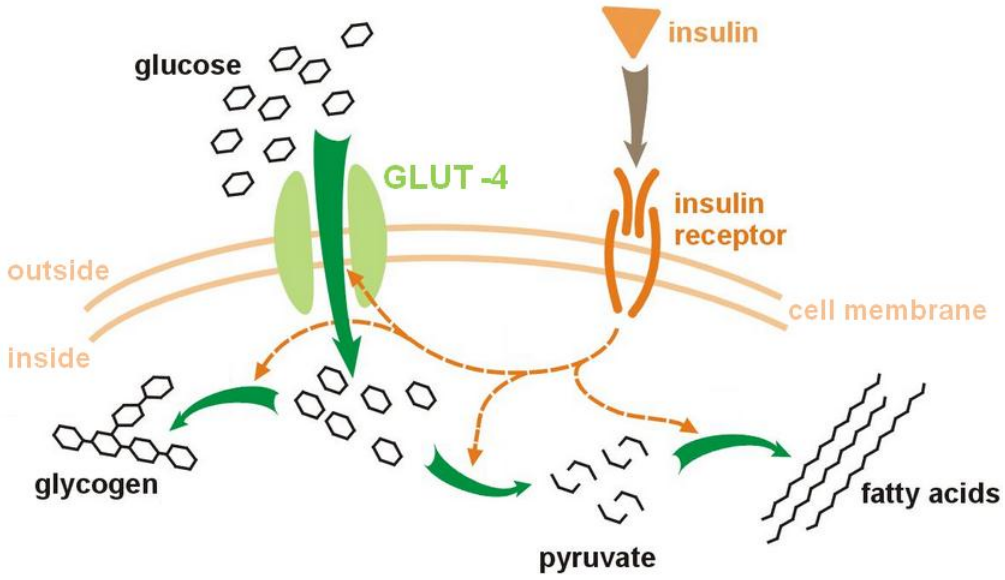

Figure 1.2: Effect of insulin on glucose uptake and metabolism. Adapted from http://biology-pictures.blogspot.co.nz/.

Once, glucose enters the cell, it undergoes energy-releasing glycolysis process that. In the case of energy abundance, insulin also plays a critical role in storing the excess energy mainly in the liver and muscles by converting the glucose into glycogen through glycogenesis. Moreover, it also promotes the synthesis of fatty acids through lipogenesis, where the energy is stored in adipose tissue as fats (Guyton & Hall 2006).

Initially, the β-cells secrete insulin into the portal vein and it travels through the liver to the heart before entering the blood circulation. From 60 – 80% of endogenous insulin secretion is extracted during this first pass extraction by the liver after being released (Cobelli & Pacini 1988; Ferrannini & Cobelli 1987; Meier *et al*. 2005; Toffolo *et al*. 2006). Later, insulin is also cleared by the kidneys, as well as through cellular degradation after mediating glucose uptake (Guyton & Hall 2006; Jefferson *et al*. 2001). Hence, there are multiple clearance paths.

In general, the secretion of insulin by the pancreatic β-cells is bi-phasic in healthy individuals (Guyton & Hall 2006; Jefferson *et al*. 2001). The strong spike of first phase secretion is released from stored and pre-produced insulin immediately after a sudden increase in glucose level within a short period of time. Later, a prolonged second phase secretion is more slowly and gradually released to eventually bring glucose levels back to normal. Importantly, the healthy pancreas secretes the right amount of insulin in response to the appearance of glucose in the blood stream. Failure to produce enough insulin to clear the excess glucose leads to elevated glucose levels in the blood and ultimately to prolonged hyperglycemia and T2D.

1.3 Pathogenesis of Type 2 diabetes mellitus (T2DM)

Diabetes mellitus is a chronic disease characterized by prolonged or uninterrupted elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both (ADA 2014). Naturally, glucose homeostasis is achieved when the level of glucose is consistently controlled to a normal basal level of approximately 4 – 5 $mmol \cdot L^{-1}$.

Regulation is accomplished via glucose uptake by the cells via insulin mediated uptake. However, as the body's ability to regulate glucose levels in blood deteriorates, the glucose stays in the blood stream until it is cleared, more slowly, through renal clearance of the kidneys (Arleth *et al*. 2000). This abnormal condition occurs in established diabetes and results in prolonged elevated blood glucose concentrations.

There are two major types of diabetes, Type 1 and Type 2 (Guyton & Hall 2006). Figure 1.3 illustrates the etiopathogenesis of these two types. In general, Type 1 diabetes (T1D) results from the body's failure or inability to produce insulin. The etiology of T1D is characterised by the destruction of the pancreatic β-cells caused by an auto-immune disorder or as a result of the action of genetic markers (ADA 2014; Bluestone *et al*. 2010). Individuals with T1D need to have exogenous insulin to maintain a safe level of glucose in the blood stream.

T2D results from the body's inability to produce enough insulin. Prior studies and definitions have characterized T2D by fasting hyperglycemia and an excessive rise in the plasma glucose concentration above baseline following glucose or meal ingestion (DeFronzo *et al*. 1983) resulting from impaired insulin utilization (insulin resistance) coupled with the body's inability to compensate with insulin production (insulin deficiency). Hence, there is a fundamental difference in the two forms of diabetes.

Theoretically, it can be said that type 2 diabetes is related to the development of *IR* and impaired β-cells. Over time, T2D is developed due to consistent and excessive insulin resistance leading to increase requirements for insulin production that, if not halted, eventually leads to loss of β-cell function and diminished secretion. Thus, loss of β-cells

eventually results in a total loss of insulin secretion. Hence, the glucose level in the blood increases, increasingly without restraint.

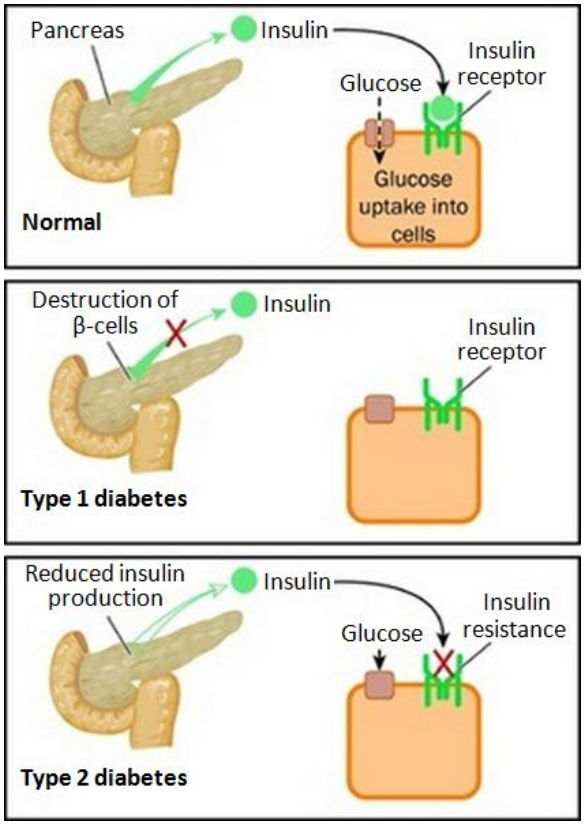

Figure 1.3: Illustration of the pathophysiological differences between normal, type 1 and type 2 diabetes. Adapted from endocrineweb.com/endocrinology/overview-pancreas.

As a result, it is generally agreed that insulin resistance is the predominant driver of the pathogenesis of T2D (Docherty 2011). A study has reported that up to 10 years ahead of a formal diagnosis of type 2 diabetes, those who developed the disease had 60% higher mean *IR* than those that did not (Martin *et al*. 1992). It is also found that amongst obese individuals, *IR* is the strongest predictor of subsequent type 2 diabetes and cardiovascular disease risk (McLaughlin *et al*. 2007). Figure 1.5 shows a clear relationship between insulin production, insulin sensitivity and glucose concentration in blood stream. The nature of insulin production increases and subsequently reaches a maximised plateau before

declining, as illustrated in Figure 1.4. This behaviour is known as Starlings curve of the pancreas (Clark *et al*. 2001; Gastaldelli *et al*. 2004). A similar curve was also presented in Ferrannini *et. al.* (Ferrannini 1997).

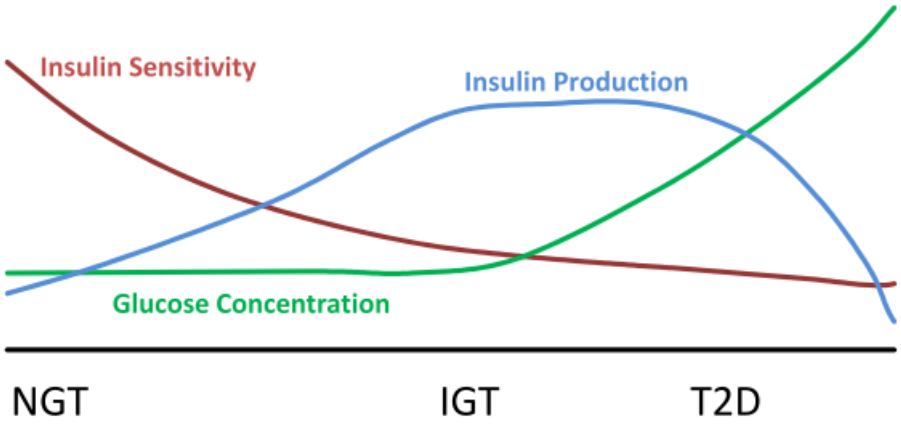

Figure 1.4: A generalisation of the inter-relationships between insulin production, insulin sensitivity and glucose concentration during the pathogenesis of type 2 diabetes (T2D). Note that NGT and IGT are normal glucose tolerance and impaired glucose tolerance, respectively. Adapted from (Docherty 2011).

As an individual moves from normal glucose tolerance (NGT) to IGT, the glucose concentration often remains the same. However, the *SI* value decreases as *IR* increases, as defined. This decrease results in higher demands on insulin production to stabilize blood glucose levels. As a result, this physiological change often goes unnoticed for a long time. Thus, the disease can be well established long before diagnosis when insulin production becomes significantly impaired and blood glucose levels rise. Hence, early identification of insulin resistance or sensitivity, as well as insulin production, could potentially ameliorate the worst long term symptoms of the disease if appropriate intervention is taken.

1.4 Preface

The main objective of this thesis is to better understand the identification of *SI* and β-cell function through the secretion of endogenous insulin. This thesis focuses on two parts: 1) accurately assessing *SI* value particularly for individuals with established T2D from the perspective of model-based *SI* assessment; and 2) identifying a smoother physiologically U_N profile based on a development of PD control model. A brief overview of the thesis includes:

Chapter 1 discusses the physiological characteristics of glucose and insulin that are associate with *IR* and the pathogenesis of T2D.

Chapter 2 reviews current and established model-based *SI* assessments.

Chapter 3 introduces a 3-parameter modelling approach for *SI* assessment. The approach is compared to a previous 2-parameter identification for individuals with established T2D.

Chapter 4 validates the importance of introducing G_B as a variable in a 3-parameter identification approach using DISST model.

Chapter 5 underlines the impact of identifying G_B as a variable towards assessing the *SI* value.

Chapter 6 explores a different modelling approach of identifying the U_N profile through the development of PD U_N model as opposed to the typical approach of deconvolution of C-peptide measurements.

Chapter 7 presents a PD model that defines U_N as a function of changes in glucose excursion.

Chapter 8 reflects how the information gathered from PD U_N model can be used in discriminating the condition state of an individual.

Chapter 9 summarises and concludes the outcomes of this thesis.

Chapter 10 defines possible future work for this research.

Chapter 2. Review of model-based insulin sensitivity (*SI*) tests

This thesis revolves around model-based assessment of insulin sensitivity and insulin secretion in the clinical evaluation of the etiology and diagnosis of diabetes. This chapter provides an overview of current model-based methods for assessing insulin sensitivity (*SI*), which is, as noted, an important metabolic marker of risk for type 2 diabetes.

2.1 Introduction

T2D is a metabolic disease that affects the body's ability to regulate blood glucose concentrations (DeFronzo & Ferrannini 1991; Ferrannini 1997; Martin *et al*. 1992). Studies show that T2D is characterized by fasting and postprandial hyperglycaemia (DeFronzo *et al*. 1983; Firth *et al*. 1986; Kirkman *et al*. 2006; Rizza 2010) and causes complications comorbidities with significant personal, social and economic cost (Bonow & Gheorghiade 2004; Gakidou *et al*. 2011; King 1999; Lam & LeRoith 2012; Santaguida *et al*. 2005). Although, this hyperglycaemia is attributed to a combination of impaired insulin utilization (insulin resistance) and a limited ability to compensate with insulin production (net insulin deficiency), many investigations found that *SI* ($SI = IR^{-1}$) is a key causative and diagnostic factor in T2D (DeFronzo & Ferrannini 1991; Ferrannini 1997; Harris *et al*. 2003; Martin *et al*. 1992) and is also associated as a major risk factor for cardiovascular disease (Hanley *et al*. 2005; McLaughlin *et al*. 2007; Santaguida *et al*. 2005; Zimmet *et al*. 1999). Thus, a practical

test that able to accurately identify *SI* would be clinically advantageous as a diagnostic and to better understand the physiological changes in T2D.

Many already developed tests measure *SI* as the efficiency in the use of insulin to reduce blood glucose (Ferrannini & Mari 1998; Pacini & Mari 2003). Each test employs a different clinical method, and thus results in a different level of accuracy or resolution. Hence, the researcher or clinician has to choose the best test in terms of intensity, cost, accuracy and physiological relevance. Therefore, a high resolution, simple, repeatable clinical measure of insulin sensitivity would have clinical and research benefits for diagnosis, research, and evaluating the impact of interventions (ADA 1998).

The aim of this chapter is to provide an overview on the most frequently used insulin sensitivity tests and, in particular, model-based *SI* assessments. However, the vast majority of these tests and models have been extensively reviewed and discusses by Lotz (Lotz 2007), Docherty (Docherty 2011) and Jamaludin (Jamaludin 2013). Hence, the focus here, in this chapter, is to give a summary of these tests in terms of diagnostic accuracy, as well as clinical burden and intensity of assessing the insulin sensitivity and secretion.

2.2 Overview of insulin sensitivity test

2.2.1 Euglycaemic hyperinsulinaemic clamp (EIC)

The EIC is regarded as the gold-standard for investigating and quantifying insulin resistance or insulin sensitivity (Ferrannini & Mari 1998; Pacini & Mari 2003). It measures the amount of glucose necessary to compensate for a hyper-physiologically increased insulin level. First

introduced in 1979 (Defronzo *et al*. 1979), this test infuses insulin at a constant rate and glucose at a variable rate to "clamp" the plasma glucose concentration at a normal fasting concentration, typically around 4.6 – 5.0 mmol·L^{-1} (McAuley *et al*. 2001).

The EIC defines an insulin sensitivity index (*ISI*) as the mean glucose infusion rate is divided by the mean insulin concentration. Both variables are measured at a steady state level at the end of the test. It has good repeatability (Defronzo *et al*. 1979; Mari *et al*. 2001; Monzillo & Hamdy 2003). However, it is very difficult to perform, time consuming, and increasingly avoided even by clinical researchers (Ferrannini & Mari 1998; Radziuk 2000). In addition, the test needs a total of 180 to 300 minutes to complete, with consistent attendance by highly trained medical personnel, making it too intensive for subjects and clinicians.

Although the EIC test is the gold standard reference for insulin sensitivity value, it does not provide an evaluation of a participant's insulin production at the same time. A second EIC test using a sustained hyper-physiological glucose concentration, instead of hyper-insulinaemia, is performed to estimate the insulin production. Thus, insulin production and sensitivity cannot be identified concurrently with the EIC test, and neither is accurately estimated at the physiological levels or actions.

2.2.2 Intravenous glucose tolerance test (IVGTT)

The IVGTT is the most well-used and common dynamic insulin sensitivity test protocol. This test protocol assesses glucose tolerance through an intravenous injection of glucose. A

series of blood samples are taken, and 20 or more samples are then assayed for glucose and insulin concentration, and, sometimes, connecting peptide (C-peptide) concentration, as well. Since the *SI* value cannot be identified directly from the data, a non-linear least-square parameter identification approach, typically using the minimal-model (MM) of glucose-insulin dynamics is used to analyse the data (Bergman *et al*. 1985; Bergman *et al*. 1979b; Bergman *et al*. 1981; Boston *et al*. 2003; Caumo *et al*. 1999).

The minimal model identifies two metabolic parameters, insulin sensitivity (S_I^{MM}) and glucose effectiveness (S_G^{MM}). S_I^{MM} defines the sensitivity of insulin to mediate glucose uptake and inhibit liver glucose production (Bergman 1989; Bergman *et al*. 1985). S_G^{MM}, on the other hand, quantifies the ability of glucose to decay from plasma at constant basal insulin (Ader *et al*. 1985; Best *et al*. 1996). In addition, the minimal model has been used with other models to identify metrics of first and second phase insulin secretion (Toffolo *et al*. 1980).

The insulin sensitivity metrics from the IVGTT have been well-validated against the EIC (Beard *et al*. 1986; Bergman *et al*. 1987; Saad *et al*. 1994). However, some studies have shown significant difference to the EIC with R = 0.44 – 0.85 (Donner *et al*. 1985; Foley *et al*. 1985; Galvin *et al*. 1992). Furthermore, the IVGTT is also known to produce ambiguous insulin sensitivity values and erratic correlations with the EIC (Bonora *et al*. 1989; Ferrannini & Mari 1998). Although, the IVGTT and minimal model are less clinically intense than the EIC, it remains a research-only application as the test is still impractical for use in wider clinical setting due to its length, intensity and complexity of parameter identification approach.

2.2.3 Oral glucose tolerance test (OGTT)

The OGTT is a simple test that involves oral consumption of a drink containing a pre-defined glucose content. A standard dose of glucose, often 75g glucose in 250 mL of flavoured water, is ingested by mouth and blood glucose levels are checked one and/ or two hours later. The OGTT is used to diagnose patients of pre-diabetes, type 1 or type 2 diabetes (ADA 2014). The two most common protocols are the 75g 2-hour trial and the 50g 1-hour trial.

The OGTT by itself, does not calculate insulin sensitivity directly and insulin production characteristics are not frequently measured or estimated. Its simplicity and accurate portrayal of the ability to dispose of glucose have made it the most common clinical diagnostic of diabetes (ADA 2014). In particular, the OGTT only measures the body's ability to remove a glucose load throughout the 1 or 2-hour trial, but provides no measure of secretion. Instead, it yields the net combination of *SI* and secretion seen as glucose removal.

A series of mathematical equations have been suggested to be applied, together with the OGTT, to assess the insulin sensitivity (Cederholm & Wibell 1990; Gutt *et al*. 2000; Matsuda & DeFronzo 1999; Piche *et al*. 2007; Stumvoll *et al*. 2000). The OGTT is rarely used to assess *SI* in clinical investigation. It is instead very well used as an accepted method for the diagnosis of diabetes (ADA 2014; WHO 2006).

2.2.4 Homeostasis model assessment (HOMA)

HOMA identifies insulin sensitivity and an index of β-cell function based on measured plasma glucose and insulin in a fasting state using a very simple physiological glucose-insulin model (Levy *et al*. 1998; Matthews *et al*. 1985; Turner *et al*. 1979). HOMA has been validated against the EIC. As this method solely depends on the precision of the fasting glucose and insulin measurements, a small error may affect the overall calculation of both indices of *SI* and β-cell function (Pacini & Mari 2003). Hence, the correlation between the EIC and HOMA varies 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; Piche *et al*. 2007), with most having on the poor side.

2.2.5 Dynamic insulin sensitivity and secretion test (DISST)

The DISST was designed to obviate the limitations of other tests. It provides quantitative measures of insulin sensitivity and insulin secretion. It is a dynamic test with a physiological model-based assessment, similar to the insulin modified IVGTT (IM-IVGTT). The DISST was developed to be an accurate IVGTT alternative that enables a shorter test duration, more physiological dosing, less frequent sampling, and higher robustness, all with a lower total cost of clinical testing (Lotz 2007; Lotz *et al*. 2010; Lotz *et al*. 2008).

The DISST uses a low dose of intravenous glucose bolus of 5 – 20 g and is followed by a low dose of intravenous insulin bolus of 0.5 – 2 U. Blood samples are measured and assayed for glucose, insulin and C-peptide concentration. The DISST identifies the participant-specific *SI*

and also U_N through a developed pharmaco-kinetic and pharmaco-dynamic model that represent the pathways and interactions between insulin, C-peptide and glucose in the human body.

The DISST produces a highly correlated metric of insulin sensitivity to the EIC test through *in-silico* study (R = 0.99) (Lotz *et al*. 2008). *In-vivo* studies also yielded a high correlation (R = 0.82) (McAuley *et al*. 2011). In addition, the DISST was able to identify participant-specific U_N profiles through deconvolution of measured C-peptide. As C-peptide is equimolarly secreted together with insulin from pancreatic β-cells (Rubenstein *et al*. 1969), the deconvolution method is considered to be effective in indentifying U_N profiles (Van Cauter *et al*. 1992). Thus, the DISST is a unique test that can quantify both insulin sensitivity and secretion at the same time with low cost but high robustness.

The DISST was further developed by Docherty (Docherty 2011), who eliminated the need for insulin and C-peptide assays via an iterative parameter identification method. The quick dynamic sensitivity test (DISTq) reduces the clinical intensity and assay cost of the DISST and correlated well to the fully sampled DISST in an *in-silico* Monte-Carlo analysis with R=0.89 (Docherty *et al*. 2009). With the low cost and high accuracy of the DISTq, it would be able to screen more patients for type 2 diabetes risk (Docherty *et al*. 2011b).

2.3 Summary

Figure 2.1 shows the relationship between accuracy and intensity of different *SI* tests. The EIC is regarded as the gold standard in assessing *SI*. However, the EIC test cannot

concurrently identify both insulin production and insulin sensitivity, both of which are required for accurate diagnosis and understanding of the etiology of diabetes. A second clamp protocol is needed to assess U_N. Hence, it is only limited to research setting, as it is too complex, intense and costly for wider clinical application.

The IVGTT with a minimal model analysis is suggested as a valid alternative to the EIC and is widely used by researchers. However, the IVGTT test has not achieved wide acceptance in a clinical application given that they are also very complex and time consuming. Additionally, relying on the minimal model in analysing and assessing *SI*, often leads to issues, particularly in over-parameterisation that causes the inability to distinguish between insulin and non-insulin mediated glucose disposal (Docherty *et al*. 2011a; McDonald *et al*. 2000; Pillonetto *et al*. 2002). Hence, the test is known to produce inconsistent *SI* values and unpredictable correlation to the EIC particularly for individual with higher insulin resistance (Docherty *et al*. 2011a; Pillonetto *et al*. 2003; Quon *et al*. 1994).

The OGTT and HOMA are cheaper and simpler compared to the IVGTT and EIC (Figure 2.1). A mathematical model is needed to analyse data obtained from the OGTT to identify an *SI* value. However, the OGTT is not often used in applications that require accurate assessment of *SI*. However, the OGTT is widely accepted and used as a method to diagnose type 2 diabetes.

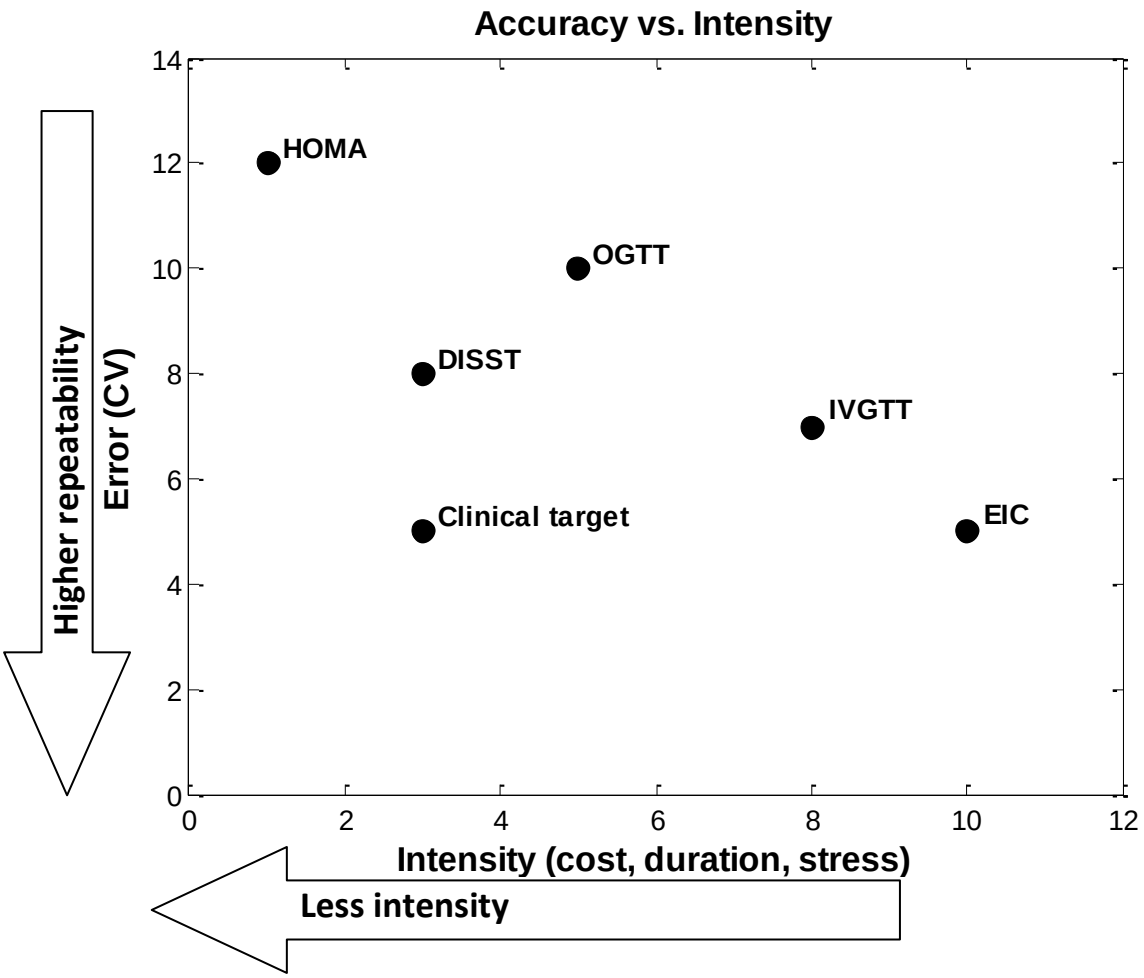

Figure 2.1: Comparison of different *SI* tests based on the test's intensity and accuracy in terms of repeatability (CV in %). The clinical target area is added for a better clinical test. Adapted from (Jamaludin 2013; Lotz 2007).

HOMA, on the other hand, identifies *SI* values based on fasting glucose and insulin values. However, with only one sample required of both glucose and insulin concentration, it does not fully represent the insulin-glucose dynamics. Thus, HOMA is used only in research that requires a simple, *SI* surrogate.

The DISST is a dynamic model-based test, similar to the IM-IVGTT. The DISST was developed to capture high resolution estimates of *SI* and also the U_N profile. With high correlation to the gold standard EIC with R = 0.82 in *in-vivo*, the DISST insulin sensitivity is a more representative measure than other insulin sensitivity metrics due to its physiological model

of pharmaco-kinetic and pharmaco-dynamic of glucose, insulin and C-peptide measurements. It also delivers a well-accepted U_N secretion profile that others do not.

Although the DISST seems to be the best protocol for assessing *SI* and U_N based on higher repeatability and lower intensity (Figure 2.1), there remains a scope better at diagnosis by improving the DISST model. The DISST, like any other model-based *SI* assessment, uses the assumption that basal glucose level is equal to the fasting glucose level when assessing insulin sensitivity. However, this assumption is untested and may be untrue for individuals with established T2D. Originally, the DISST identifies *SI* together with a glucose distribution volume (V_G) in a 2 parameter approach employing the iterative integral method (IIM). However, if basal glucose is considered as a variable, a 3 parameter approach is needed, where basal glucose is identified together with the *SI* and V_G value. Hence, *SI* can be potentially assessed with better accuracy in this case, particularly for T2D individuals.

In addition, the DISST identifies the participant-specific U_N profile based on deconvolution of C-peptide measurement. Although it remains to be the best method in identifying endogenous insulin, due to the fact that C-peptide and insulin are co-secreted from β-cells, these C-peptide measurements are relatively sparse. Hence, while diagnostically effective, there is room for improvement and to reduce sampling and thus cost, which is the focus of this research.

Chapter 3. 3-parameter identification of model-based insulin sensitivity assessment

This chapter discusses an adaptation to enable identification of 3 parameters, comprising G_B, *SI* and V_G, when assessing *SI* value for individuals particularly with established T2D.

3.1 Introduction

The pathogenesis of T2D is well known, and is characterized by *IR* and β-cell dysfunction (Kahn 2003). β-cell dysfunction is associated with reduced and inconsistent endogenous insulin production, while *IR* as a condition refers to a state in which the body becomes less effective or unable to use secreted endogenous insulin for lowering blood glucose. An individual can have *IR* with, or without, β-cell dysfunction and reduced insulin secretion. These conditions contribute to major metabolic disorders, such as glucose intolerance or hyperglycemia, and, if left untreated, ultimately lead to the emergence of T2D.

In particular, *IR* is a strong early predictor of subsequent T2D, up to 10 years in advance (DeFronzo & Ferrannini 1991; Ferrannini 1997; Harris *et al*. 2003; Martin *et al*. 1992). It is also associated with increased cardiovascular disease risk (Hanley *et al*. 2005; McLaughlin *et al*. 2007; Zimmet *et al*. 1999) as part of an overall syndrome of conditions that can emerge. Early identification of *IR* would thus benefit diagnosis and offer the opportunity to reduce subsequent risk and its social and economic impact.

The ability to quantify *SI* and U_N is essential to improving the understanding of the complex physiology underlying type 2 diabetes. The first is associated with *IR*, while the second is associated with the ability to endogenously secrete insulin to reduce glucose level. Thus, it is necessary to assess both to enable complete diagnosis.

Throughout the years, many experimental tests have been designed to quantitatively assess *SI* and U_N (Bergman *et al*. 1985; Hovorka & Jones 1994). Mathematical models of glycaemic dynamics, in particular, have been coupled with clinical data to identify these 2 key aspects in the pathogenesis of type 2 diabetes. However, none have yet been accepted as a clinical standard, and are primarily used as research tools.

SI is defined as the ability of insulin (exogenous or endogenous) to lower blood glucose concentration by stimulating glucose uptake and suppressing its glucose production (Pacini & Mari 2003). The EIC is regarded as the gold standard method for identifying *SI* (Ferrannini & Mari 1998; Pacini & Mari 2003), due to its good accuracy and high repeatability (Defronzo *et al*. 1979; Monzillo & Hamdy 2003). The EIC measures *SI* through the amount of glucose necessary to compensate for a hyper-physiologically increased insulin level while maintaining a normal, fasting glucose concentration of about 5 $mmol \cdot L^{-1}$ (Pacini & Mari 2003).

However, the EIC is increasingly avoided by clinical researches due to its experimental complexity, the need for clinical expertise in administering the test, and its time consuming design (Ferrannini & Mari 1998; Radziuk 2000). Thus, the IVGTT with minimal model assessment was proposed as a potential way to mitigate the clinical intensity of the EIC

(Pacini & Bergman 1986). However, the IVGTT is known to produce ambiguous insulin sensitivity values and erratic correlations with the EIC (Bonora *et al*. 1989; Ferrannini & Mari 1998). This difficulty is particularly true for those with low *SI* (higher *IR*) (Pillonetto *et al*. 2002; Quon *et al*. 1994), which is, paradoxically, the target population requiring the most accuracy in an *SI* test. It is also costly and clinically intensive due to the frequent, typically 1-3 min, sampling.

The DISST is a low intensity test that incorporates a clinical protocol similar to the IM-IVGTT (Bergman *et al*. 1979b; Ward *et al*. 2001). The DISST data modelling and data fitting methods were customized to the clinical protocol to allow a robust measurement of *SI* that avoids the problems encountered with FS-IVGTT assessment in insulin resistant patients (Caumo *et al*. 1999; Cobelli *et al*. 1986; Docherty *et al*. ; Krudys *et al*. 2006). The DISST also compares very favourably with EIC in assessing *SI* with strong correlation of R = 0.82, and produced highly repeatable *SI* and U_N metrics (Lotz 2007; Lotz *et al*. 2010; Lotz *et al*. 2008; McAuley *et al*. 2011). In addition, it provides U_N and *SI* resolution where the supra-physiological EIC does not (Lotz 2007; McAuley *et al*. 2011).

The pharmacokinetics and pharmacodynamics of the DISST model relates the rate of glucose decay to the concentration of insulin available in the interstitium to provide a metric of *SI* (Lotz 2007; Lotz *et al*. 2010). Like all other model-based assessments of *SI* (Bergman *et al*. 1979b; Bergman *et al*. 1981; Bergman *et al*. 1987; Boston *et al*. 2003; Caumo *et al*. 1999), the DISST model-based approach uses the participant's measured G_0 as their modelled G_B so that $G_0 = G_B$. Hence, *SI* is identified together with V_G in a 2-parameter identification approach.

Although, the DISST model, with the assumption of $G_0 = G_B$, produces an *SI* value that is highly correlated to the EIC value. However, the model was developed in a relatively healthy, normoglycemic cohort (Lotz ; Lotz *et al*. 2010). Understandably, G_0 is defined as a glucose concentration taken early in the morning after an overnight fast of 8 hours or more. While, G_B, on the other hand, is defined as the stable overnight glucose concentration (Holman & Turner 1981). Hence, the assumption of $G_0 = G_B$ might be untrue for some cases, particularly those involving low *SI* value. More specifically, prior studies show that G_0 levels and insulin concentrations are slightly higher in the morning than their overnight "basal" levels, especially for participants with diabetes (Holman & Turner 1977,1978,1979,1981).

In addition, the evidence suggests that G_B and G_0 should be treated as separate entities for individuals with established diabetes as the levels are determined by relative insufficiencies in *SI,* U_N and rates of gluconeogenesis (Cahill 1971; Cahill *et al*. 1959; Steele *et al*. 1968). Therefore, identifying G_B as a variable may provide more precise information particularly when assessing *SI* value especially for individuals with T2D. Hence, a new 3-parameter identification approach is developed in this chapter, where G_B is identified in concert with *SI* and V_G.

3.2 DISST Model

The DISST provides quantitative measures of both *SI* and U_N (Lotz *et al*. 2010; McAuley *et al*. 2011; McAuley *et al*. 2007). The DISST is similar to the insulin modified IVGTT, which typically uses an alternative modelling approach that requires a higher intensity test. The

DISST model describes the U_N secretion profile from the deconvolution of C-peptide concentration (Van Cauter *et al*. 1992):

C-peptide Pharmaco-kinetic Model

$$\dot{C} = -(k_1 + k_3)C + k_2 Y + \frac{U_N}{V_p} \tag{3.1}$$

$$\dot{Y} = -k_2 Y + k_1 C \tag{3.2}$$

The insulin kinetics are described (Lotz *et al*. 2010):

Insulin Pharmaco-kinetic Model

$$\dot{I} = -n_k I - n_L \frac{I}{1 + \alpha_I I} - \frac{n_I}{V_p}(I - Q) + \frac{U_{ex}}{V_p} + (1 - x_L)\frac{U_N}{V_p} \tag{3.3}$$

$$\dot{Q} = -\left(n_C + \frac{n_I}{V_Q}\right)Q + \frac{n_I}{V_Q} I \tag{3.4}$$

Finally, *SI* is identified from a glucose insulin pharmacodynamic (PD) model (Chase *et al*. 2005):

Glucose-Insulin Pharmaco-dynamic Model

$$\dot{G} = -p_{gu}(G - G_B) - SI(GQ - G_B Q_B) + \frac{P_t}{V_G} \tag{3.5}$$

All values for Equations (3.1) – (3.5) are described in Table 3.1.

Table 3.1: Nomenclatures of DISST model

Variable	Unit	Description	Role
C	$pmol \cdot L^{-1}$	Plasma C-peptide concentration	measured
I	$mU \cdot L^{-1}$	Plasma insulin concentration	measured
G	$mmol \cdot L^{-1}$	Blood glucose concentration	measured
Y	$pmol \cdot L^{-1}$	Interstitial C-peptide concentration	simulated
Q	$mU \cdot L^{-1}$	Interstitial insulin concentration	simulated
Q_B	$mU \cdot L^{-1}$	Basal interstitial insulin concentration	simulated
U_N	$mU \cdot min^{-1}$	Endogenous insulin secretion	simulated/ deconvoluted
k_1, k_2, k_3	min^{-1}	C-peptide transport rates	*a-priori*
V_p	L	Plasma insulin distribution volume	*a-priori*
V_q	L	Interstitial insulin distribution volume	*a-priori*
n_k	min^{-1}	Renal insulin clearance rate	*a-priori*
n_I	$L \cdot min^{-1}$	Plasma-interstitial diffusion rate	*a-priori*
n_C	min^{-1}	Interstitial insulin degradation rate	*a-priori*
U_{ex}	$mU \cdot min^{-1}$	Exogenous insulin input rate	*a-priori*
P_t	$mmol \cdot min^{-1}$	Exogenous glucose input rate	*a-priori*
p_{gu}	min^{-1}	Non-insulin mediated glucose disposal rate	*a-priori*
α_I	$L \cdot mU^{-1}$	Hepatic insulin clearance saturation parameter	*a-priori*
G_B	$mmol \cdot L^{-1}$	Basal blood glucose concentration	identified
V_g	L	Glucose distribution volume	identified
n_L	min^{-1}	Hepatic insulin clearance rate	identified
x_L	1	Fractional first-pass hepatic insulin extraction	identified
SI	$L \cdot mU^{-1} \cdot min^{-1}$	Insulin sensitivity	identified

3.3 Parameter identification

3.3.1 2-parameter identification approach

Initially, the DISST model uses a 2-parameter (x = [*SI*, V_G]) identification approach when assessing the *SI* value, having made the assumption of $G_0 = G_B$. Previously, these participant-specific parameter values of *SI* and V_G were identified based on the physiological simulation of interstitial insulin and measured glucose data using the iterative integral method (Docherty *et al*. 2012). The glucose samples that were taken in the first 5 minutes after the glucose bolus were disregarded by the identification methods, as this period is heavily influenced by mixing kinetics that are not captured by the whole body model of glucose metabolism (Edsberg *et al*. 1987; Lotz *et al*. 2010). Linear interpolation was then used as an estimate of the glucose response to the test stimulus.

In the identification procedure, the integral formulation of Equation 3.5 was rearranged and separated into the coefficients of the known and unknowns parameters, yielding:

$$-SI \underbrace{\int_0^t (GQ - G_B Q_B)\, dt}_{CSI} + \frac{1}{V_G} \underbrace{\int_0^t P_t dt}_{CV_G} = \underbrace{G_t - G_0 + \int_0^t p_{gu}(G - G_B) dt}_{C} \tag{3.6}$$

These coefficients of *CSI*, CV_G and *C* were evaluated over the sample times from t = 0 until $t = t_1, t_2, \cdots, t_{end}$ at the end of the test. These sample periods were chosen to minimise the fitting error and the variability of the identified insulin sensitivity (Docherty *et al*. 2011a). Subsequently, a matrix formulation was arranged to define the *SI* and V_G terms, as defined:

$$\begin{bmatrix} CSI_{t=0}^{t=t_1} & {CV_G}_{t=0}^{t=t_1} \\ CSI_{t=0}^{t=t_2} & {CV_G}_{t=0}^{t=t_2} \\ \vdots & \vdots \\ CSI_{t=0}^{t=t_{end}} & {CV_G}_{t=0}^{t=t_{end}} \end{bmatrix} \begin{bmatrix} -SI \\ 1/V_G \end{bmatrix} = \begin{bmatrix} C_{t=0}^{t=t_1} \\ C_{t=0}^{t=t_2} \\ \vdots \\ C_{t=0}^{t=t_{end}} \end{bmatrix} \quad (3.7)$$

where t_i $(i = 1, 2\ \ldots) < t_{end}$ capture different integration intervals.

The values of V_G are limited to identification of values within 12 to 25% of the participant's bodyweight to reduce the effect incomplete mixing might have on the *SI* term (Defronzo *et al*. 1979; Ferrannini & Mari 1998; Lotz 2007; Lotz *et al*. 2010). However, while this 2-parameter identification approach might be true for a more normoglycemic, healthy cohort, it may not hold in T2D or high insulin resistance.

3.3.2 3-parameter identification approach

Previous studies have suggested that G_B and G_0 should be treated as separate entities especially for individuals with established diabetes (Holman & Turner 1977,1978,1979,1981). Hence, the participant-specific parameter values of G_B, *SI* and V_G can be identified in a 3-parameter identification approach by adapting the Gauss Newton parameter identification method (Björck 1996). The iteration function is defined:

$$\mathbf{x}_{i+1} = \mathbf{x}_i - (\mathbf{J^T J})^{-1}\mathbf{J^T \psi} \quad (3.8)$$

where $\mathbf{x}_i = [G_{Bi}, SI_i, V_{Gi}]^T$ and *i* is the iteration number. The Jacobian matrix (**J**) and the residual matrix (**ψ**) are defined:

$$\mathbf{J}(\mathbf{x}_i) = \begin{bmatrix} \frac{\delta\psi_1}{\delta G_{Bi}} & \frac{\delta\psi_1}{\delta SI_i} & \frac{\delta\psi_1}{\delta V_{Gi}} \\ \frac{\delta\psi_2}{\delta G_{Bi}} & \frac{\delta\psi_2}{\delta SI_i} & \frac{\delta\psi_2}{\delta V_{Gi}} \\ \vdots & \vdots & \vdots \\ \frac{\delta\psi_n}{\delta G_{Bi}} & \frac{\delta\psi_n}{\delta SI_i} & \frac{\delta\psi_n}{\delta V_{Gi}} \end{bmatrix}, \qquad \boldsymbol{\psi}(\mathbf{x}_i) = \begin{bmatrix} G(\mathbf{x}_i, t_1) - G_S(t_1) \\ G(\mathbf{x}_i, t_2) - G_S(t_2) \\ \vdots \\ G(\mathbf{x}_i, t_n) - G_S(t_n) \end{bmatrix} \tag{3.9}$$

where n is the number of measured samples, $\frac{\delta\psi_1}{\delta G_{Bi}}$ is the derivative of the residual matrix with respect to G_B, $G(\mathbf{x}_i, t_1)$ is the modelled glucose concentration at $t = t_1$ given $\mathbf{x}_i$, $G_S(t_1)$ is the measured glucose level at $t = t_1$.

V_G is similarly limited to within the range of 12% to 25% of participant's body weight to avoid any parameter estimation issues. Equation 3.8 is iterated using Equation 3.9 until convergence to a tolerance.

3.4 Summary

With an assumption of $G_0 = G_B$, like most model-based assessments of *SI* employed, the *SI* value is well addressed by DISST model using a 2-parameter identification approach. However, previous studies have demonstrated that G_0 is not equal to G_B especially for individual with established diabetes. Hence, the original DISST model, while appropriate for more normoglycaemic cohorts, needs to model basal glucose level as a variable for assessing individuals with established T2D. This chapter presents the equations and rationale for a novel 3-parameter identification approach.

Chapter 4. The necessity of identifying the basal glucose set-point in the Type 2 Diabetes

This chapter underlines the importance of identifying G_B as a variable, as opposed to other typical model-based *SI* assessments, where G_B is set equal to G_0. In particular, the impact of this approach is assessed for individuals with established T2D, where assessing *SI* accurately is important.

4.1 Introduction

The DISST is an alternative modelling approach that allows a lower intensity test compared to the IM-IVGTT that is often used to assess those with T2D and those with high insulin resistance (Bergman *et al*. 1979b; Ward *et al*. 2001). The DISST *SI* value is highly correlated to the EIC *SI* value (R=0.82), which is widely regarded as the reference method (McAuley *et al*. 2011). In particular, the DISST is able to produce highly repeatable SI metrics and also provides quantitative measures of U_N via deconvolution of C-peptide data (Lotz 2007; Lotz *et al*. 2010; Lotz *et al*. 2008; McAuley *et al*. 2011).

However, the DISST model and identification methods use the participant's measured G_0 as their G_B setting $G_B = G_0$. The G_B term in the DISST model effectively has the role of determining the set-point towards which the modelled glucose response moves. This choice thus matches assumptions in all other model-based tests (Bergman *et al*. 1979b; Bergman *et*

al. 1981; Bergman *et al*. 1987; Boston *et al*. 2003; Caumo *et al*. 1999). However, it is important to note that the DISST model was developed in a relatively healthy, normoglycemic cohort (Lotz ; Lotz *et al*. 2010). Hence, re-defining the role of G_B as a variable in the DISST based approach to assessing *SI* could more clearly capture the observed glycaemic behaviour especially in T2D.

More specifically, studies have shown that G_0 levels and insulin concentrations are slightly higher in the morning than their overnight "basal" levels, especially for participants with established diabetes (Holman & Turner 1977,1978,1979,1981). Thus, the assumption of $G_B = G_0$ needs to be reviewed. In addition, prior evidence suggests that G_B and G_0 should be treated as separate entities for individuals with established diabetes as the levels are determined by relative insufficiencies in *SI,* U_N, and rates of gluconeogenesis (Cahill 1971; Cahill *et al*. 1959; Steele *et al*. 1968).

Hence, this chapter presents a novel modelling approach that identifies G_B as a variable in a 3-parameter identification (x = [G_B, *SI*, V_G]). The goal is to capture more accurate glucose dynamics, particularly for individuals with established type 2 diabetes. Results are thus expected to be improved over the 2-parameter identification approach, especially for T2D and highly insulin resistant cohorts.

4.2 Methods

4.2.1 Participants

Fourteen individuals with established type 2 diabetes mellitus were recruited from the Wellington region of New Zealand to take part in an Atkins-Based low carbohydrate dietary intervention study. Recruited participants were aged between 30 and 65 with a BMI range of 34 to 46 $kg \cdot m^{-2}$ at baseline. Participants were excluded if they had major physiological or psychological illness at the time of testing. Pregnant or lactating females were also excluded. Two participants discontinued the intervention, the first citing personal reasons, and the second left the study due to a renal stone. Twelve participants each underwent three IM-IVGTTs over a 24 week period resulting in a total of 36 data sets for the study. Participants had their age and BMI recorded (median [IQR]; 47.5 [42.5, 54.5] and 40.40 [37.48, 43.48], respectively). Full demographic details and results of the intervention study have been previously described (Krebs *et al*.). Table 4.1 provides some further demographic data. Ethics approval for this study was provided by the New Zealand Ministry of Health, Central Regional Ethics Committee.

Table 4.1: Participant characteristics at baseline (W0) week 12 (W12) and the end of the trial at week 24 (W24) as well as their duration of diabetes.

Participant	Gender (M/ F)	Age (yrs)	Height (m)	Weight (kg)			Duration of diabetes (years)
				W0	W12	W24	
1	M	46	1.81	142.6	133.1	137.0	10
2	M	56	1.79	130.8	121.1	114.4	1
3	F	52	1.55	82.0	77.6	76.2	11
4	F	55	1.71	121.7	111.7	108.7	6
5	F	35	1.61	119.5	106.2	104.9	2
6	M	56	1.79	145.3	140.6	135.9	5
7	F	38	1.57	113.5	110.0	107.0	3
8	F	45	1.59	95.6	87.6	86.6	7
9	M	49	1.75	134.0	118.6	115.9	2
10	M	41	1.71	128.0	127.0	132.3	4
11	F	54	1.56	96.3	86.2	81.5	2
12	M	44	1.80	130.7	123.5	122.8	0.5
25%	6 M 6 F	42.5	1.58	104.9	96.9	95.8	2.0
Median		47.5	1.71	124.9	115.2	111.6	3.5
75%		54.5	1.79	132.4	125.3	127.6	6.5

4.2.2 Clinical procedure

The IM-IVGTT clinical protocol utilised in this study was similar to the protocol defined by Ward *et al.* (Ward *et al*. 2001). A 0.2 $g \cdot kg^{-1}$ glucose bolus was administered at t = 1 minute and then an infusion of insulin that was intended to replicate the insulinaemic response of a normoglycaemic individual was administered. An insulin infusion was started at t = 2 minutes at a rate of 3.5 $mU \cdot kg^{-1} \cdot min^{-1}$ and was reduced to 0.5 $mU \cdot kg^{-1} \cdot min^{-1}$ at t = 7 minutes.

Further reductions occurred at t = 17 minutes, to 0.25 mU·kg^{-1}·min^{-1}, and at t = 50 minutes, to 0.1 mU·kg^{-1}·min^{-1}. The infusion of mU·kg^{-1}·min^{-1} was maintained for the remainder of the procedure. Venous blood samples were taken and put into fluoride oxalate tubes at times: t = -10, -5, -1, 0, 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. Blood samples were assayed for glucose and insulin concentration using standard commercial assays (Roche Diagnostics, New Zealand) at an accredited laboratory (Diabetes and Lipid Laboratory, University of Otago, Dunedin, New Zealand).

4.2.3 Physiological model

4.2.3.1 DISST Model

With only glucose and insulin concentrations available from the clinical trial, the interstitial insulin kinetics and glucose dynamics of the DISST models was used (Lotz ; Lotz *et al*. 2010):

$$\dot{Q} = -\left(n_C + \frac{n_I}{V_Q}\right)Q + \frac{n_I}{V_Q}I \tag{4.1}$$

$$\dot{G} = -p_{gu}(G - G_B) - SI(GQ - G_B Q_B) + \frac{P_t}{V_G} \tag{4.2}$$

where the equation nomenclature is well defined in Chapter 3, Section 3.2, Table 3.1. Note that V_Q, n_I and n_C are defined a-priori based on anatomical functions (Barrett *et al*. 2009; Docherty 2011; Lotz *et al*. 2010; Van Cauter *et al*. 1992), while the DISST model sets p_{gu} as a constant at 0.004 min^{-1} (Lotz *et al*. 2010).

4.2.4 Parameter identification

Q was simulated via integrating factors and a linear interpolation of I.

$$Q = e^{-\int_{t_0}^{t_1} n_C + \frac{n_I}{V_Q} dt} \left(Q_0 + \int_{t_0}^{t_1} e^{\int_{t_0}^{t_1} n_C + \frac{n_I}{V_Q} dt} \frac{n_I}{V_Q} I dt \right) \tag{4.1a}$$

where Q_0 is determined assuming a steady state at *t* = -10 minutes:

$$Q_0 = \frac{\frac{n_I}{V_Q} I_0}{n_C + \frac{n_I}{V_Q}} \tag{4.3}$$

The DISST model typically sets $G_B = G_0$ (Lotz ; Lotz *et al*. 2010). Hence, G_0 acts as a surrogate basal glucose concentration level. However, for individuals with elevated fasting glucose, this assumption may not be accurate (Holman & Turner 1977,1978,1979,1981), and is tested in this study.

In this analysis, G_B was identified in concert with SI and V_G. The typical approach used with the DISST model identifies only *SI* and V_G. Thus, the outcomes of the 3-parameter identification approach (x = [G_B, *SI*, V_G]) model can be compared to the outputs of the typical 2-parameter identification approach (x = [*SI*, V_G]). A Gauss Newton parameter identification method was used to identify these 3 parameters. A full detailed description of the identification methodology via Gauss Newton method was previously discussed in Chapter 3, Section 3.3.2.

The Jacobian was numerically evaluated using perturbations of $[\delta G_B, \delta SI, \delta V_G] = [10^{-3}, 10^{-8}, 10^{-3}]$. These perturbation values were 0.1% of the order of magnitude of the expected parameter values. Glucose samples between t = 1 and t = 10 minutes were disregarded by the identification methods, as this period is heavily influenced by mixing kinetics that are not captured by the whole body model of glucose metabolism (Edsberg *et al*. 1987; Lotz *et al*. 2010).

Identifying G_B in concert with SI and V_G can cause identified parameter trade off in some cases (Docherty *et al*. 2011a). The value of V_G was thus limited to physiologically measured bounds from other studies (Defronzo *et al*. 1979; Ferrannini & Mari 1998; Lotz 2007; Lotz *et al*. 2010). In particular, V_G was limited to the range of [0.12Bw, 0.25Bw] where bodyweight (Bw) is measured in kg and the coefficients have units of l·kg^{-1}, which is a standard estimation approach linking volume to an easily measured value. Similarly, G_B was limited to a minimum of 3 mmol·L^{-1}. Both sets of limits are physiologically outside normal published bounds, but do not allow non-physiological results, such as negative or very small G_B or volume.

4.3 Statistical analysis

Identified model residuals compared to the clinical data and interpretation of population trends were used to assess the performance of the G_B identified - DISST model. The *p*-values are defined with signed ranksum (p_{rs}) and Kolmogrov Smirnov test (p_{ks}) to assess median and variability of non-parametric non-Gaussian distributions. A Pearson correlation (*R* value)

is used in the analysis to show the linear relationship between two sets of data. All analysis was undertaken using MATLAB (R2013b, Mathworks, Inc., Natick, MA, USA).

4.4 Results

Table 4.2 shows the tabulated data comparing the 2- and 3- parameter identification approaches across all 36 tests over 24 weeks of the clinical study. It can be clearly seen that adopting the 3-parameter identification approach in assessing the *SI* value lead to significant differences between the measured G_0, identified basal glucose ($G_{B\text{-}ID}$), and resulting identified *SI* values between the two identification approaches. Although, there is no significant different in variability for V_G values as p_{ks} (Kolmogorov Smirnov) = 0.2975, the medians are significantly difference since p_{rs} (Signed-ranksum) < 0.00001.

Figures 4.1 and 4.2 show the individual relationships between G_0, $G_{B\text{-}ID}$ and identified *SI* from the 2- and 3- parameter identification approaches for the DISST model across all participants and tests. Note the bias about the 1:1 line indicating that on average, the identified, model-based basal set point for glucose ($G_{B\text{-}ID}$) was significantly lower than the fasting rate (G_0) for this cohort with diabetes. Figure 4.1 shows there were significant differences in median and variability between the G_0 and $G_{B\text{-}ID}$ values in this cohort (p_{rs}<0.0001, p_{ks}<0.0001). In general, G_0 was higher than the $G_{B\text{-}ID}$ value, with only 4 exceptions over 36 results (11.1%). Although there was a significant difference in the levels of $G_{B\text{-}ID}$ and G_0, they were relatively well correlated (R=0.70), indicating a moderately consistent bias in the relationship between values.

Table 4.2: Tabulated data of G_0, $G_{B\text{-}ID}$, SI and V_G identified from two modelling approaches across 36 tests.

	2-parameter identification			3-parameter identification		
	G_0 [mmol·L^{-1}]	SI [×10^{-4} L·mU^{-1}·min^{-1}]	V_G [L]	$G_{B\text{-}ID}$ [mmol·L^{-1}]	SI [×10^{-4} L·mU^{-1}·min^{-1}]	V_G [L]
	16.85	3.27	35.65	9.59	0.34	35.65
	6.67	3.14	26.03	7.54	3.08	32.70
	16.33	7.80	11.38	11.48	2.71	14.99
	7.73	7.88	28.14	5.38	4.35	30.43
	8.50	9.27	28.23	5.57	4.39	29.88
	7.51	2.18	30.50	8.53	2.71	33.48
	14.42	6.01	23.13	9.29	1.95	28.38
	6.64	22.85	14.95	3.95	11.56	16.48
	9.07	1.92	33.50	6.24	0.78	33.50
	6.62	5.19	31.30	4.27	2.29	32.00
	7.25	8.61	12.14	4.67	3.59	16.25
	8.58	5.23	24.08	5.76	2.31	29.15
	11.36	1.93	29.04	9.14	0.96	33.28
	6.05	4.89	23.52	5.60	4.13	25.57
	10.33	8.17	11.10	3.00	1.32	12.14
	6.62	11.59	20.40	4.29	6.57	23.79
	6.75	8.51	26.55	3.57	3.15	26.55
	7.09	2.12	33.82	6.38	2.73	33.14
	12.62	5.95	23.29	9.42	2.82	27.50
	6.51	26.11	13.45	3.43	11.33	15.25
	5.12	4.85	29.65	4.11	3.57	29.65
	5.60	6.21	31.75	5.77	7.05	28.31
	6.96	7.87	13.08	4.64	5.20	13.98
	6.64	10.27	22.81	5.30	7.09	22.75
	11.72	2.96	26.83	9.69	1.15	34.25
	5.91	6.01	17.76	3.86	3.82	18.75
	10.80	11.17	9.14	3.51	2.50	11.91
	6.69	10.48	27.17	5.94	8.50	27.18
	7.60	9.50	20.51	3.51	2.62	25.52
	6.74	3.21	29.70	7.57	3.89	33.98
	9.96	6.50	23.01	6.58	1.79	26.75
	6.76	16.67	11.87	4.65	10.24	14.08
	6.09	5.34	28.97	5.33	4.70	27.15
	9.92	6.26	21.37	5.53	1.47	25.23
	6.39	12.00	11.83	5.43	7.62	15.52
	7.22	7.82	21.30	5.37	4.47	26.37
25%	6.63	4.87	16.36	4.28	2.30	17.61
Median	7.16	6.38	23.41	5.48	3.36	26.95
75%	9.94	9.39	29.01	7.06	4.95	31.21

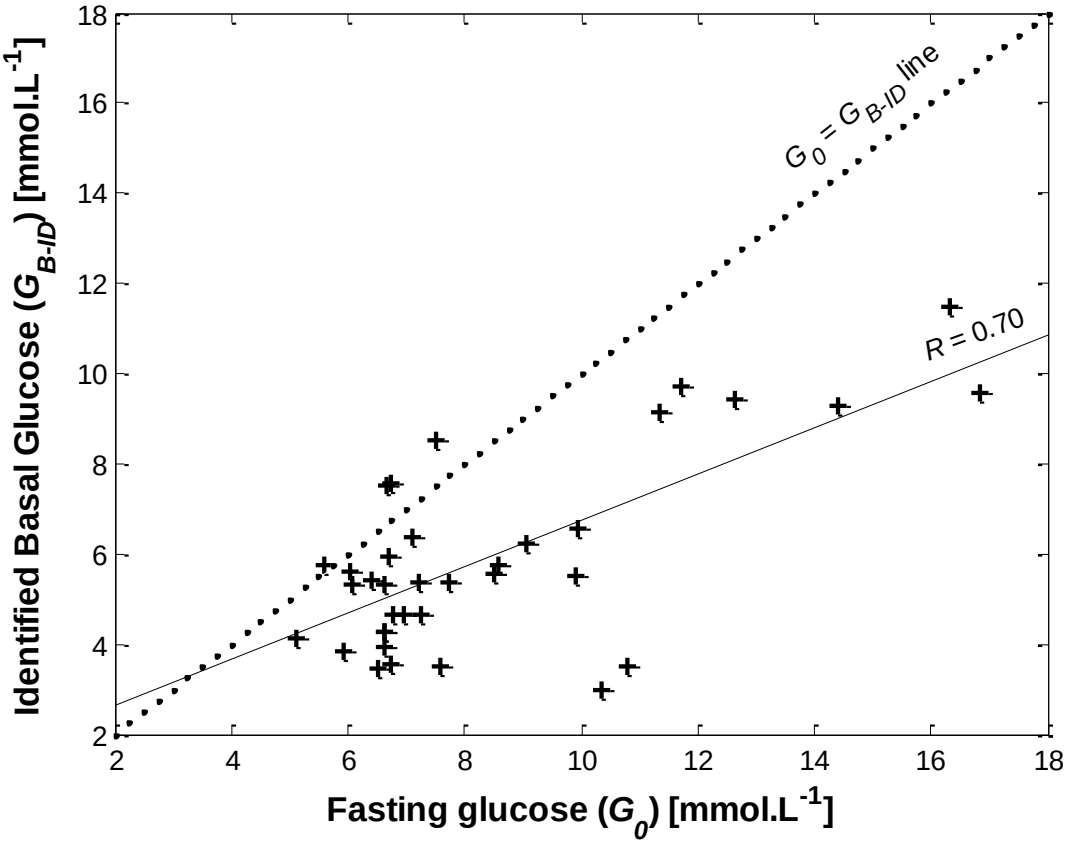

Figure 4.1: Relationship between G_0 and $G_{B\text{-}ID}$ across tests. The 1:1 G_0 = $G_{B\text{-}ID}$ line (dots) is to show the bias between approaches. The solid line has R=0.70.

Figure 4.2 shows the effect that identifying $G_{B\text{-}ID}$ has on the identified *SI* values in the 3-parameter approach. There is a reasonably strong correlation between the *SI* values between the 2- and 3- parameter identification of the DISST model (R=0.83). The bias indicates that by identifying basal glucose, the model captures consistently lower *SI* values for those with established T2D.

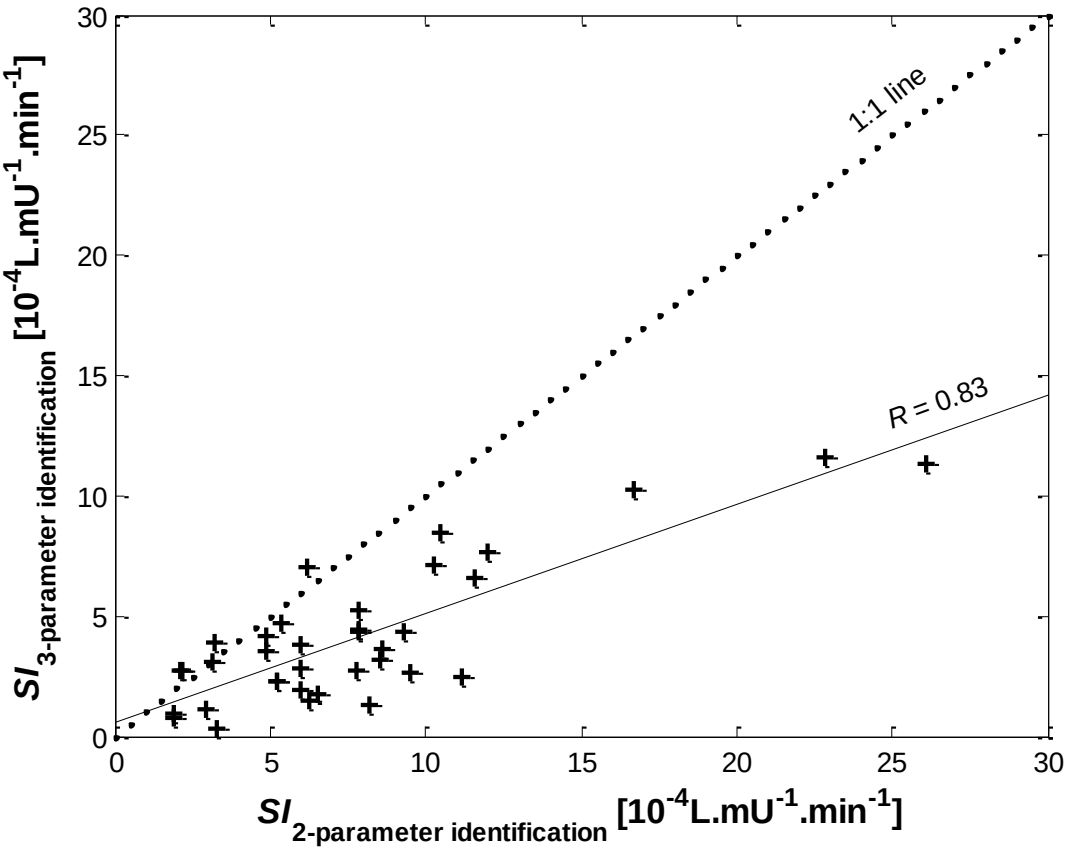

Figure 4.2: Relationships between *SI* values of 2- and 3- parameter identification approach across tests. The 1:1 line (dots) is to show the bias between approaches. The solid line has R=0.83.

Figure 4.3 shows the fitted glucose profiles and measured glucose data from 3 different participants. It also shows that the identified $G_{B\text{-}ID}$ levels are well below the measured G_0 values, as depicted in Figure 4.1. Figure 4.4 illustrates the residual errors for all 36 tests of both the typical DISST model and the proposed three parameter identified model that identifies basal glucose. Note again that the glucose samples taken within 10 minutes of glucose injection were ignored due to un-modelled mixing effects.

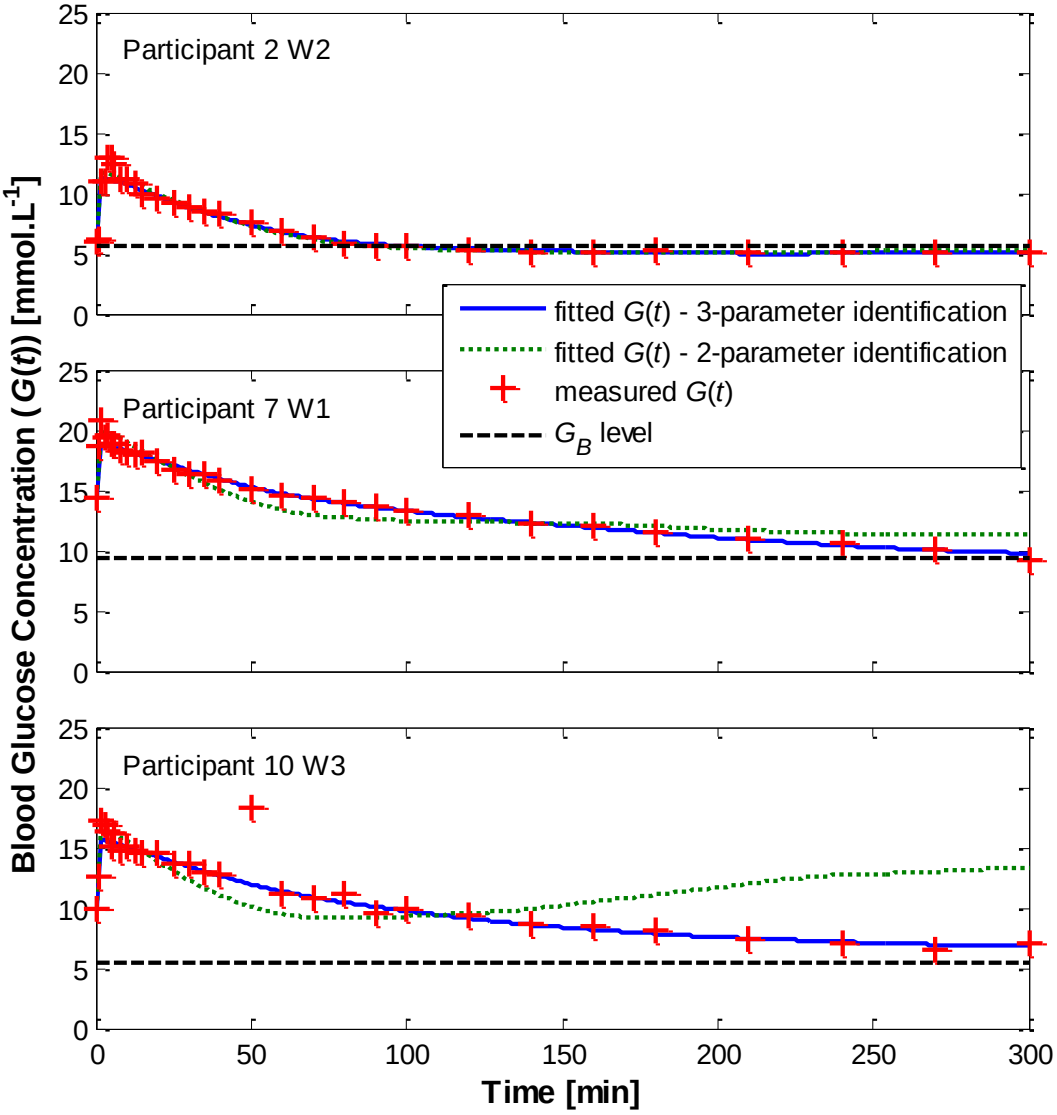

Figure 4.3: Blood glucose participant-specific profile ($G(t)$) for participants 2, 7, 10 with 2- and 3- parameter identification approach.

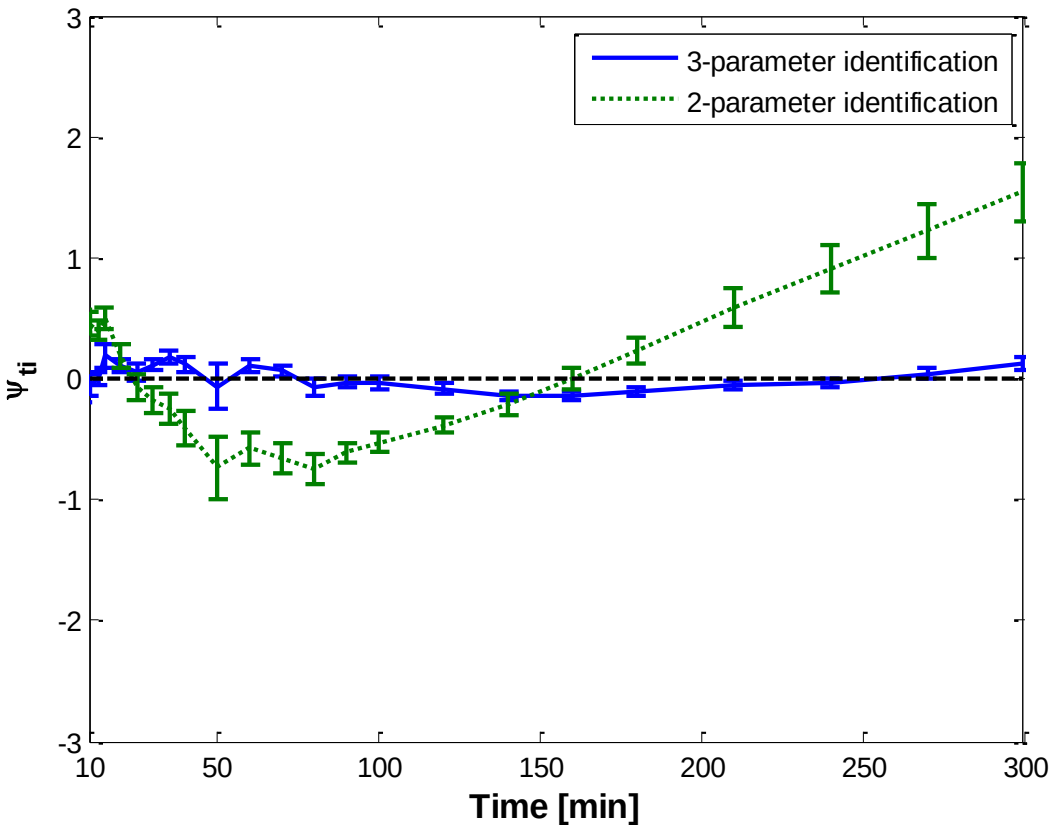

Figure 4.4: Residual error (mean and standard error, $SE = \frac{SD}{\sqrt{N}}$) between the measured glucose data and the response model by Equation 2 for all 36 tests. The residuals reflect model accuracy after bolus dosing and mixing errors are passed.

4.5 Discussion

This analysis is the first study to demonstrate that glucose excursions are more accurately modelled using basal glucose as an identified variable in the DISST model, rather than assuming fasting glucose $G_0 = G_B$ for individuals with established type 2 diabetes. The typical approach employed when using the DISST model defines the G_0 as equal to the G_B. Hence, the glucose response defined by the original model typically tends towards the measured basal value (G_0).

However, this analysis has shown that this assumption is not valid for a cohort with established diabetes. This discrepancy in the assumption is evidenced by the significant distinction in the values of G_0 and G_B. In addition, this discrepancy reduces as participant glycaemic control improved across the 24 weeks of the dietary intervention study (Krebs *et al*.). Figure 4.1 shows that while most participants had elevated G_0 levels, the $G_{B\text{-}ID}$ was often much closer to the lower value seen in healthy subjects. In particular, 14 of the 36 identified G_B values were in the normal reference range of 4-5.6 $mmol \cdot L^{-1}$ (ADA), while only 2 of 36 measured G_0 values were in that range.

However, there were some participants for whom $G_{B\text{-}ID}$ remained very high throughout the intervention. Of the three participants that exhibited $G_{B\text{-}ID}$ values greater than 9 $mmol \cdot L^{-1}$, two were first diagnosed 10 years prior to this trial. In contrast, the mean duration of diabetes for the whole cohort was 4.4 years (SD=1.0 year) and median was 3.5 years. This outcome indicates a possible mechanism of dysfunction in type 2 diabetes that develops during the course of the disease, and matches the well-known growth of dysfunction over time in T2D individuals. However, while this study lacks the numbers required for conclusive proof of this trend, it does show the ability of the improved identification approach to better capture expected metabolic behaviour.

Figure 4.2 shows the effects of *SI* values when identified the G_B values used are significantly lower than the measured G_0 values for these type 2 diabetes participants. Hypothetically, if models set $G_B = G_0$, *SI* will be used in the model to account for low glucose levels, rather than G_B and is thus identified in the 2-parameter case as a higher *SI* value. A recent study shows that type 2 diabetes subjects have *SI* values in the magnitude of $1\text{-}4\times10^{-4}$ $L \cdot mU^{-1} \cdot min^{-1}$

(Lotz 2007). Although, there is not enough evidence to prove a precise range of *SI* value for type 2 diabetes participants, it is understandable that lower *SI* value contributes to the pathogenesis of type 2 diabetes as *SI* is inversely proportional to *IR* (Ferrannini 1997). Hence, there is good reason to believe that the lower $G_{B\text{-}ID}$ values are real and realistic based on current knowledge.

Figure 4.3 shows the blood glucose profiles of three participants as modelled by the typical 2-parameter (x = [SI, V_G]) and 3-parameter (x = [G_B, SI, V_G]) DISST identification approach. While the typical 2-parameter DISST identification fails to fully capture the responses of these participants, the amended 3-parameter identification method captures the observed behaviours more closely. This outcome is confirmed by the residual plots in Figure 4.4 that indicate a much smaller, more consistent trend about the measured data. This change implies the modified model captures previously un-modelled effects due to poor a-priori estimates in the model that are rectified by identifying 3 parameters instead of 2.

The original DISST model was developed (Lotz *et al*. 2010) and validated (McAuley *et al*. 2011) primarily in relatively normoglycaemic and glucose tolerant cohorts. In these cohorts, the incidence of impaired fasting glucose was relatively low, and thus, the assumption $G_B = G_0$ was well founded. However, the glycaemic behaviour of the cohort used in this analysis showed that this assumption was most not valid in these participants. In particular, the lower glucose levels achieved in the later part of the test would be falsely attributed to increased insulin sensitivity, rather than a G_B value that was lower than G_0. The significantly biased residuals in Figure 4.4 validate the outcome where they show that the typical DISST model cannot capture all the dynamics of this T2D cohort without identifying G_B directly.

Overall, these results indicate that the original 2-parameter approach, while appropriate for normoglycaemic and mild intolerant cohorts (Lotz ; Lotz *et al*.), is less suitable for highly insulin resistant individuals with established type 2 diabetes. Furthermore, it is unknown how much the outcomes of this study would be applicable to pre-diabetic individuals that have elevated blood glucose. Hence, this assertion remains to be determined.

Neither DISST identification approach accurately captures the peak value of the measured blood glucose data. This particular result was due to the disregarded glucose data within 10 minutes of glucose injection. This data was rejected due to the unmodelled effects of intravascular mixing (Edsberg *et al*. 1987). A second compartment to model local/global mixing kinetics could be added. However, this addition was deemed unnecessary, as such compartments do not add value to the DISST modelled outcomes (Lotz *et al*. 2010). The approach used was intended to avoid over-fitting and/ or over-modelling of mixing effects.

Although, this analysis was done in a small test cohort, the outcomes are significant as it has shown that G_B is an important variable when modelling the glycaemic behaviour in established type 2 diabetes. It also showed that G_B can be quite different to the typically assumed G_0 value used in all other studies, and that it may also have some diagnostic value. These findings suggest that the G_B value should be treated as a variable in DISST model identification for this cohort. Further validation in a much larger cohort will provide a broader foundation for these findings.

4.6 Summary

This analysis has shown the presence of a dysfunction in the basal (set-point) glucose in individuals with type 2 diabetes. The magnitude of the dysfunction has been shown to be linked to insulin sensitivity and the degree of fasting glucose. This analysis suggests that the basal glucose is a more appropriate variable for individuals with type 2 diabetes, as using the fasting glucose measurement as the basal set-point was shown to be a poor assumption for this cohort - although this requires confirmation in a larger study with a clamp as the reference.

Chapter 5. Impact of identifying the basal glucose set-point on model-based assessment of insulin sensitivity

This chapter portrays the influence of identifying the basal glucose set point as a variable towards assessing *SI*. It is particularly targeted for individuals with established T2D, where model-based *SI* tests often fail or suffer poor resolution.

5.1 Introduction

SI is widely regarded as an important index that quantifies the body's ability to lower blood glucose concentration with insulin and is one of the key element in the pathogenesis of type 2 diabetes (Pacini & Mari 2003). The pathogenesis of T2D progresses through 3 distinct stages: 1) NGT; 2) IGT; and 3) T2D (Pories & Dohm 2012). Typically, *SI* reduces during the progression of type 2 diabetes (Hanley *et al*. 2003; Ingelsson *et al*. 2005; Martin *et al*. 1992; Zethelius *et al*. 2004). A NGT individual will have a high value of *SI*, while a T2D will have a lower value of *SI*.

The DISST was designed to capture high resolution estimates of participant-specific *SI* and U_N profiles (Lotz 2007; Lotz *et al*. 2010; McAuley *et al*. 2011). The DISST compares favourably with the gold standard EIC in assessing *SI* with strong correlation of R = 0.82 (McAuley *et al*. 2011). Like most model-based assessments of *SI* and *IR* (Bergman *et al*.

1979b; Bergman *et al*. 1981; Bergman *et al*. 1987; Boston *et al*. 2003; Caumo *et al*. 1999), the DISST model approach uses the participant's measured G_0 as their modelled G_B so that $G_0 = G_B$. However, previous studies have shown that G_0 levels and insulin concentrations are slightly higher in the morning than their overnight levels, especially for diagnosed diabetes participants (Holman & Turner 1977,1978,1979,1981). This evidence suggests that G_B is not fully represented by G_0, especially for these individuals.

However, whether G_0 and G_B should be treated as separate entities has yet to be determined for any cohort. In addition, it is not known whether identifying G_B in the DISST model would lead to a better result in assessing the *SI* value. This chapter uses a new parameter identification approach to evaluate the impact on the identified *SI* value from a model-based *SI* assessment when acknowledging G_B as an identified model variable in a cohort of individuals with type 2 diabetes.

5.2 Methods

A total of 36 full test IM-IVGTT data sets, recorded from 12 participants in a 24-week dietary intervention study, were used to further analyse the efficacy of SI value between measured G_0 and identified G_B in a 2- and 3- parameter identification approaches. A full detailed description of the identification methodology was well discussed in Chapter 4, Section 4.2.

5.3 Statistical analysis

The median and variability of *SI* and G_B values are compared using the signed ranksum (p_{rs}) and Kolmogrov Smirnov (p_{ks}) tests, respectively. The signed ranksum test compares the specific participant matched *SI* results from the 2- and 3- parameter identification approaches. In addition, the Kolmogrov Smirnov (KS) test is sensitive to differences in both location and shape of the empirical cumulative distribution functions of *SI* distributions from the two approaches. P-values less than $p < 0.05$ were considered significant. All analysis was undertaken using MATLAB (R2013b, Mathworks, Inc., Natick, MA, USA).

5.4 Results

Table 5.1 contrasts measured G_0 values and identified G_B values across the participants and weeks of testing as well as the *SI* values identified across the two identification approaches. There were significant differences between the pooled patient- and week- specific G_0 and identified G_B values in this cohort (Signed ranksum: $p_{rs}<0.0001$, Kolmogorov Smirnov: $p_{ks}<0.0001$). Although there was a significant difference in the magnitudes of G_B and G_0, they were relatively well correlated (R=0.70), indicating a moderately consistent bias in the relationship between the measured and identified values. A similar trend can be seen for the identified *SI* values from the typical 2-parameter ($x = [SI, V_G]$) and 3-parameter ($x = [G_B, SI, V_G]$) identified models. In particular, there were significant differences across the two identification approaches in identified *SI* values (Signed ranksum: $p_{rs}<0.0001$, Kolmogorov Smirnov: $p_{ks}<0.0001$) with a stronger correlation of R=0.83.

Table 5.1: Tabulated data of initial G_0, G_B, SI identified from two modelling approaches across all participants.

Participant	G_0 [mmol·L^{-1}]			G_B [mmol·L^{-1}]			$SI_{2\text{-parameter DISST}}$ [×10^{-4} L·mU^{-1}·min^{-1}]			$SI_{3\text{-parameter DISST}}$ [×10^{-4} L·mU^{-1}·min^{-1}]		
	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	16.85	11.36	11.72	9.59	9.14	9.69	3.27	1.93	2.96	0.34	0.96	1.15
2	6.67	6.05	5.91	7.54	5.60	3.86	3.14	4.89	6.01	3.08	4.13	3.82
3	16.33	10.33	10.80	11.48	3.00	3.51	7.80	8.17	11.17	2.71	1.32	2.50
4	7.73	6.62	6.69	5.38	4.29	5.94	7.88	11.59	10.48	4.35	6.57	8.50
5	8.50	6.75	7.60	5.57	3.57	3.51	9.27	8.51	9.50	4.39	3.15	2.62
6	7.51	7.09	6.74	8.53	6.38	7.57	2.18	2.12	3.21	2.71	2.73	3.89
7	14.42	12.62	9.96	9.29	9.42	6.58	6.01	5.95	6.50	1.95	2.82	1.79
8	6.64	6.51	6.76	3.95	3.43	4.65	22.85	26.11	16.67	11.56	11.33	10.24
9	9.07	5.12	6.09	6.24	4.11	5.33	1.92	4.85	5.34	0.78	3.57	4.70
10	6.62	5.60	9.92	4.27	5.77	5.53	5.19	6.21	6.26	2.29	7.05	1.47
11	7.25	6.96	6.39	4.67	4.64	5.43	8.61	7.87	12.00	3.59	5.20	7.62
12	8.58	6.64	7.22	5.76	5.30	5.37	5.23	10.27	7.82	2.31	7.09	4.47
25%	6.96	6.28	6.54	5.02	3.84	4.25	3.20	4.87	5.67	2.12	2.78	2.14
Median	8.11	6.69	6.99	6.00	4.97	5.40	5.62	7.04	7.16	2.71	3.85	3.86
75%	11.75	8.71	9.94	8.91	6.07	6.26	8.24	9.39	10.82	3.97	6.81	6.16

Figure 5.1 shows the individual relationships between G_0 used in the 2-parameter identification and identified G_B from the 3-parameter identification for all participants and weeks. Note the bias about the 1:1 line indicating that, in almost all cases, the identified G_B value was significantly lower than G_0 for this cohort with diagnosed type 2 diabetes. There were only 4 exceptions over 36 results (11.1%) where $G_0 < G_B$.

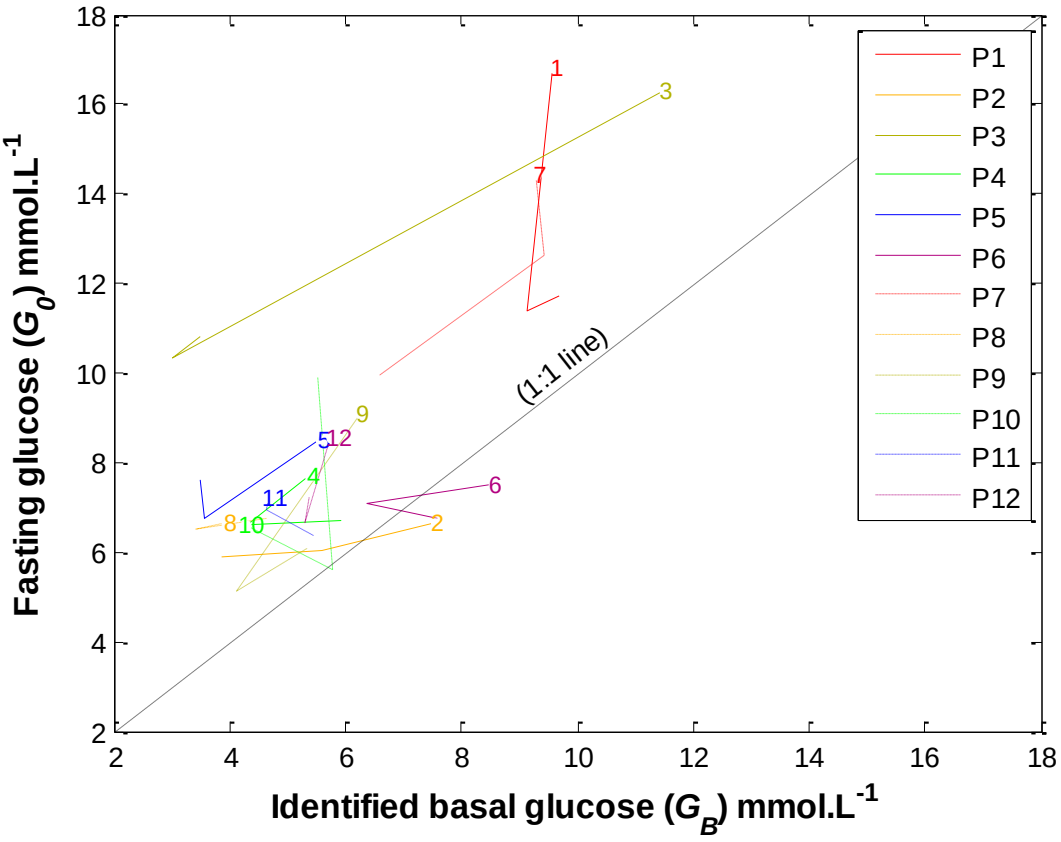

Figure 5.1: Relationship between G_0 and identified G_B across all participants and weeks.

Figure 5.2 exhibits a significant reduction in *SI* values from the 3-parameter identification compared to the typical 2-parameter identification approach. Table 5.1 shows that the highest *SI* value identified by the 3-parameter case was 11.56×10^{-4} L·mU^{-1}·min^{-1}, compared to 26.11×10^{-4} L·mU^{-1}·min^{-1} for the 2-parameter case. Figure 5.3 shows the distribution of G_0 and identified G_B across *SI* values from the two modelling approaches.

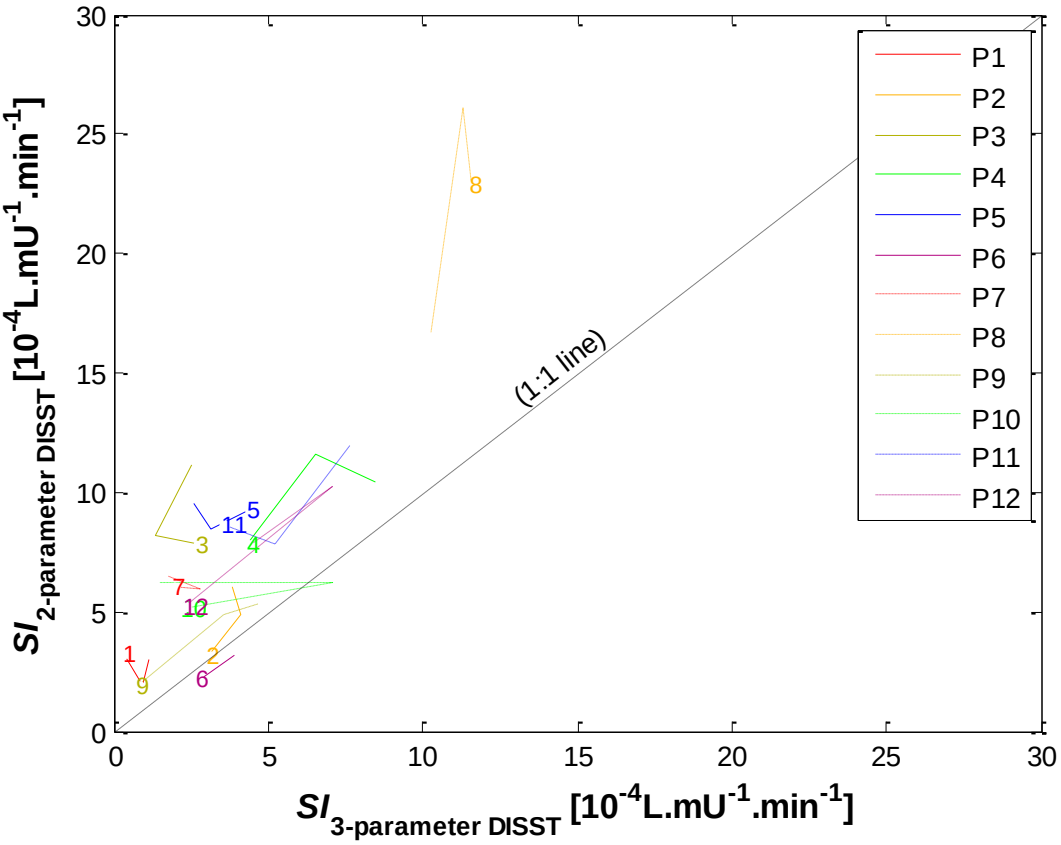

Figure 5.2: Relationships between *SI* values identified from the typical 2-parameter (x = [*SI*, V_G]) and 3-parameter (x = [G_B, *SI*, V_G]) DISST model across all participants and weeks.

Figure 5.4 shows the fitted blood glucose models against the measured glucose data for Participants 2 and 10. It shows the impact of adopting $G_0 = G_B$ in the 2-parameter case and identifying G_B in the 3-parameter case. For Participant 2 in week 12 the identified G_B value was similar to the G_0 value and thus, there was minimal difference in the simulations or *SI* values (left panel vs right panel). In contrast, Participant 10 exhibited a much larger discrepancy between G_B and G_0. Hence, this participant's simulations and *SI* values across models were quite different.

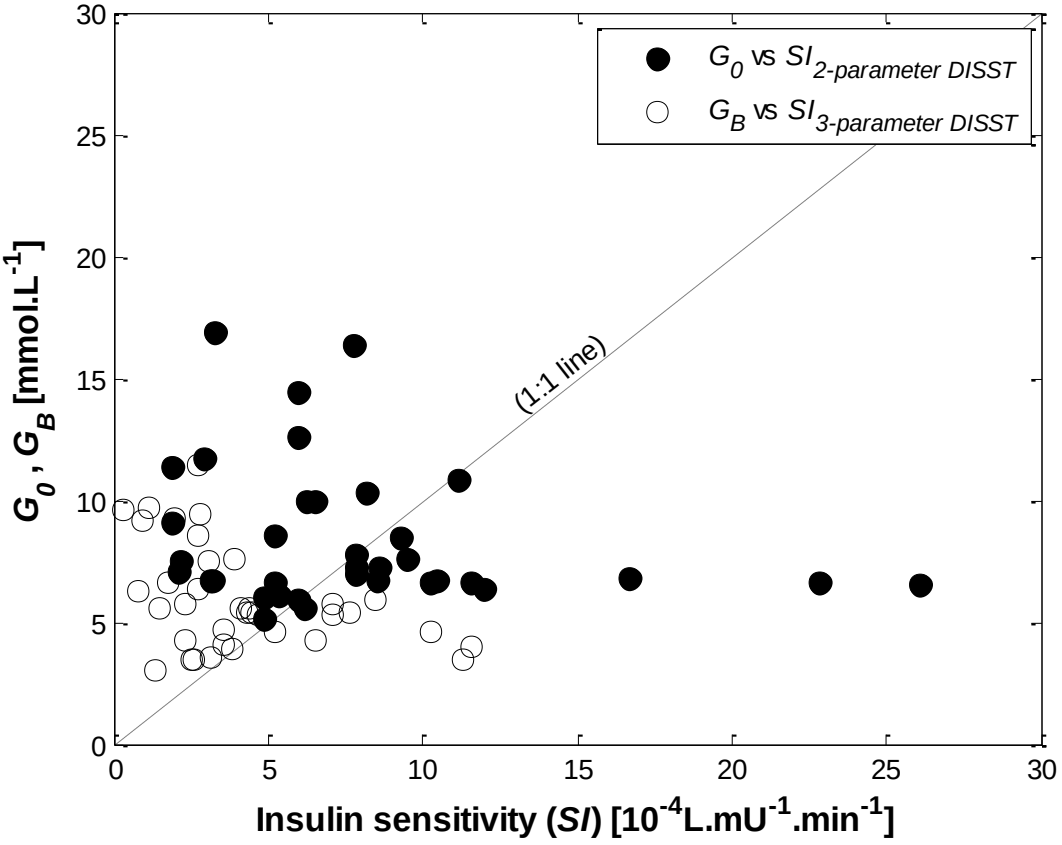

Figure 5.3: Distribution of fasting and identified basal glucose concentration and identified insulin sensitivity between the 2-parameter (x = [SI, V_G]) and 3-parameter (x = [G_B, SI, V_G]) DISST model.

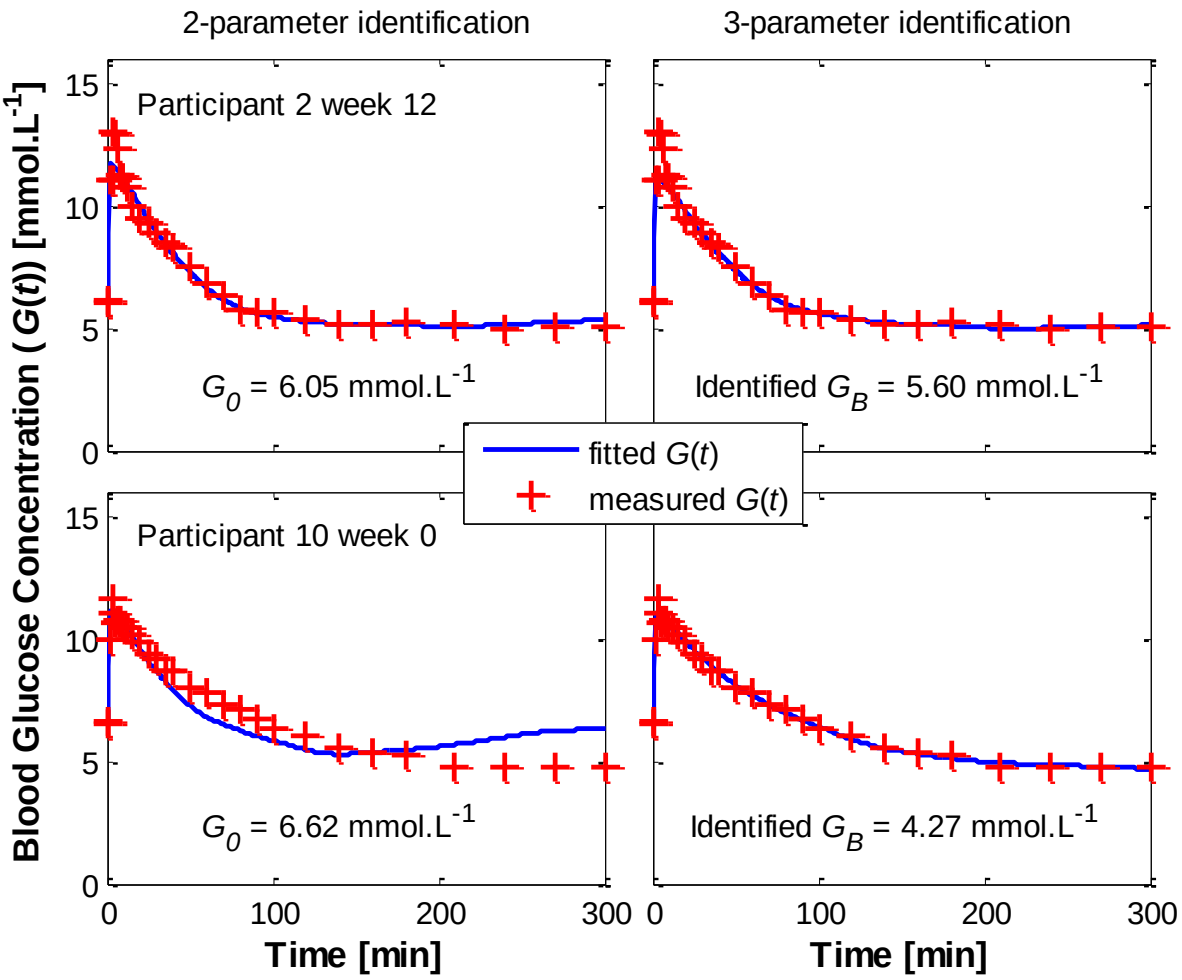

Figure 5.4: Blood glucose participant-specific profile ($G(t)$) for Participants 6 and 10 with typical 2-parameter and 3-parameter identification of the DISST model.

Figure 5.5 shows the discrepancy in *SI* values across the two identification approaches. The bias in *SI* values across the two model approaches was -3.16 x10^{-4}L·mU^{-1}·min^{-1} (IQR: -10.50 to 0.66 x10^{-4}L·mU^{-1}·min^{-1}).

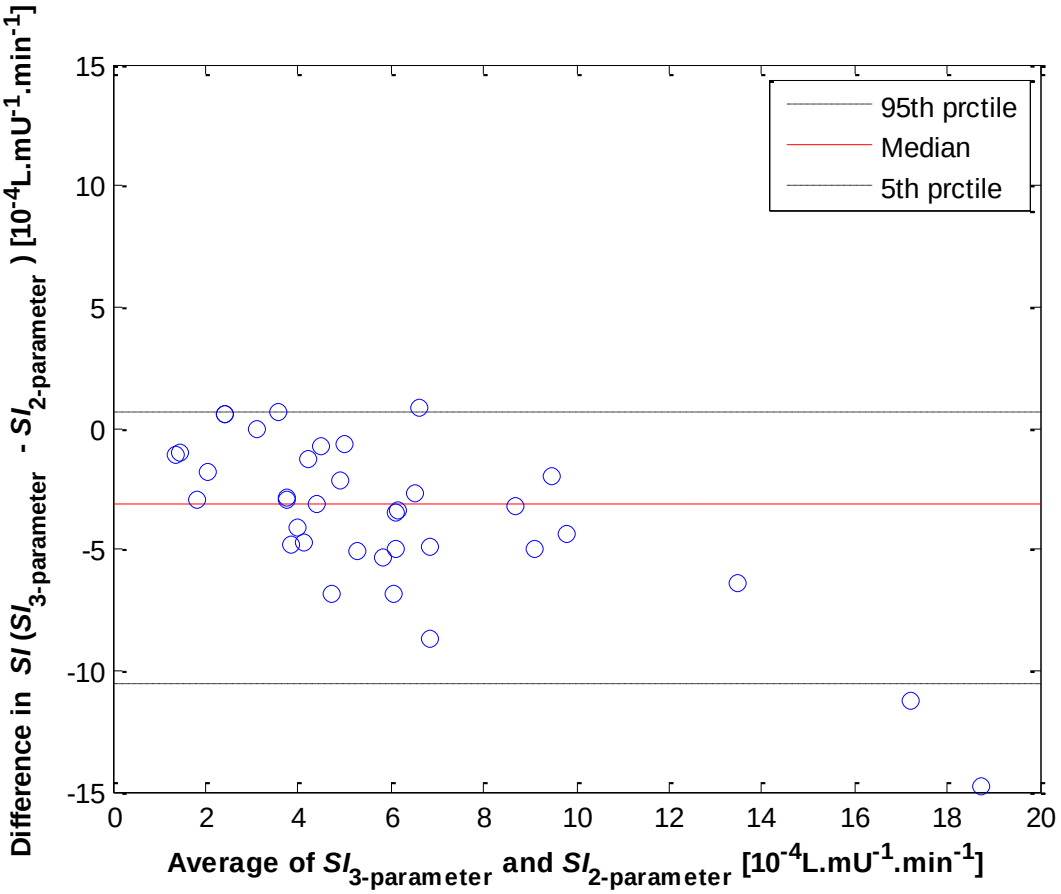

Figure 5.5: Bland Altman plot of insulin sensitivity values from 2- and 3- parameter identification approaches of DISST model.

5.5 Discussion

Typical model-based identification of *SI* sets G_0 as the basal blood glucose level, G_B, that the glucose level tends towards (Bergman *et al*. 1979b; Bergman *et al*. 1981; Bergman *et al*. 1987; Boston *et al*. 2003; Caumo *et al*. 1999; Docherty 2011; Lotz 2007; Lotz *et al*. 2010; Lotz *et al*. 2008; McAuley *et al*. 2011). However, this study finds a significant distinction between fasting G_0 and "set-point" G_B glucose concentration for these insulin resistant participants. The results suggest that G_B should be identified as a variable during the modelling of glycaemic data from individuals with type 2 diabetes, when using the DISST or similar

model. Furthermore, the findings indicate a possible mechanism of dysfunction for individuals with longer term established type 2 diabetes.

Figure 5.3 shows the impact of G_0 and G_B in assessing the *SI* value. In broad terms, when the model is fit to the clinical data, increasing G_B is compensated by higher *SI* values. Hence, setting $G_B = G_0$, where this analysis finds $G_B < G_0$ ($p < 0.001$), will yield higher *SI* values. The typical model thus effectively captures a low glucose level as an effect of *SI*, rather than (a lower than assumed) G_B.

Identification of G_B thus proves crucial in identifying a more representative value of *SI* for this cohort using this model. In particular, for this insulin resistant cohort, use of a lumped parameter *SI,* that is representative of both peripheral and hepatic insulin sensitivity, is best modelled in conjunction with G_B as an identified variable. However, there was no evidence to support the necessity of identifying G_B in healthy cohorts, where the assumption of $G_B = G_0$ by other model-based identification of *SI* is much more accurate (Beard *et al*. 1986; Pacini *et al*. 1998).

Figure 5.4 shows blood glucose profiles ($G(t)$) fitted by the two identification approaches. As Participant 10 has $G_0 > G_B$, the fit-to-data is much better for the 3-parameter identification (Median absolute residual error (RE) = 0.0800 mmol·L^{-1}) than the 2-parameter case (RE = 0.5233 mmol·L^{-1}) where the RE was ~7× larger. Hence, it is clear that assuming $G_B = G_0$ in the model has not captured the behaviour of this dataset particularly well, and the identified model with the 2-parameter approach is thus not fully representative of the patient state.

However, if $G_0 \approx G_B$ as in Participant 2, the fitted $G(t)$ profiles from both models were well calibrated against the measured glucose data. The RE of fitted ($G(t)$) profile and measured glucose data is 0.1657 mmol·L^{-1} compared to 0.1350 mmol·L^{-1} for the 2- and 3-parameter cases, respectively. This result implies that, when the $G_0 \approx G_B$ the adapted model identification approach provides minimal benefit and no impediment and thus, the *SI* values across the approaches are equivalent. However, it is important to note, that one does not know if $G_0 \approx G_B$ until after the 3-parameter identification is performed. Thus, the approach should be used in either case.

Table 5.1 shows that while most participants had elevated G_0 glucose levels, G_B was identified closer to a normal reference range of 4-5.6 mmol·L^{-1} (ADA). In particular, 14 of the 36 G_B values (38.9%) identified were in the normal range, while only 2 of 36 G_0 values (5.6%) were in that range. Figure 5.1 shows that G_0 was typically higher than identified G_B, with only 4 exceptions over 36 results (11.1%). This result implies that most individuals with type 2 diabetes have a lower set point G_B level than assumed by G_0 and would thus attain lower glucose levels if sufficient exogenous insulin and/or longer-term fasting was introduced (Ciampolini *et al*. 2010).

The Bland Altman plot in Figure 5.5 implies that the bias is not equal to a constant value of -3.16 x10^{-4}. A random variation of data around slope of -0.64 can be clearly seen. However, it can be said that, the *SI* value identified by 3-parameter is significantly lower compare to 2-parameter identification approach particularly at *SI* value > 10 ×10^{-4} L·mU^{-1}·min^{-1}. However, this figure is well affected by Participant 8. *SI* values for Participant 8 across the clinical study in Table 5.1 shows that while 2-parameter approach identified *SI* in wider range of 16 –

26×10^{-4} $L\cdot mU^{-1}\cdot min^{-1}$, the 3-parameter approach identified in much narrow range of 10 – 12×10^{-4} $L\cdot mU^{-1}\cdot min^{-1}$. This indicates an inconsistent value of *SI* will be identified when adopting the assumption $G_0 = G_B$ particularly for established T2D participant.

Throughout the study, Participant 8 maintained relatively high values of *SI* compared to other participants. This participant had been diagnosed with diabetes 7 years prior to the study. The high values of *SI* imply that their diabetes is predominantly driven by an inability to produce sufficient insulin. This lack of insulin ultimately led to high fasting glucose levels (G_0 in Table 5.1, Figure 5.1). However, when increased levels of insulin were introduced by the IM-IVGTT, the participant's glycaemic response tended towards a value much lower than the fasting value of G_0 and towards the identified $G_B \approx 4 mmol\cdot L^{-1}$ and $G_B < G_0$ that was found for that participant.

Table 5.1 shows that Participant 10 experienced a modest weight loss between week 0 and week 12 (0.8%) that was concurrent with an improved in *SI* value. However, after week 12 the participant failed to comply the diet and gained 4.2% body weight over the second period. Hence, the *SI* of Participant 10 fell between week 12 and week 24. This result aligns with the general trend of improved *SI* due to weight loss (Camastra *et al*. 2005; Ferrannini *et al*. 2005). Throughout the study, Participant 10 had consistent identified G_B values within the healthy range. In contrast, their G_0 values at week 0 and 12 were slightly elevated, and the week 24 G_0 was a highly elevated 9.92 $mmol\cdot L^{-1}$. This result shows that while the change in diet had a quick effect on the fasting glucose, the G_B remained healthy throughout the intervention. Hence, the adapted 3-parameter model identification approach captured an underlying and important aspect of this participant's metabolic response.

Participant 11 achieved improvements in every aspect as a result of the intervention. In particular, their body weight dropped, *SI* improved, and G_0 improved. Changes in G_B were minimal for this participant but $G_B < G_0$ was true throughout the intervention. However, by identifying G_B, the *SI* values of this participant were altered. Inspection of the *SI* values from the simple 2-parameter identification of the DISST model does not exhibit the expected improvement in insulin sensitivity for this participant. Thus, setting $G_B = G_0$ potentially obscured improvements in *SI* that were elucidated by the 3-parameter model identification.

Glycaemic responses of healthy individuals tend toward the fasting level. Nine of the 12 participants had glycaemic responses that tended towards a healthy basal G_B value, despite all participants having impaired fasting glucose at week 0. Two of the 3 individuals (Participants 1, 3, 7) that had elevated identified G_B values had long-term diabetes. These latter results may imply that the basal set point of glycaemia could be a late onset dysfunction of the disease – although greater participant numbers would be required to confirm this outcome.

Figure 5.2 shows that the identified *SI* values from the 3-parameter identification are significantly lower than for the typical 2-parameter identification, with only 4 exceptions over 36 results (11.1%). Although, there is no accepted range of *SI* values used to diagnose participants with T2D, the 3-parameter model identified values in the range of 2 - 4×10^{-4} $L\cdot mU^{-1}\cdot min^{-1}$ are more in line with previous findings (Lotz 2007). This outcome shows that when G_B is not an identified variable, the participants' glycaemic dynamics are potentially wrongly attributed to *SI*, and that identification of G_B provides a more clinically representative result.

5.6 Summary

Identifying G_B, SI and V_G as model variables in a 3-parameter identification allows greater descriptive ability of the metabolism of individuals with type 2 diabetes and is thus a necessary modification for using the DISST model for this cohort, and in general since no accuracy is last when $G_B \approx G_0$. The assumption of $G_B = G_0$ effects the typical 2-parameter identification and results by leading to potentially erroneous SI values or SI changes from an intervention. This analysis has shown that it is important to model the basal glucose value as a variable when assessing the SI value for individuals with type 2 diabetes, and presented a new effective 3-parameter identification approach to accomplish that task.

Chapter 6. Model-based identification of endogenous insulin secretion

This chapter discusses the development of a model to identify patient-specific U_N parameters. The modelling approach will link U_N to changes in glucose concentration in a feedback control modelling framework. In contrast, U_N profiles are typically derived via direct inversion or deconvolution of interpolated measured values of C-peptide concentration.

6.1 Introduction

Although the pathogenesis of type 2 diabetes varies, the common route includes failure of pancreatic β-cells to compensate for *IR* (Breda *et al*. 2002; Ferrannini 1997; Kahn 1998; Mari *et al*. 2002; Pories & Dohm 2012). The inability to produce sufficient insulin to meet demand results in elevated glucose concentrations. However, this elevation in glucose concentration does not occur until the insulin demand exceeds the maximal possible insulin secretion rate, which only arrives in the later stages of the pathogenesis of type 2 diabetes and well after initial pathological changes in U_N has occurred (Ferrannini 1997; Pories & Dohm 2012). Thus, diagnosis based solely on *IR* can miss the early stages of dysfunction.

Thus, measuring endogenous insulin secretion may enable earlier diagnosis of metabolic dysfunction, long before elevated glucose occurs, which in turn would allow earlier, possibly

more effective intervention. Many studies have been conducted to determine the best technique for identifying the participant's glycemic condition by measuring their insulin secretion and insulin sensitivity (Albareda *et al*. 2000; Bergman *et al*. 2002b; Lotz *et al*. 2010; Mari *et al*. 2005; McAuley *et al*. 2007). Unlike *SI*, there is no gold standard for the measurement of β-cell function or U_N. Thus, most secretion studies use deconvolution of C-peptide concentration measurements to identify participant-specific U_N profiles (Eaton *et al*. 1980; Polonsky *et al*. 1986; Van Cauter *et al*. 1992), which is a de-facto standard approach.

The deconvolution method is effective due to the fact that insulin and C-peptide are co-secreted in an equimolar fashion from the β-cells in the pancreas (Rubenstein *et al*. 1969). While the pharmacokinetics of insulin are complex, the pharmacokinetics of C-peptide are much simpler. Hence, the process of identifying U_N via C-peptide concentration is crucial. In particular, insulin undergoes a substantial first pass hepatic extraction before reaching the peripheral circulation. In addition, clearance via glucose uptake in the cells is another clearance route that is highly variable. This extraction inhibits the modelling of U_N directly from insulin measurements due to the added, unknown variability and may lead to misidentification of U_N (Hovorka & Jones 1994; Polonsky & Rubenstein 1986). Thus, the single, well defined kidney clearance of C-peptide provides a more suitable basis for estimating U_N.

This chapter presents a novel PD control model of U_N to estimate participant-specific U_N profiles, as opposed to identification based on a deconvolution approach, which relies on frequent, costly C-peptide sampling to capture all the dynamics. The PD control U_N model is developed to provide an estimation of participant-specific U_N profiles based on a

physiological model of human body's closed-loop system in controlling the set of rate of insulin secretion in respond to glucose concentration. It also allows for more sparse C-peptide sampling by providing smooth, physiologically relevant dynamics between measurements.

6.2 Existing deconvolution approach

As the concentration of glucose in bloodstream rises, it triggers a response to the β-cell in the islet of Langerhans within the pancreas (Figure 6.1) to produce and secrete insulin (Cherrington 1999). The release of insulin expedites glucose uptake from blood stream into the tissue cells. This endogenous insulin secretion (U_N) plays a key role in regulating blood glucose concentration to a normal, healthy level of 4 – 5.6 mmol.L^{-1} (ADA 2014).

Prior studies have shown that insulin is formed from proinsulin, a single-chain polypeptide precursor (Steiner *et al*. 1967; Steiner & Oyer 1967). However, like insulin, C-peptide, is also produced by the pancreatic β-cells (Despopoulos & Silbernagl 2003; Guyton & Hall 2006). In addition, C-peptide is also originated from the proinsulin and is recognised as a by-product of insulin (Chevenne *et al*. 1999).

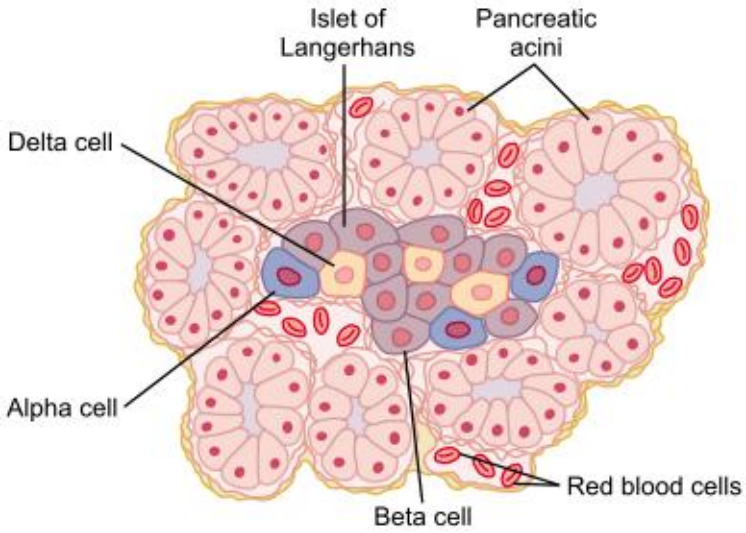

Figure 6.1: Physiologic anatomy of an islet of Langerhans in the pancreas. Adapted from (Guyton & Hall 2006).

Figure 6.2 illustrates the 86-amino-acid precursor proinsulin. This precursor proinsulin is cleaved internally to yield C-peptide (31 amino acids) and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulfide bonds (Chevenne *et al*. 1999). Thus, both insulin and C-peptide are secreted in equimolar amounts (Rubenstein *et al*. 1969). C-peptide has a lower clearance rate than plasma insulin due to having fewer, less variable clearance routes (Rubenstein *et al*. 1969).

In particular, the fact that C-peptide is only cleared by the kidney and not degraded in the liver or tissues, means that it can provide valuable information of endogenous insulin secretion via C-peptide models. Thus, by exploiting particular mathematical models of insulin kinetics, glucose-insulin dynamics and C-peptide kinetics, models can be used to provide a direct estimate of pancreatic β-cell insulin secretion and also of hepatic insulin extraction (Mari *et al*. 2002; Pacini & Mari 2003; Polonsky *et al*. 1986; Watanabe *et al*. 1989).

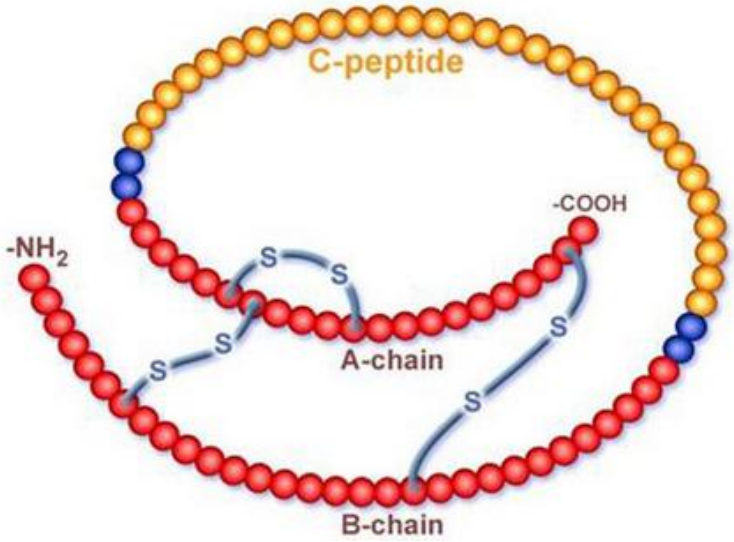

Figure 6.2: Schematic representation of human proinsulin. (Figure taken from cebix.com/index.php/science/c-peptide-biology).

The DISST provides quantitative measures of a participant-specific *SI* value and U_N profile (Lotz 2007; Lotz *et al*. 2010; McAuley *et al*. 2011; McAuley *et al*. 2007). The physiological model used in the DISST is illustrated in Figure 6.3. The DISST *SI* value is highly correlated to the EIC (R = 0.82), and the test can contrast U_N characteristics across patient groups with different levels of *IR* (McAuley *et al*. 2011). The DISST uses the endogenous insulin secretion 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), which is widely used in the field.

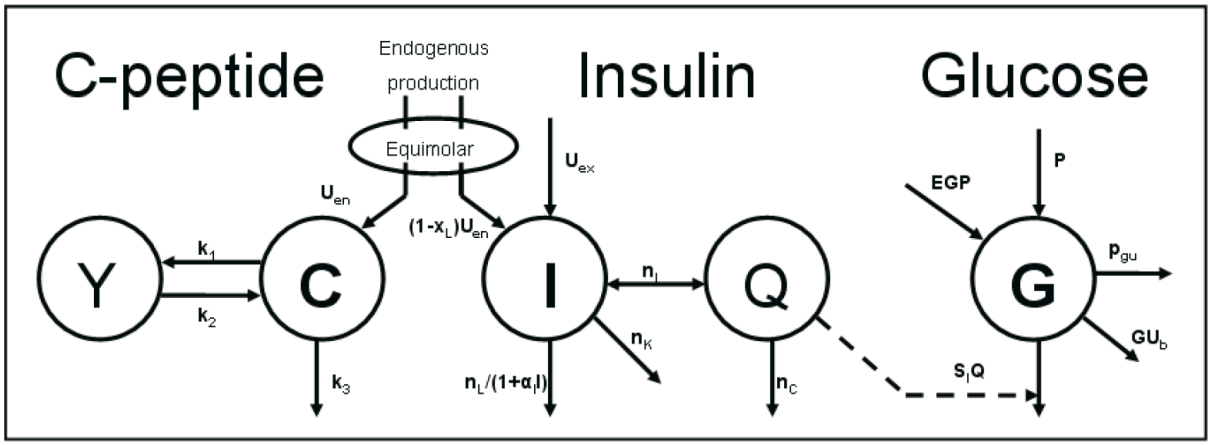

Figure 6.3: The physiological model of DISST. Adapted from (Docherty *et al*. 2009).

The DISST model describes the U_N profile from the deconvolution of C-peptide concentration (Lotz *et al*. 2010; McAuley *et al*. 2011):

$$\dot{C} = -(k_1 + k_3)C + k_2 Y + \frac{U_N}{V_p} \tag{6.1}$$

$$\dot{Y} = -k_2 Y + k_1 C \tag{6.2}$$

where C and Y are the plasma and interstitial C-peptide concentrations [pmol·L^{-1}] respectively; k_1, k_2 and k_3 are the C-peptide transport rate constants [min^{-1}]; U_N is the endogenous insulin secretion [mU·min^{-1}] with a conversion factor between pmol and mU of 6.94 pmol·mU^{-1} and V_p is the volume of plasma distribution [L].

Table 6.1 shows the derivation of the rate constants of k_1, k_2 and k_3 and V_p that are defined *a-priori* based anatomical functions, as proposed by Van Cauter *et al.* (Van Cauter *et al*. 1992).

Table 6.1: *A-priori* identification of C-peptide kinetics parameters.

Steps:	Normal	Obese	T2D
1. Short half-life ($t_{1/2}$-short) [min]	4.95	4.55	4.52
2. Fraction (*F*)	0.76	0.78	0.78
3. Long half-life ($t_{1/2}$-long) [min]	$t_{1/2} - long = 0.14 \times age[year] + 29.2$		
4. C-peptide transport rates constants (k_1, k_2 and k_3)	$k_2 = F \times (b - a) + a$ $k_3 = \frac{ab}{k_2}$ $k_1 = a + b - k_2 - k_3$ where $a = \frac{log_{10}(2)}{t_{1/2}-short}$ and $b = \frac{log_{10}(2)}{t_{1/2}-long}$		
5. Plasma distribution volume (V_p)	If male: $V_p = 1.11 \times BSA + 0.64$ If female: $V_p = 1.11 \times BSA + 2.04$ where body surface area (BSA) is defined as: $BSA = \sqrt[2]{\frac{weight\ [kg] \times height\ [m]}{36}}$		

The DISST uses the integral-based estimation method to identify U_N (Hann *et al*. 2005). Initially, the interstitial C-peptide concentration is determined using the analytical solution of Equation 6.2 and a linear interpolation between the measured C-peptide data:

$$Y_t = k_1 \int_0^t C_{interp} e^{-k_2(t-\tau)} d\tau \tag{6.3}$$

where C_{interp} represents the linear interpolated of measured C-peptide values.

Later information of *Y* is incorporated into the integral of Equation 6.1:

$$\int_{t_0}^{t_1} \dot{C}dt = -(k_1 + k_3)\int_{t_0}^{t_1} Cdt + k_2\int_{t_0}^{t_1} Y_t dt + \int_{t_0}^{t_1} \frac{U_N}{V_p}dt \tag{6.4}$$

Rearranging known parameters yields:

$$\frac{U_N(t_1) - U_N(t_0)}{V_p} = C_{t_1} - C_{t_0} + (k_1 + k_3)\int_{t_0}^{t_1} Cdt - k_2\int_{t_0}^{t_1} Y_t dt \tag{6.5}$$

Then, U_N was defined using Equation 6.5 at a 1-minute resolution between *t*=0 and *t*=end, by interpolating between more sparsely measured C-peptide points.

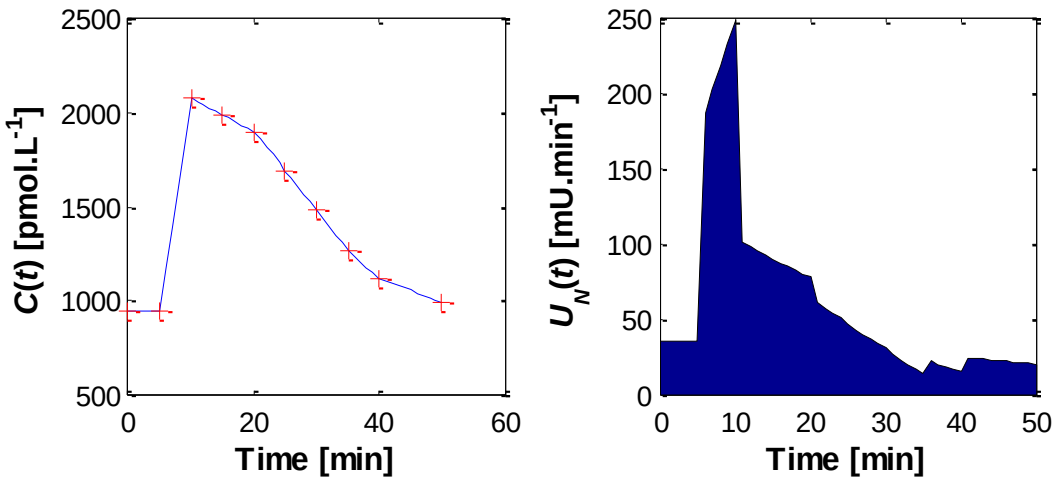

Figure 6.4: Measured C-peptide concentration and U_N profile identified by DISST model.

Figure 6.4 shows that the estimated participant-specific U_N profile from deconvoluted C-peptide concentrations. This estimation can thus be used to provide an indication of the

secretion of insulin. In specific cohorts it can then be used to gain insight into the pathophysiology of type 2 diabetes.

6.3 U_N model

Normally, insulin is secreted by the pancreatic β-cells in response to sudden or gradual increases in blood glucose level. The amount of insulin secreted is dependent on the glucose level that needs to be lowered to a normal range value of 4 – 5.6 mmol·L^{-1}. Although, there is no gold standard in identifying insulin secretion, the insulin secretion identification method validated by Van Cauter *et al.* (Van Cauter *et al*. 1992) has been widely used by many leading insulin sensitivity research groups (Bock *et al*. 2006; Ferrannini & Mari 2004; Jones *et al*. 1997; Mari 1998). However, this model does not account for the pharmacodynamic reaction on the β-cells to glucose.

Secreted insulin can be quantified by 3 metrics: 1) basal endogenous insulin production (U_B); 2) first phase insulin production (U_1); and 3) second phase insulin production (U_2) (Lotz *et al*. 2010; McAuley *et al*. 2011). U_B is defined as the insulin required by an individual to maintain a constant fasting glucose measurement. U_1 quantifies the dependence of insulin secretion on the positive rate of change of glucose concentration. Finally, in contrast to U_1, U_2 is quantifies the U_N reaction to the glucose concentration over the basal glucose concentration at steady state.

In modelling terms, the regulation of blood glucose by insulin secretion is controlled by a physiological closed-loop feedback-control system (Cherrington 1999). Hence, a nonlinear

PD model is proposed to identify the U_N profile based on blood glucose behaviour. The proposed PD control U_N model estimates the endogenous insulin secretion as a function of increasing glucose (derivative control, ϕ_D) and glucose above basal (proportional control, ϕ_P):

$$U_N = U_B + \phi_P(G - G_B) + \phi_D\langle\dot{G}\rangle \tag{6.6}$$

where U_N is the modelled endogenous insulin secretion [mU·min^{-1}]; U_B is basal insulin [mU·min^{-1}]; G and G_B is glucose and basal glucose concentration, respectively [mmol·L^{-1}]; ϕ_P and ϕ_D are the proportional, and derivative gains (mU·L·mmol^{-1}·min^{-1} and mU·L·mmol^{-1}, respectively). Finally, it is important to note that $\langle\dot{G}\rangle$ indicates the negative rate of change in glucose is equal to zero, adding a nonlinear component that is physiologically relevant.

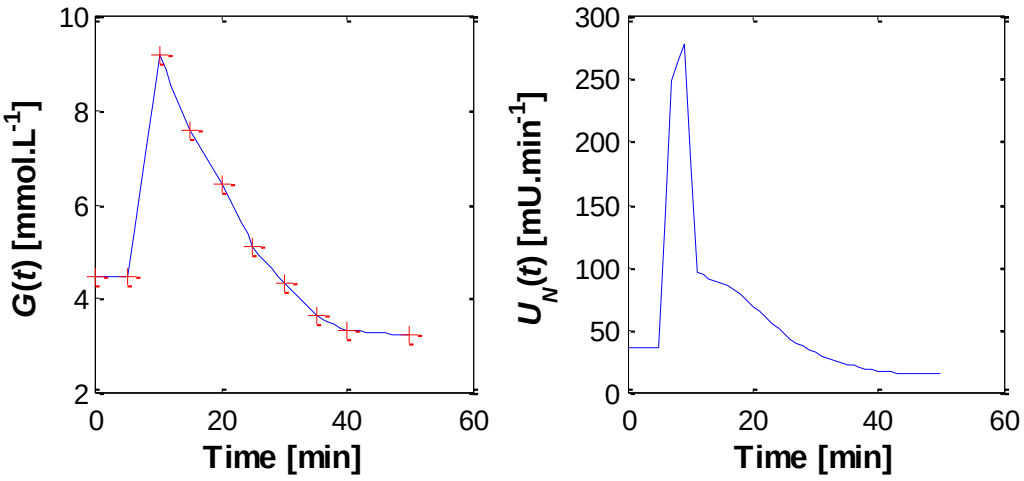

Figure 6.5: Measured glucose concentration and U_N profile identified from the PD U_N model.

Figure 6.5 shows the example of a U_N profile identified from PD U_N model. The proposed U_N model directly links insulin secretion to glucose concentration. The derivative gain (ϕ_D), determines the first phase (U_1) of U_N as a function of positive glucose gradient. Identifying the first phase insulin secretion is crucial as prior studies have shown that loss of first phase

insulin secretion is an independent predictor of T2D (Bunt *et al*. 2007; Del Prato & Tiengo 2001; Pratley & Weyer 2001; Vranic *et al*. 1971; Weyer *et al*. 1999). The proportional gain (ϕ_P) determines the second phase (U_2) of U_N and is thus, an important characteristic in the prediabetic state (McAuley *et al*. 2011; Pories & Dohm 2012).

A similar control approach has been applied previously by Cobelli *et al.* and Ferrannini *et al.* (Breda *et al*. 2001; Dalla Man *et al*. 2010; Mari *et al*. 2002; Toffolo *et al*. 2001). It has also been used by Doran *et al* but with fixed PD gains (Chase *et al*. 2003). However, the proposed PD U_N model offers model simplicity compared to previous models. The PD U_N model allows a direct interpretation of physiological pattern of β-cell functions to glucose excursions in a simple, readily identifiable model. A rise in glucose level can be easily captured by the derivative gain whereas the proportional gain provide valuable information of U_N in suppressing the glucose level to a normal, healthy level.

6.4 Summary

The proposed U_N model is based on the physiological closed-loop control of insulin secretion in response to increasing glucose (derivative control, ϕ_D) and glucose above basal (proportional control, ϕ_P). By defining the model-based U_N profiles as dependent on glucose levels, the modelling approach is more physiologically representative. Such a model might be particularly advantageous where samples were infrequent and deconvolution could not provide good resolution, by providing a structured continuous approximation to fit available data.

Chapter 7. Development of a proportional-derivative control model for the endogenous insulin secretion response to glucose

This chapter presents a simple PD control model to define insulin secretion as a function of increasing glucose rate, derivative control, ϕ_D, and glucose level above basal, proportional control, ϕ_P.

7.1 Introduction

Insulin is secreted by pancreatic β cells to maintain normoglycemia. Impaired U_N contributes to metabolic disorders, such as glucose intolerance or hyperglycemia. Hyperglycemia, if left untreated, ultimately leads to T2D. Understanding U_N secretion characteristics is thus a critical aspect of characterizing this metabolic disorder (Ferrannini *et al*. 2005; Pacini & Mari 2003).

Assessing insulin secretion through mathematical modelling received considerable attention during the 1970s (Bergman & Urquhart 1971; Cerasi *et al*. 1974; Grodsky 1972). Unlike insulin sensitivity (*SI*) (Defronzo *et al*. 1979), there is no gold standard for the measurement of β cell function or U_N. However, modelling insulin secretion as a function of peripheral C-peptide levels by mathematical deconvolution is a widespread approach (Eaton *et al*. 1980; Van Cauter *et al*. 1992). This method proves more accurate than direct measurement of

insulin levels. In particular, insulin and C-peptide are co-secreted at equimolar rates from β cells (Rubenstein *et al*. 1969). However, the rate of insulin clearance is more variable than the rate of C-peptide clearance due to its greater number and variability of clearance routes.

Relationships between insulin sensitivity and insulin secretion have been defined by a number of previous studies (e.g. (Bergman *et al*. 2002a; Bergman *et al*. 1981; Cobelli *et al*. 2007; Cretti *et al*. 2001; Docherty *et al*.)). The IVGTT with minimal model has been the most frequently used model-based approach (Breda & Cobelli 2001; Toffolo *et al*. 1999). However, the minimal model is known to produce ambiguous *SI* values and erratic correlation with the gold standard of *SI* measurement, EIC (Pillonetto *et al*. 2002; Saad *et al*. 1994), particularly for those with low *SI* (higher *IR*) (Pillonetto *et al*. 2002; Quon *et al*. 1994). The DISST provides a model-based *SI* metric that is highly correlated to the ISI metric from the EIC with R=0.82 (McAuley *et al*. 2011). The DISST also provides quantitative measures of U_N via deconvolution of C-peptide data (Lotz *et al*. 2010), yielding significant added diagnostic insight.

The physiological model used to evaluate DISST test data typically uses a deconvolution of measured C-peptide data to generate a participant specific U_N profile. However, it is known that the body uses closed-loop, feedback-control to set the rate of insulin secretion in response to glucose and insulin concentrations (Cherrington 1999). Hence, this chapter discusses a simple PD control model that defines insulin secretion as a function of rate of change of glucose, derivative control, and glucose level above basal, proportional control. Understanding the discriminatory ability of these U_N gains relative to metabolic status may

enable greater insight into the etiology of type 2 diabetes and the metabolic syndrome than deconvolved U_N secretion rates alone.

7.2 Methods

7.2.1 Participants and Data

A total of 94 female participants were recruited from the Otago region of New Zealand to take part in a 10-week dietary intervention trial. Full trial details are available in Te Morenga *et al* (2010). Inclusion criteria required a body mass index (BMI) greater than 25, or BMI > 23 and a family history of T2D, or ethnic disposition toward T2D. Participants were excluded if they had a major illness, including established diabetes, at the time of testing. In total, 68 participants provided 204 full test DISST data sets at week 0, week 4 and week 10 of the intervention. All participants had their age and BMI recorded (median [IQR]; 42.5 [34.5, 50.5] and 32.34 [27.92, 36.94], respectively).

7.2.2 Clinical procedure

Participants reported in the morning after an overnight fast. Each participant had a cannula inserted in the ante-cubital fossa, a vein in inner elbow, for blood sampling and administration of glucose and insulin boluses. Blood samples were drawn at t=0, 5, 10, 15, 20, 25, 30, 35, 40 and 50 minutes. A 10g IV glucose bolus (50% dextrose and 50% normal saline) was administered intravenously at t=6 minutes. A 1U IV insulin bolus was administered intravenously at t=16 minutes. Blood samples were assayed for plasma glucose (Enzymatic glucose hexokinase assay, Abbot Labs, Illinois USA), insulin and C-

peptide concentration (ELISA Immunoassay, Roche, Mannheim, Germany). All experimental procedures were approved by the University of Otago Human Ethics Committee.

7.2.3 Physiological models

7.2.3.1 DISST model

The DISST provides quantitative measures of both *SI* and the U_N profile (Lotz *et al*. 2010; McAuley *et al*. 2011; McAuley *et al*. 2007), and is similar to the insulin modified IVGTT, which uses alternative measurement and dosing, as well as a typical modelling approach (Bergman *et al*. 1979a; Ward *et al*. 2001). The DISST model identifies the U_N profile via the deconvolution of C-peptide assays (Van Cauter *et al*. 1992). A full detailed description of the DISST model was well discussed in Chapter 3, Section 3.2.

7.2.3.2 Proportional-derivative (PD) endogenous insulin secretion (U_N) model

The body uses closed-loop, feedback-control to set the rate of endogenous insulin secretion in response to glucose and insulin concentrations to maintain glucose homeostasis (Cherrington 1999). The proposed PD controller defines a patient-specific U_N profile as a function of the rate of change of glucose, derivative control, ϕ_D, and glucose level above basal, proportional control, ϕ_P. The proposed PD control model is well defined in Chapter 6, Section 6.3.

U_B is derived from Equations 3.1 and 3.2 (in Chapter 3, Section 3.2), assuming a steady state at t = 0 minutes:

$$U_B = k_3 C_0 V_p \tag{7.1}$$

where C_0 denotes the C-peptide measured at $t = 0$.

7.2.4 Parameter identification

Most *a-priori* parameters are quantified as functions of the participant anatomical characteristics (weight, height, sex, age) defined by Van Cauter *et al.* (Van Cauter *et al*. 1992). The DISST methodology typically sets p_{gu} as a constant equal to 0.004 min^{-1} (Lotz *et al*. 2010).

A 7 parameter identification approach adapting the Gauss Newton method is developed to identify the participant-specific parameters of G_B, SI, V_G, ϕ_P, ϕ_D, n_L and x_L from the measured data. The function is defined:

$$\mathbf{x}_{i+1} = \mathbf{x}_i - (\mathbf{J}^\mathbf{T}\mathbf{J})^{-1}\mathbf{J}^\mathbf{T}\boldsymbol{\psi} \tag{7.2}$$

where $\mathbf{x}_i = \left[G_{B_i}, SI_i, V_{G_i}, \phi_{D_i}, \phi_{P_i}, n_{L_i}, x_{L_i}\right]$ is the parameter vector and i is the iteration number. The Jacobian matrix (**J**) and the residual matrix ($\boldsymbol{\psi}$) are defined:

$$\mathbf{J}(\mathbf{x}_i) = \begin{bmatrix} \frac{\delta\psi_1}{\delta G_B} & \frac{\delta\psi_1}{\delta SI} & \cdots & \frac{\delta\psi_1}{\delta x_L} \\ \frac{\delta\psi_2}{\delta G_B} & \frac{\delta\psi_2}{\delta SI} & \cdots & \frac{\delta\psi_2}{\delta x_L} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\delta\psi_n}{\delta G_B} & \frac{\delta\psi_n}{\delta SI} & \cdots & \frac{\delta\psi_n}{\delta x_L} \end{bmatrix}$$

$$\boldsymbol{\psi}(\mathbf{x}_i) = \begin{bmatrix} \left(G(\mathbf{x}_i, t_1) - G_{M,1}\right)/\overline{G_M} \\ \vdots \\ \left(G(\mathbf{x}_i, t_n) - G_{M,n}\right)/\overline{G_M} \\ \left(C(\mathbf{x}_i, t_1) - C_{M,1}\right)/\overline{C_M} \\ \vdots \\ \left(C(\mathbf{x}_i, t_n) - C_{M,n}\right)/\overline{C_M} \\ \left(I(\mathbf{x}_i, t_1) - I_{M,1}\right)/\overline{I_M} \\ \vdots \\ \left(I(\mathbf{x}_i, t_n) - I_{M,n}\right)/\overline{I_M} \end{bmatrix} \tag{7.3}$$

where $I(\mathbf{x}_i, t_n)$, $G(\mathbf{x}_i, t_n)$ and $C(\mathbf{x}_i, t_n)$ are the simulated values at $t = t_n$ given $\mathbf{x}_i$; $I_{M,n}$, $G_{M.n}$ and $C_{M,n}$ are the measured values at $t = t_n$; n is the number of measured samples; and $\overline{I_M}$, $\overline{G_M}$ and $\overline{C_M}$ are the mean measured values of these species.

To avoid model misidentification issues, insulin samples taken within 10 minutes of insulin administration and glucose samples taken within 10 minutes of glucose injection in the DISST protocol were ignored in the model identification process to minimize errors introduced by the confounding effects of intravascular mixing (Caumo *et al*. 1999; Edsberg *et al*. 1987; Lotz 2007). The value of V_G was limited to physiological bounds to reduce the effect that incomplete mixing might have during the parameter identification process. In particular, V_G is constrained within the range of $0.12Bw$ to $0.25Bw$ where Bw is measured in kg and the coefficients have units of L·kg^{-1} (Defronzo *et al*. 1979; Ferrannini & Mari 1998; Lotz 2007; Lotz *et al*. 2010).

7.3 Statistical analysis

Model residuals and interpretation of population trends were used to assess the performance of the PD based U_N model by comparing fitted C-peptide versus measured C-peptide values. The residual error of C-peptide determines the performance of the U_N profile of this PD model against the de-convoluted U_N profile, as defined by Equations 7.4 – 7.6.

Mean Residual error of C-peptide (μ) is defined:

$$\mu(t) = \frac{1}{n}\sum \frac{C_{fitted}(t) - C_{measured}(t)}{C_{measured}(t)} \tag{7.4}$$

Standard error of C-peptide (*SE*) is defined:

$$SE(t) = \frac{SD(t)}{\sqrt{n}} \tag{7.5}$$

where standard deviation (*SD*) is defined as:

$$SD = \sqrt{\frac{\sum\left(C_{fitted}(t) - \mu(t)\right)^2}{n}} \tag{7.6}$$

where blood samples were collected at *t* = 0, 5, 10, 15, 20, 25, 30, 35, 40 and 50 min for the measurement of C-peptide and *n* is the number of tests conducted amongst 68 participants.

Correlations were used to describe the relationship between ratio of $\frac{\phi_D}{\phi_P}$ and SI, as well as ϕ_D against ϕ_P. All analysis was undertaken using MATLAB (R2013b, Mathworks, Inc., Natick, MA, USA).

7.4 Results

Figure 7.1 shows the simulated versus measured plasma insulin, glucose, C-peptide and U_N profiles from one typical participant. Note again that the insulin and glucose samples taken within 10 minutes of bolus injection were ignored due to unmodelled mixing effects. In general, using the DISST model with a PD derived U_N model and a Gauss Newton identification method shows that the simulated data fits well against the measured data.

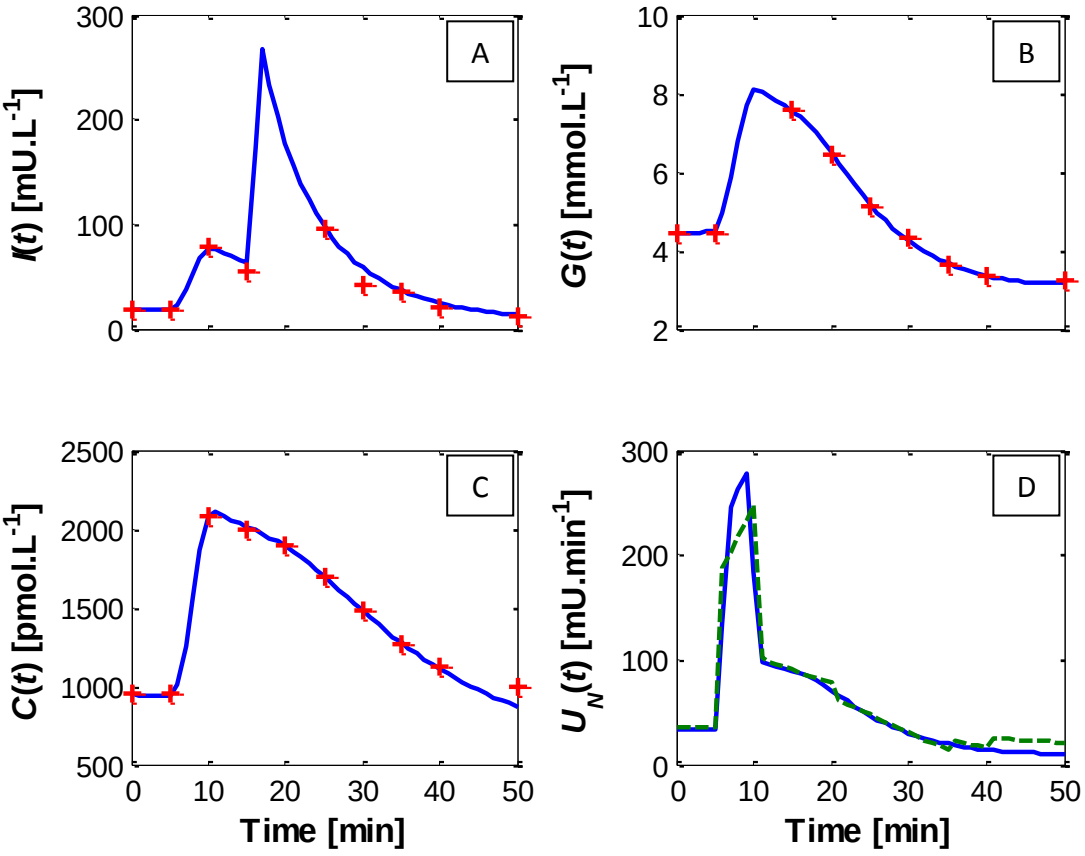

Figure 7.1: Simulated (solid blue line) and measured (red '+' symbol) of; (A) plasma insulin, (B) glucose, (C) C-peptide for a typical participant response to the DISST model, and (D) Endogenous insulin secretion profile identified from the PD modelled U_N model (solid blue line) and from deconvoluted C-peptide measurement (dashed green).

Figure 7.2 shows the residual error between the measured C-peptide data and the response modelled by PD U_N model on Equation 7.1 for all 204 tests. Figure 7.3 shows the dependence of the ratio $\frac{\phi_D}{\phi_P}$ to *SI* with R = 0.33. Figure 7.4 shows the distribution of ϕ_P as a function of ϕ_D with weak correlation of *R* = 0.32. Although the correlation was weak, it elucidates greater information on β-cell functionality in maintaining glucose level in bloodstream.

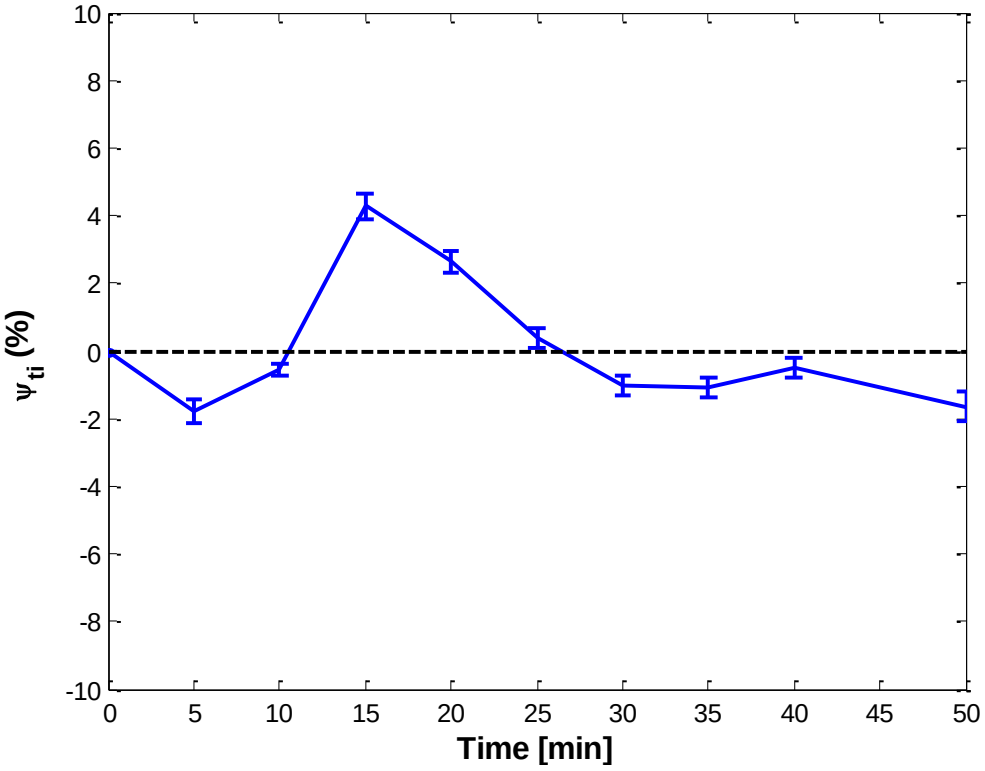

Figure 7.2: Residual error (mean and standard error, $SE = \frac{SD}{\sqrt{N}}$) between the measured C-peptide data and the response modelled by PD U_N model.

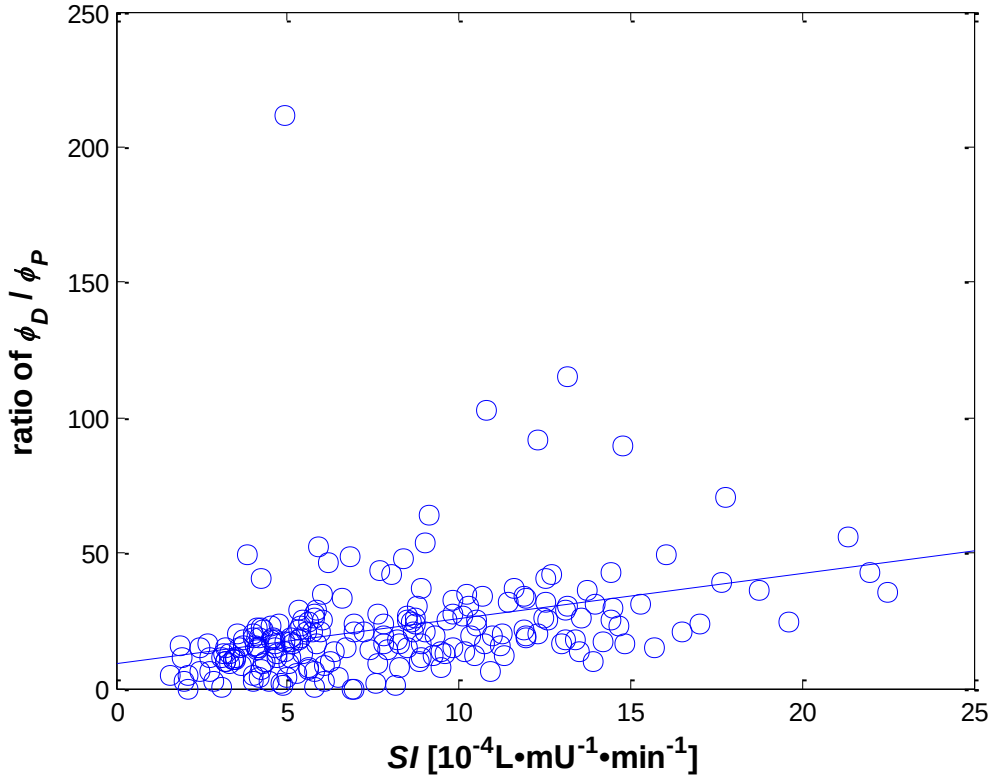

Figure 7.3: Ratio of $\frac{\phi_D}{\phi_P}$ as a function of *SI*, with line defined for R = 0.33.

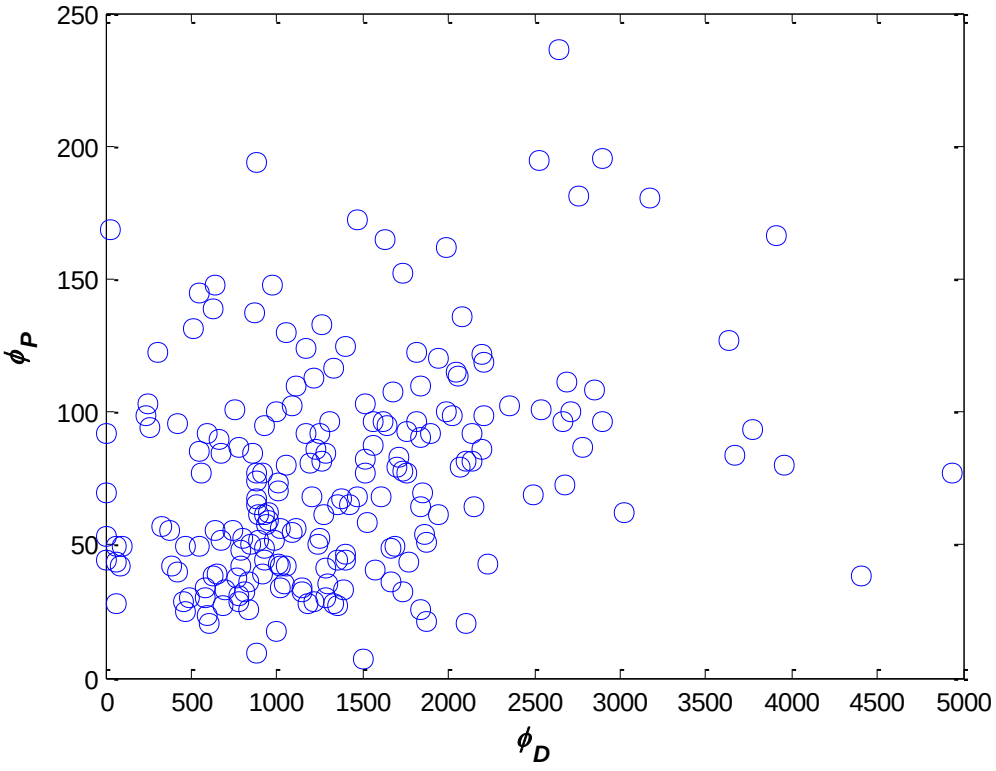

Figure 7.4: Distribution of ϕ_D against ϕ_P for all tests.

7.5 Discussion

The DISST validation study used deconvolution of measured C-peptide data to determine participant-specific U_N profiles (Lotz *et al*. 2010). It clearly showed that similar *SI* values did not necessarily have similar UN profiles. Thus, it was able to differentiate clearly different states in the progressive of T2D.

However, regulation of blood glucose by endogenous insulin is effectively a closed-loop feedback-control system (Cherrington 1999). Secretion responds to level and rate of change of glucose. Hence, a PD model is proposed that mimics this behaviour to identify the U_N profile.

Studies have also shown that the insulin secretory response to glucose is multiphasic in nature (Cerasi 1967; Curry *et al*. 1968). The first phase of insulin is secreted immediately in response to sudden increases in the glucose level. Hence, it causes a rapid elevation of plasma insulin with at peak value achieved within five minutes (Lerner & Porte 1971). The second phase of insulin is slowly and gradually released in response to sustained elevation in plasma glucose (Lerner & Porte 1971).

The PD U_N model developed here defines U_N based on three physiological stages: 1) U_B is the basal endogenous insulin production rate; 2) U_1, the magnitude of the first phase response to glucose appearance; and 3) U_2 is the second phase response to glucose. U_1 is thus, mathematically defined as linearly dependent on the positive rate of change of glucose

concentration. Whereas, the magnitude of the U_2 is defined as proportional function over the basal glucose concentration at steady state (Docherty 2011).

Figure 7.1 shows the plasma insulin, glucose, C-peptide and U_N profile of simulated and measured data from one participant. In particular, it elucidates the difference between identified U_N from the PD control and the for less smooth and less realistic deconvoluted U_N profile. It also shows that the general trends of U_N from the PD control U_N model were in accordance with the deconvoluted U_N profile providing a measure of validation. This behaviour indicates that the PD U_N model is capable of capturing the first and second phases of insulin secretion responses to the glucose bolus encountered in dynamic tests. Moreover, the proposed model provides a direct physiological link between glucose concentration and resultant insulin secretion, which is physiologically accurate and provides a means to model this behaviour with limited data. Hence, the main benefit of the proposed model may be found when a lack of resolution in the C-peptide samples reduces the accuracy of the resulting deconvolved profile.

Figure 7.2 shows the residual error between measured C-peptide data and the response modelled by PD U_N model and Equation 7.1. It can be said that higher residual error at the forth C-peptide assay may be due to timing as this sample attempts to capture peak first phase response in the DISST before full mixing may have occurred. However, the residual error value tended to stay within the 10% of the measured data.

Figure 7.3 shows the distribution of $\frac{\phi_D}{\phi_P}$ against the *SI* value. Although the correlation of between $\frac{\phi_D}{\phi_P}$ and *SI* was weak (*R* = 0.33), it elucidates a trend between *SI* and $\frac{\phi_D}{\phi_P}$. The trend indicates that those with low ratios typically had lower insulin sensitivity than the general cohort. In general, as the ratio of $\frac{\phi_D}{\phi_P}$ decreases, *SI* value will decrease. Hypothetically, *IR* participants typically have limited first phase and high basal secretion, requiring a different set of ϕ_P and ϕ_D values than a healthy person with high first phase and low basal.

Clinically, an increased basal insulin secretion and blunted first phase response indicates an early stage in the progression of impaired glucose tolerance (Lotz *et al*. 2010; McAuley *et al*. 2011). Further, an individual that has established T2D can have low basal insulin and virtually no first phase secretion. Equally, a healthy athletic person may generally also have low basal secretion, and high or low first phase secretion. Thus, *IR* participants relied more heavily on the second phase or proportional gain in maintaining the glucose homeostasis. This latter point was inferred by the diagnostic value of U_2 in (McAuley *et al*. 2011), and matches clinical expectations (Ferrannini 1997). Higher second phase secretion is also well captured by this PD modelling approach.

Figure 7.4 shows the distribution value of ϕ_D and ϕ_P. It can be seen that ϕ_D has a greater value than ϕ_P. ϕ_D was typically an order of magnitude (×20) ϕ_P. Although both gains play an important role in glucose homeostasis, it is thought that the U_1 provides greater influences in suppressing the sudden elevated plasma glucose level back to a normal level. Studies have shown that the loss in U_1 is a strong predictor of type 2 diabetes (Bunt *et al*.

2007; Del Prato & Tiengo 2001; Pratley & Weyer 2001; Vranic *et al*. 1971; Weyer *et al*. 1999).

This study was undertaken in a cohort of adult female participants that were considered 'at-risk' of type 2 diabetes and related metabolic disorders. Hence, the outcomes of this study may be isolated to cohorts of this type. However, it may be reasonably assumed that gender does not play a significant role in the modulation of insulin secretion as a function of glucose excursions in adults, and no prior literature in a large field suggests otherwise. Furthermore, this at-risk cohort is a cohort of greatest clinical interest to the mitigation of glycaemic and other metabolic disorders. However, further confirmation must be undertaken in various other cohorts.

7.6 Summary

This chapter presents a thorough analysis of a simple, but effective PD control model of insulin secretion. The proposed model links insulin secretion to glucose concentration and is able to deliver a good compromise between model simplicity and accuracy. Although the proposed model requires further validation, it is likely to be useful for analysis of the pathogenesis of T2D as it captures the physiological determinants of patient-specific U_N profile.

Chapter 8. The efficacy of the PD U_N model in identifying the condition stage of a participant

This chapter discuss the efficacy of the proposed PD U_N model in identifying the condition stage of each participant based on the U_N profile derived from PD U_N model.

8.1 Introduction

Deconvolution of C-peptide concentration measurements is regarded as the best identification method in quantifying the U_N profiles, and is used by most studies (e.g. (Eaton *et al*. 1980; Polonsky *et al*. 1986; Van Cauter *et al*. 1992)). This method is assumed to be accurate due to the fact that insulin and C-peptide are co-secreted in an equimolar fashion from the pancreatic β cells (Rubenstein *et al*. 1969). Unlike C-peptide, acquiring plasma insulin measurements to precisely predict U_N will lead to false information as insulin undergoes a substantial, subject-specific first pass hepatic extraction before reaching the peripheral circulation (Hovorka & Jones 1994; Polonsky & Rubenstein 1986). In addition, insulin is cleared subsequently by the liver, kidney and peripheral uptake, all of which can be variable and hard to quantify. In contrast, C-peptide is only cleared by the kidney, which is a reasonably low variability pathway. Thus, C-peptide data is the best, most robust means of estimating endogenous insulin secretion.

Although the use of C-peptide has proven a better means of estimating U_N (Pacini & Mari 2003), the need for C-peptide measurement during study is time-consuming and expensive (Lin *et al*. 2010). The added cost can significantly reduce the economic viability of effective diabetes screening tests (Docherty *et al*. 2010; Docherty *et al*. 2011b). Hence, there remains significant scope and impact in better identifying a U_N profile without the use of many C-peptide measurements.

The PD control model discussed in Chapter 7 provides the capability of using the PD model to link the patient-specific U_N profile to glucose excursions. Physiologically, the amount of insulin to be secreted is determined most prominently by glucose level and the change in glucose level (gradient). The ϕ_D and ϕ_P identified by a PD U_N model is thus able to capture the physiological characteristics of first and second phase of insulin secretion, respectively.

Studies have shown that the insulin secreted by pancreatic β-cells is secreted in a biphasic, if not multiphasic, pattern (Cerasi 1967; Cerasi & Luft 1967; Curry *et al*. 1968). The U_N profile in response to glucose challenge or appearance is typically quantified into 2 phases; 1) first phase secretion and 2) second phase secretion. The first phase occurs rapidly due to a sudden change in glucose level after glucose stimulation and only lasts for few minutes (Curry *et al*. 1968). Unlike first phase, the second phase secretion lasts longer, as it is gradually released by the pancreatic β-cells to reduce the remaining elevated glucose level to a safe, normal level (Curry *et al*. 1968). Figure 8.1 shows a schematic U_N with first and second phase secretion.

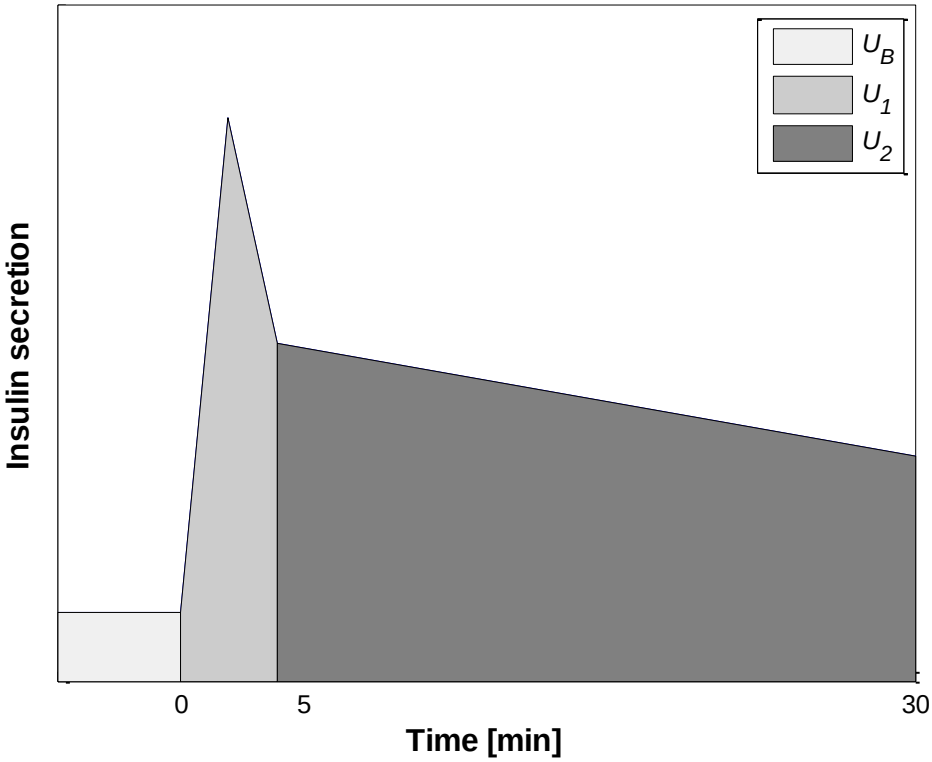

Figure 8.1: Illustration of insulin secretion from pancreatic β-cell. U_B is defined as basal insulin, U_1 is first phase and U_2 is the second phase of insulin secretion.

The relationship between the characteristics of first and second phase U_N with T2D is well founded by prior studies (Bunt *et al*. 2007; Del Prato & Tiengo 2001; Pratley & Weyer 2001; Weyer *et al*. 1999). It is thought that the loss of first phase secretion and reduced second phase secretion define the U_N characteristics of T2D (Cerasi & Luft 1967; Davis *et al*. 1993). Since the PD U_N model captures the pancreatic response to glucose, associating ϕ_D and ϕ_P to the first and second phase secretion provides an insight gain towards understanding the pathogenesis of type 2 diabetes. In particular, the changes in ϕ_D and ϕ_P as diabetes develops should illustrate these observed changes in secretion pattern. Hence, this chapter investigates the accuracy of this previously proposed PD control U_N model in identifying and discriminating the U_N profile for NGT and IFG participants, particularly in relation to changes

in PD model parameters between these two groups. Successful outcome would indicate the diagnostic potential of subject-specific ϕ_D and ϕ_P values.

8.2 Methods

A total of 204 full test DISST data sets, recorded from 68 female participants in a 10-week dietary intervention trial, were used to further analyse the efficacy of PD based U_N model. The ϕ_D and ϕ_P values were identified in a seven parameter identification approach ($x = [G_B, SI, V_G, \phi_D, \phi_P, n_L, x_L]$) using the Gauss Newton parameter identification method (Björck 1996). A full detailed description of the identification methodology was discussed in Chapter 7, Section 7.2.

8.3 Statistical analysis

In this study, the PD U_N model accuracy was assessed via the produced residual matrix ($\boldsymbol{\psi}$). The results of ϕ_P and ϕ_D are reported in median and interquartile range (IQR) for 3 participant categories: All, NGT, and IFG. All analyses were undertaken using MATLAB (R2013b, Mathworks, Inc., Natick, MA, USA).

8.4 Results

Among 204 full DISST test data sets, 17 were classed as IFG based on a G_0 cut-off value of 5.56 mmol·L^{-1} (100 mg·dL^{-1}) as defined by the American Diabetes Association (ADA) criteria

(ADA 2014). Figure 8.2 shows the distribution of ϕ_D/ϕ_P ratio against G_0 across NGT and IFG group sets of data. It also shows that the median value of ϕ_D/ϕ_P for NGT is higher than for IFG with 19.11 min and 2.76 min, respectively. In addition, the distribution data of the ratio of ϕ_D/ϕ_P versus G_0 are group into A, B and C, and are qualitatively cross-referenced with the insulin resistance and first phase secretion plot from Ferrannini *et. al.* (Ferrannini 1997).

Figure 8.3 shows the gain distribution of ϕ_D versus ϕ_P across both groups. It shows that ϕ_D values are greater value than ϕ_P. In addition, the dotted lines of ϕ_D/ϕ_P = 5, 10, and 100 show the distribution of both gains with relations to the state of participants as portrayed in Figure 8.2. Furthermore, it shows that as the ratio decreases, the profile of the participant moves from NGT to IFG, as expected and seen in Figure 8.2. A full statistical summary of both gains are presented in Table 8.1. It can be seen that while the median of ϕ_P remains equal across both groups of participant, ϕ_D remains significantly different where ϕ_DNGT ≈ $4 \times \phi_D$ IFG, (p < 0.0001). Thus, as expected, ϕ_D and first phase response is reduced significantly in IFG subjects, which is a pre-cursor to T2D.

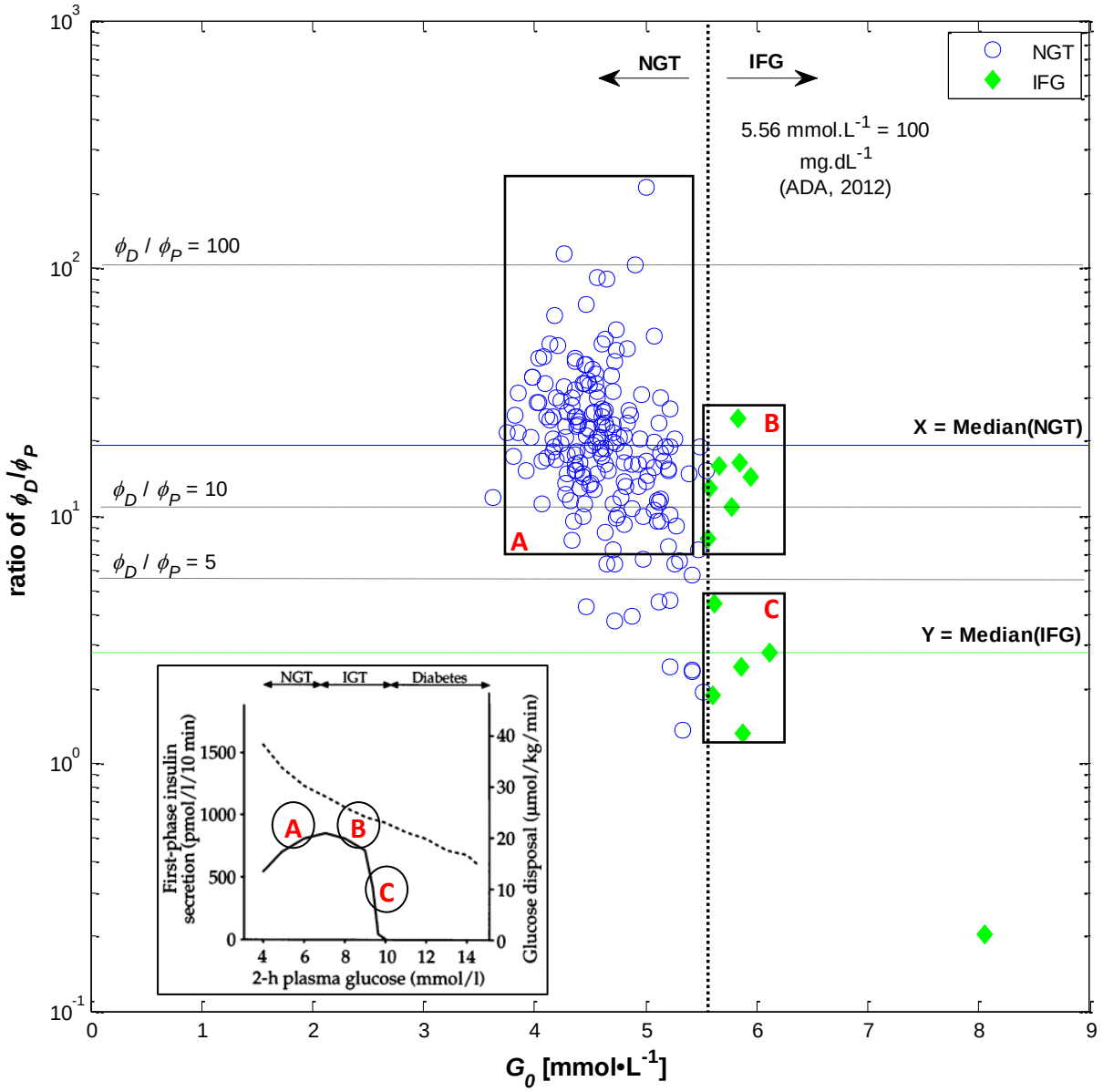

Figure 8.2: Distribution of ϕ_D/ϕ_P against G_0 where X = 19.11 min and Y = 2.79 min. Insert picture is the insulin resistance (as measured by glucose disposal) and first phase insulin secretion plot versus 2 hour glucose levels in NGT, IGT and T2D (adapted from (Ferrannini 1997)).

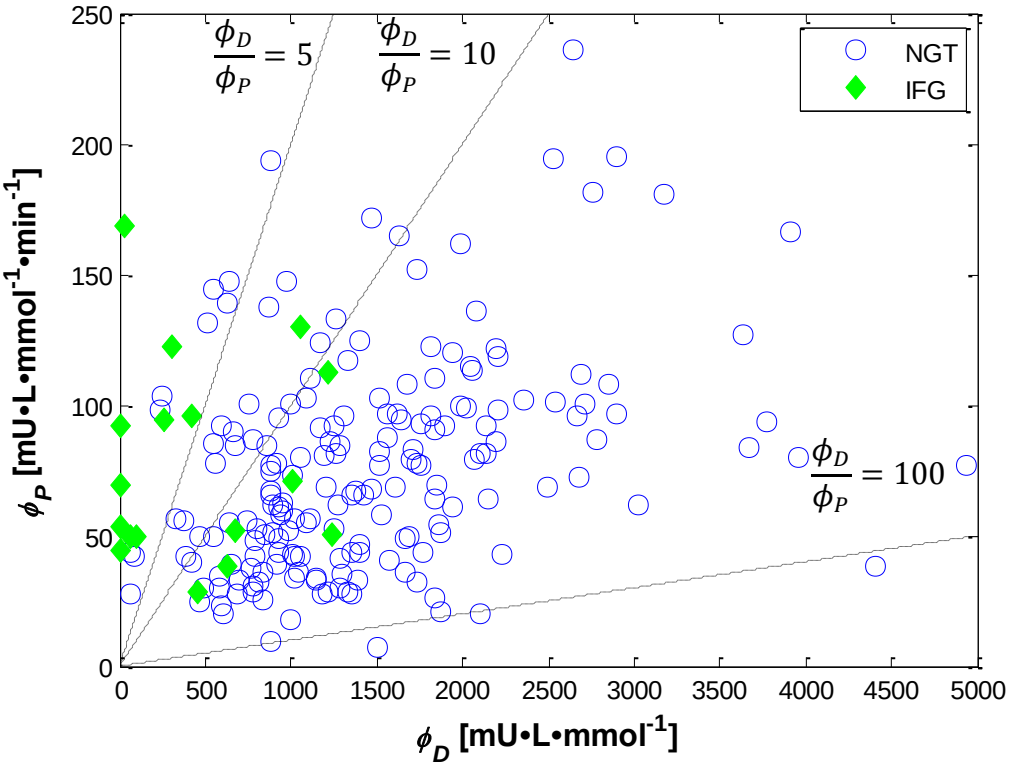

Figure 8.3: Distribution of ϕ_D over ϕ_P during the intervention study. The ϕ_D/ϕ_P = 5, 10, and 100 dotted lines are shown for context.

Table 8.1: Summary statistics of derivative ($\boldsymbol{\phi_D}$) and proportional ($\boldsymbol{\phi_P}$) gains.

		Median [IQR]		
Group	Total	ϕ_P [mU·L·mmol^{-1}·min^{-1}]	ϕ_D [mU·L·mmol^{-1}]	$\frac{\phi_D}{\phi_P}$ [min]
NGT	187	69.58 [43.06, 96.41]	1283.37 [879.35, 1848.09]	19.11 [13.19, 27.61]
IFG	17	69.47 [49.51, 100.07]	302.55 [25.72, 756.46]	2.79 [0.15, 13.25]

Figure 8.4 shows the PD model simulated versus measured plasma glucose and U_N profiles from 3 different participants. The 3 participants have high, average and low *SI* values, and

thus cover the range of participants. Note again that the glucose samples taken within 10 minutes of bolus injection were ignored due to unmodelled mixing effects. It can be seen that as a participant's G_0 rises above the 5.56 mmol·L^{-1} diagnostic threshold for IFG, the value of ϕ_D drops and becomes approximately equal to ϕ_P.

Figure 8.5 depicts a relationship between the ratio of ϕ_D/ϕ_P against BMI value, which is another risk factor for IFG and T2D (Kahn *et al*. 2006). In particular, 16 out of 17 IFG participants are obese and have a lower ratio of ϕ_D/ϕ_P. Thus, as the BMI of the participant increases, there is a general trend for their profile tends to shift from NGT to IFG. This was expected from clinical literature (Kahn *et al*. 2006).

In addition, Figure 8.6 shows the correlation between each of the gains versus the BMI value for both groups. It can be seen that, at BMI > 30, which defines obese (WHO 2000), ϕ_D for the IFG group is significantly lower than for the NGT with median value of each group equals to 283 mU·L·mmol^{-1}·min^{-1} and 1568 mU·L·mmol^{-1} ($p < 0.0001$) respectively. Additionally, the ϕ_P value for IFG subjects remains closer to the NGT value with median values equal to 70 mU·L·mmol^{-1}·min^{-1} and 85 mU·L·mmol^{-1} ($p = 0.3601$) respectively.

Figure 8.7 shows the distribution of the ratio of ϕ_D/ϕ_P against the participant's age. Although, NGT participants cover most of the range from 20 to 65 years old, the IFG participants are aged 35 to 61 years old. However, Figure 8.7 shows that there is no clear relationship between age and the ratio of ϕ_D/ϕ_P, which is expected given similar ages and different insulin resistance and diabetes status. Thus, the correlations hold with expected

and reported clinical observations, and, equally importantly, are poor where no prior clinical observation has been found.

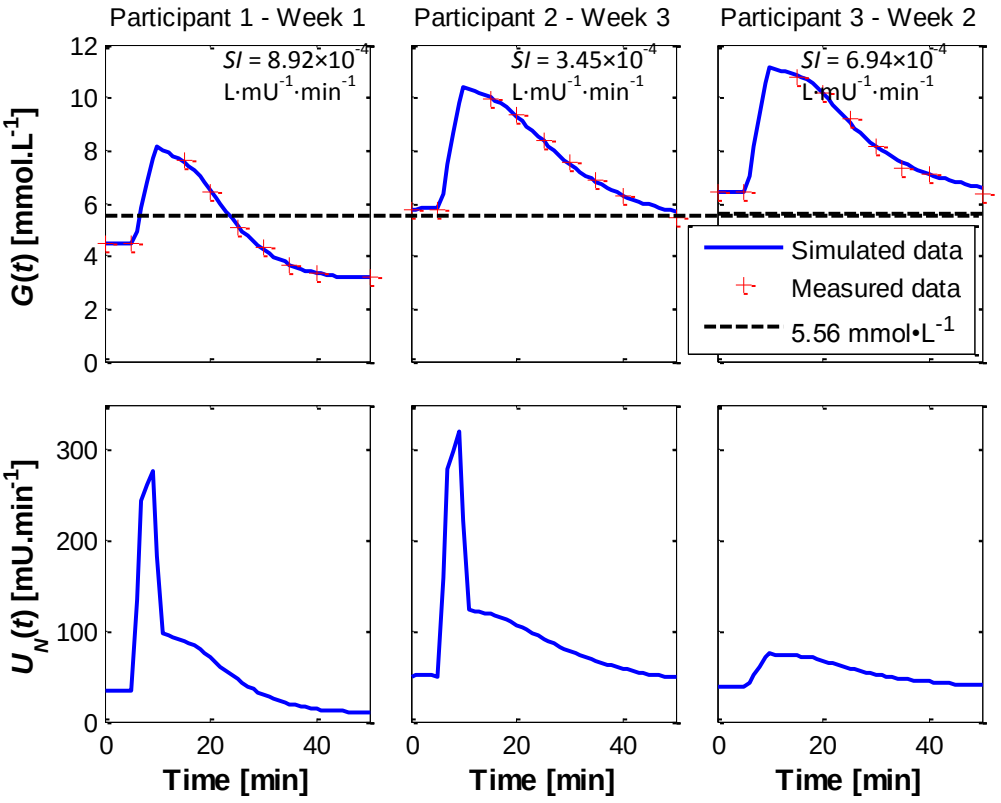

Figure 8.4: Relationship between plasma glucose concentration and U_N profile from 3 different participant response to the DISST and PD U_N model.

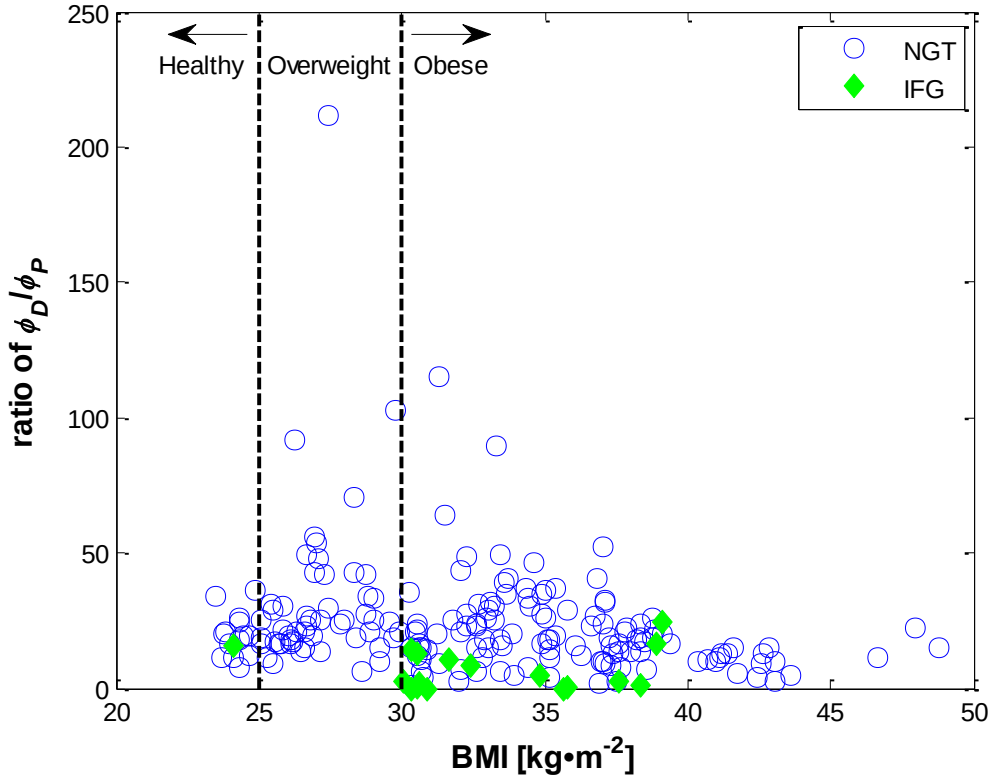

Figure 8.5: Distribution of the ratio of ϕ_D/ϕ_P against BMI value for all 204 tests.

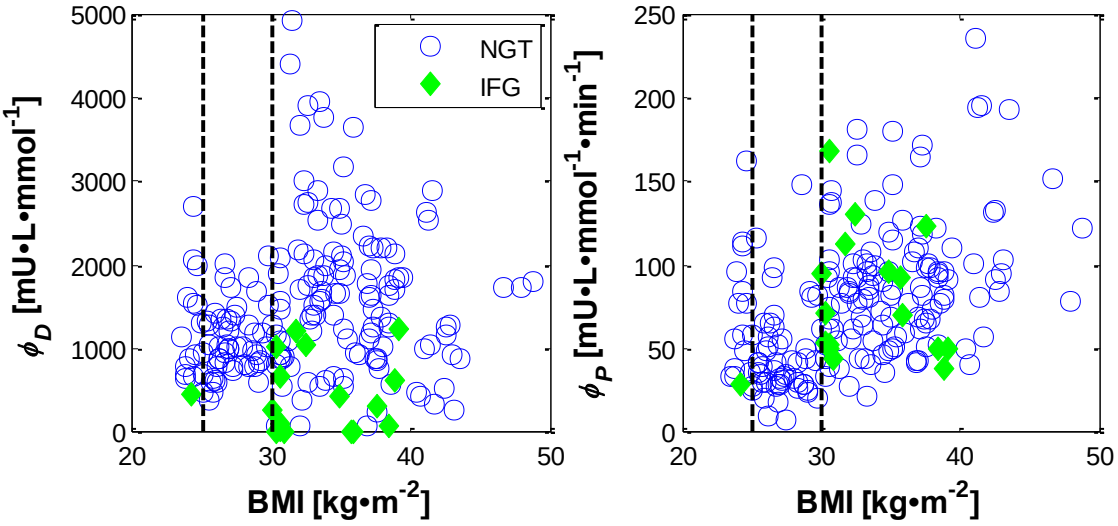

Figure 8.6: Distribution of each derivative and proportional gain versus BMI value.

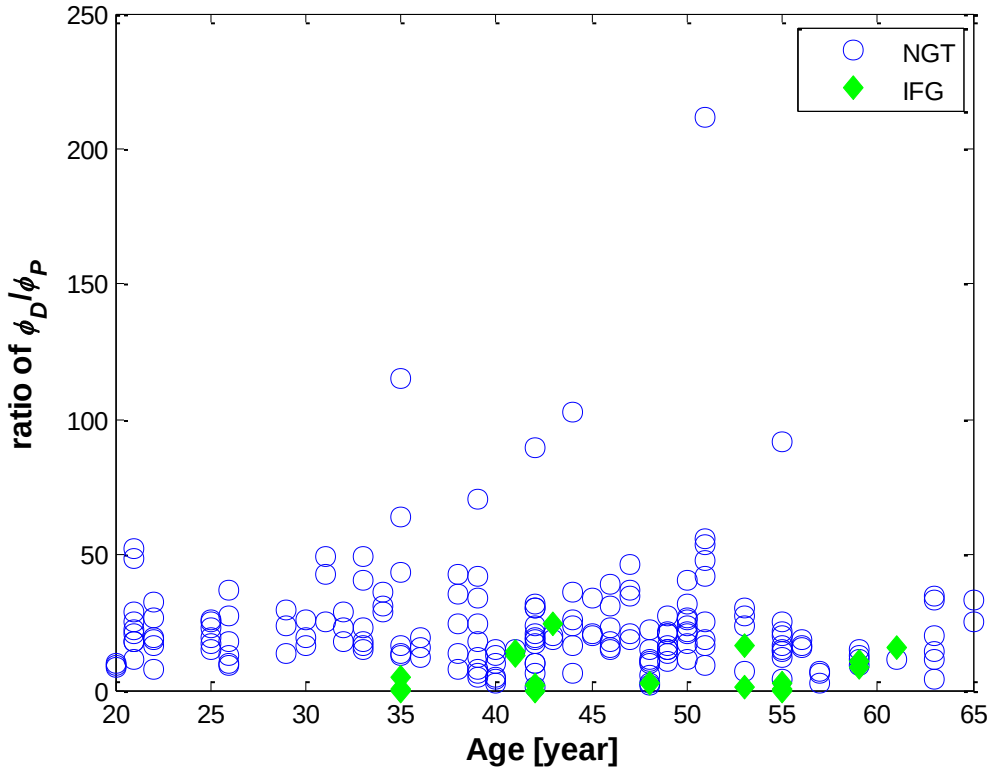

Figure 8.7: Distribution of ϕ_D/ϕ_P against participant's age.

8.5 Discussion

Like most secretion studies (Eaton *et al*. 1980; Polonsky *et al*. 1986; Van Cauter *et al*. 1992), the DISST model-based approach was developed to use deconvolution of C-peptide data to determine participant specific U_N profiles (McAuley *et al*. 2011). Although, the identification of U_N based on the deconvolution of C-peptide data is relatively accurate, due to the fact that insulin and C-peptide are equimolarly secreted by pancreatic β-cells, there remains scope to better identify U_N as C-peptide measurements are often sparse. It is known that the regulation of blood glucose concentrations is effectively a closed-loop feedback-control system (Cherrington 1999). Thus, a subject-specific PD model is used that directly mimics this behaviour to identify a smoother more, physiologically U_N profile. However, the main

purpose of this chapter was to further validate the PD U_N model by its ability to differentiate NGT and IFG participants.

The proposed PD U_N model distinguishes the U_N profile into 3 major roles; U_B, first phase insulin secretion and second phase insulin secretion. The ϕ_D captures the first phase of U_N (U_1) based on the dependence of insulin secretion on the positive rate of change of glucose concentration. The ϕ_P effectively determines the second phase of U_N (U_2), as well as the basal level when there is no challenge, based on a proportional function over the basal glucose concentration at steady state.

Figure 8.2 shows the distribution of ϕ_D/ϕ_P against G_0 on a log scale with the ADA guideline. It can be seen that the NGT group has higher gain ratio compared to the IFG group ($p < 0.0001$), where the median value of gain ratio was~7× higher. Only 5 out of 187 NGT results are below the IFG median value showing clear separation. Theoretically, an individual with higher insulin resistance will have a limited first phase secretion, based on many clinical observations (Ferrannini 1997), causing a much lower ϕ_D/ϕ_P ratio than a healthy participant with a high first phase insulin secretion. Hence, this resultant difference in median ratios is expected.

The development of T2D is a more gradual process compared to type 1 diabetes. The pathogenesis of T2D progresses through 3 distinct stages: 1) NGT; 2) IGT; and 3) T2D (Ferrannini 1997; Pories & Dohm 2012). In addition, IFG is defined by an elevation fasting plasma glucose between 100 to 125 mg·dL^{-1}, while IGT, on the other hand, is defined by an elevated 2-h post load of OGTT plasma glucose between 140 to 199 mg·dL^{-1} (ADA 2014;

Nathan *et al*. 2007). Like IGT, IFG also represents as an intermediate metabolic state between normal glucose homeostasis and diabetes (Alberti & Zimmet 1998; Ferrannini 1997; Nathan *et al*. 2007). In general, determining the value of the ϕ_D and proportional gain ϕ_P is crucial when assessing which stage the participant belongs to. Studies have shown that loss of first phase insulin secretion is an independent predictor of eventual type 2 diabetes (Bunt *et al*. 2007; Del Prato & Tiengo 2001; Pratley & Weyer 2001; Vranic *et al*. 1971; Weyer *et al*. 1999). In addition, second phase insulin secretion is an important characteristic in the prediabetic state (McAuley *et al*. 2011; Pories & Dohm 2012). However, for early diagnosis, it is the changes in ϕ_D that appear most important, and are captured at intermediate stages in the patho-physiology of T2D.

In addition, Figure 8.2 also shows a clear relationship between the ratio of both gains of the PD U_N model against participant's condition based on ADA guidelines (ADA 2014). Previous study has shown that changes in the first phase secretion reflects directly to the movement of the state of individuals from NGT to IGT, and drops drastically as it reaches diabetes (Ferrannini 1997). Evidently, if ϕ_P is assumed to be constant, based on the result of median value in Table 8.1 for both groups of participant, ϕ_D plays a critical role in deciding the ratio of ϕ_D/ϕ_P. As the value of ϕ_D increases, the ratio increases until it reaches a plateau before it drops significantly. Furthermore, it can be seen that, the ratio of ϕ_D/ϕ_P at the early stage of IFG is almost equal to ratio for the NGT group. Prior studies show that U_N did not differ significantly from normal individuals at early stage of IGT, but later drops abruptly as diabetes develops fully (Ferrannini 1997; Groop *et al*. 1993).

Figure 8.3 shows that while ϕ_D gains are scattered across a wider range from ~0 to 4.93×10^3 mU·L·$mmol^{-1}$, ϕ_P remains in a narrow range from 7.09 to 236.06 mU·L·$mmol^{-1}$·min^{-1}. In addition, Table 8.1 shows that although ϕ_P holds similar values across both groups, ϕ_D remains significantly different between the NGT and IFG groups (p < 0.0001). Thus, as ϕ_D decreases, the metabolic state of the participant could be hypothesized to move from NGT toward IFG and the first known symptoms of diabetes. Figure 8.3 also shows the lines of ϕ_D/ϕ_P ratio discriminating different participant types for the most part, as well as this trajectory of developing diabetes.

Figure 8.4 elucidates that as a participant is diagnosed with IFG (G_0 > 5.56 mmol·L^{-1} (ADA 2014)), the value of ϕ_D tends to reduce and becomes almost equal to ϕ_P. However, for NGT participant (G_0 < 5.56 mmol·L^{-1}), ϕ_D has a greater value than ϕ_P. Hence, by judging the pattern of each U_N profile, it can be said that when ϕ_D decreases or moves closer to ϕ_P, the participant is losing burst secretion for first phase as *IR* rises (Ferrannini 1997).

In addition, studies show that the intermediate metabolic state between normal and diabetes is more associated with decreased insulin sensitivity, rather than insulin secretion itself (Ferrannini 1997). The latter point is portrayed in Figure 8.4. In addition, Participants 1 and 2 have almost similar U_N profiles. However, Participant 2 is diagnosed as IFG. Hence, by examining the U_N profile itself, without considering the fasting glucose condition, this Participant 2 could be interpreted as a having normal, healthy pancreatic response to the glucose challenge. However, the *SI* value shows that Participant 2 has a lower *SI* value, 3.45 × 10^{-4} L·mU^{-1}·min^{-1} compared to *SI* = 8.92 × 10^{-4} L·mU^{-1}·min^{-1} for Participant 1. Although,

there is no agreed diagnostic *SI* value, prior studies have shown that lower *SI* values are a major factor of the pathogenesis of T2D (Ferrannini 1997; Ferrannini & Mari 1998).

While both gains play an important role in defining the participant-specific U_N profile, the ϕ_D appears to be more important in defining the early changes in the metabolic state of the participant. Theoretically, if ϕ_P is fixed to a certain value, ϕ_D will vary when quantifying the participant-specific U_N profile depending on the metabolic state of the participant. A value of $\phi_D \approx 0$ is predicted for participants with type 2 diabetes. Furthermore, down sampling measured glucose data when assessing U_N characteristics over a limited period of time from 0 to 30 min will result in significantly reduced clinical cost and clinical attention during the trial. With fewer samples, the outcome result would provide less effective information compared to a full data set. However, further validation is needed to prove both assumptions and to determine the degree to which the findings of this study can be interpolated in a down-sampling exercise to reduce test cost and intensity.

Figure 8.5 shows the correlation between the ϕ_D/ϕ_P ratio against the BMI value for both NGT and IFG with R = -0.21 and R = 0.05, respectively. Although, the correlation was weak, the outcome elucidates the ratio of ϕ_D/ϕ_P for the IFG group is significantly lower than for NGT, particularly for obese participants. In addition, judging from Figure 8.6, particularly on the distribution of ϕ_D against BMI value, it can be postulated that obese participants with higher fasting glucose have reduced first phase secretion compared to normal, healthy participant, matching many clinical studies and the strong association of obesity with IFG and T2D. Further, studies have found that the level of first phase insulin secretion is reduced in individuals with higher plasma glucose than normal and essentially absent in individuals

with fasting hyperglycemia (Brunzell *et al*. 1976; Kahn *et al*. 1969). Thus, these results match clinical results. Thus, given the loss in first phase secretion for the IFG group, Figure 8.6 also shows that the IFG participants rely heavily on second phase secretion to restore normoglycemia, again matching known clinical observations.

Figure 8.7 shows age brings little impact during the identification of U_N profile. Although glucose tolerance decreases with advancing age in general, the aging process generates more impact on insulin sensitivity than secretion (DeFronzo 1981). Prior study also shows that there is a variability in associating the effects of aging on insulin secretion particularly with confounding factors associated with obesity and concomitant insulin resistance (Adelman 1989). Thus, the failure to associate age and impaired U_N matches clinical results, and further validates the diagnostic value of the PD model parameters.

While this PD control U_N model requires further validation, it is likely to be useful for analysis of the pathogenesis of T2D as it captures the physiological determinants of participant-specific U_N profiles. Ultimately, this model provides a direct physiological link between insulin secretion to glucose concentration, as well as to eventual insulin sensitivity.

8.6 Summary

This chapter discussed a further analysis of the proposed PD U_N model adapting a Gauss Newton parameter identification method. The proposed model offers model simplicity as well as a link between insulin secretion and glucose concentration. In addition, the U_N profile response from PD U_N model provides clear information in determining the condition

stage of each participant, and this status is reflected and captured by the values of the PD model gains ϕ_D and ϕ_P.

Chapter 9. Conclusions and future work

9.1 Conclusions

Type 2 diabetes mellitus (T2DM) is a metabolic disease that affects the body's ability to regulate glucose levels in blood. T2DM is characterized by fasting hyperglycemia and an excessive rise of glucose levels in the blood above baseline following glucose or meal ingestion resulting from impaired insulin utilization (insulin resistance) coupled with the body's inability to compensate with insulin production (insulin deficiency). Thus, identification of these two main factors can offer the clinical opportunities to ameliorate the worst symptoms of the disease.

The broad relationship between insulin sensitivity and insulin secretion is well-known. Throughout years, studies have developed test protocols consists of mathematical modelling coupled with clinical analysis to identify pathogenesis of T2D through a quantitative assessment of insulin sensitivity and estimation of U_N. The application of modelling to clinical research has since been slow, but the IVGTT with minimal model has been the most frequently used model-based approach for measuring glycemic metabolism. However, like most model-based assessment, IVGTT is limited to research-only application due to its intensity, length and complexity to perform. Furthermore, the IVGTT is generally known to produce ambiguous *SI* values and erratic correlations with the gold standard. Hence, this thesis develops a more comprehensive glucose-insulin pharmacokinetics and

pharmacodynamics model that can be assessed with high accuracy, high mathematical robustness from a novel low cost and duration test protocol for type 2 diabetes patients.

This thesis improves two key aspects that to benefit the analysis of the pathogenesis of T2D. More specifically, the identification approach that accurately assesses *SI* values, especially for individuals with established T2D by redefining the role of basal glucose level and identifying it directly. Second, this thesis proposed a PD model that able to identify a smoother physiologically U_N profile that mimic the behaviour of a closed-loop feedback control human body system when regulating the blood glucose levels, and enabling for more precise estimates of *SI* from data. Both outcomes also deliver potential real diagnostic and monitoring metrics, as well.

With the assumption of G_0 equals to G_B, like most model-based assessments of *SI*, the *SI* value is well addressed by DISST model using a 2-parameter identification approach (x = [*SI*, V_G]). However, evidence suggests that $G_B \neq G_0$, and thus, should be treated as separate entities especially in individuals with established diabetes. Hence, the original DISST model, while appropriate for more normoglycaemic cohorts, needs to model G_B as a variable for assessing individuals with established T2D. A 3-parameter identification approach is developed where G_B is identified in concert with SI and VG, where (x = [G_B, *SI*, V_G]).

Result in Chapter 4 showed a significant differences between the G_0 and identified G_B values in this cohort (p_{rs} and $p_{ks} < 0.0001$), although both values were well correlated ($R = 0.70$). This analysis has shown that G_B is an important variable for modelling the glycaemic behaviour in T2D. This analysis suggests that the identified basal glucose is a more

appropriate variable for individuals with type 2 diabetes, as using the fasting glucose measurement as the basal set-point was shown to be a poor assumption for this cohort - although this requires confirmation in a larger study with a clamp as the reference.

Clinically, identifying G_B, SI and V_G as model variables in a 3-parameter identification also allows greater descriptive ability of the metabolism of individuals with T2D. The assumption of $G_B = G_0$ effects the typical 2-parameter identification employed by DISST model and results by leading to potentially erroneous SI values or SI changes due an intervention, which could lead to ineffective interventions being seen as effective, and vice versa. Chapter 5 showed that the SI value identified by the 3-parameter modelling approach were significantly lower ($p<0.05$) than the 2-parameter modelling approach. The findings were in-line with expected participant physiology. Thus, the typical modelling approaches, with an assumption of $G_B = G_0$, can over-estimate sensitivity in this cohort by lumping G_B dynamics into SI. Finally, the identified G_B tracked well with T2D pathogenesis, offering a new monitoring metric that in future could augment the well known HbA1C.

Modelling insulin secretion as a function of peripheral C-peptide levels by mathematical deconvolution has become a widespread approach. Although it remains to be the best method in identifying endogenous insulin due to the fact that C-peptide and insulin are co-secreted from β-cells, these C-peptide measurements are relatively sparse, costly to obtain and time-consuming. Hence, while diagnostically affective, there is room for improvement and to reduce sampling and thus cost.

The proposed U_N model is based on the physiological closed-loop control of insulin secretion in response to increasing glucose (derivative control, ϕ_D) and glucose above basal (proportional control, ϕ_P). By defining the model-based U_N profiles as dependent on glucose levels, the modelling approach is more physiologically representative. Although the proposed model requires further validation, it is likely to be useful for analysis of the pathogenesis of T2D as it captures the physiological determinants of patient-specific U_N profile.

The simplicity of PD U_N model provides clear relationship between the U_N profile and the metabolic state of each participant. This metabolic status is reflected and captured by the values of the PD model gains ϕ_D and ϕ_P. An individual with higher insulin resistance will have a limited first phase secretion, based on many clinical observations, causing a much lower ϕ_D/ϕ_P ratio than a healthy participant with a high first phase insulin secretion. Result in Chapter 8 showed that as a participant diagnoses with IFG (G_0 > 5.56 mmol·L^{-1}), the value of ϕ_D tends to reduce and becomes almost equal to ϕ_P. However, for NGT participant (G_0 < 5.56 mmol·L^{-1}), ϕ_D has a greater value than ϕ_P. Thus, as the metabolic state of a participant moves from NGT to pre-diabetes state, the participant is losing burst secretion for first phase results in decrease in ϕ_D.

Overall, this thesis has thus delivered these main results to improve the mathematical and clinical precision of model-based tests to monitor and diagnosis T2D and its pathogenesis. These outcomes resulted in first of their kind models and observations. The methods created offer significant future potential as both diagnostic or monitoring, model-based biomarkers.

9.2 Future work

The outcomes presented in this thesis provide good ground breaking findings in understanding the pathogenesis of type 2 diabetes particularly in insulin sensitivity and secretion. However, further optimisation can be implemented to improve the early diagnosis capability on pre-diabetes stage.

9.2.1 The role of basal glucose (G_B)

Chapter 3, 4 and 5 elucidate the importance of defining the role of G_B when assessing the *SI* value particularly in individuals with established type 2 diabetes. Result shows that when G_B is not an identified variable, the participants' glycaemic dynamics are potentially wrongly attributed to *SI*, and that identification of G_B provides a more clinically representative result. However, more validation on different cohort, healthy and hyperglycemia, as well as different model-based *SI* assessment approach is required to confirm this outcome.

As $G_B \neq G_0$, it is suggested that the need to fast overnight before undertaking the clinical trial is reviewed. The definition of $G_0 = G_B$ is already argued as study shows that walking in the morning or coming up to the clinic while fasting have G_0 slightly higher than overnight G_B levels (Holman & Turner 1981). Thus, while suggesting the participant or volunteer to monitor their food consumption, a much relax environment would potentially provide better assessment on the *SI* value.

9.2.2 Endogenous insulin secretion model (U_N)

Although the main focus of developing this PD U_N model is to eliminate the use of C-peptide measurements, there is a need for a thorough validation for accuracy for this proposed model. Theoretically, U_N is based on three physiological responses: U_B is the basal endogenous production rate; U_1, the magnitude of the first phase response to glucose appearance; and U_2 is the second phase response to glucose (Cheng *et al*. 2013; Docherty 2011). However, U_B is still dependent on C-peptide measurement at steady state.

Additionally, the interpretation of PD gains of ϕ_D and ϕ_P provides a direct relationship between U_N profile and the metabolic state of participant. However, characterising the ϕ_D and ϕ_P as a function of participants' anatomical characteristic (weight, height, age, sex) would provide better estimation of U_N profile. Further validation with larger and different cohort is required to quantify the accuracy of this PD U_N model.

9.2.3 Real time assessment of insulin sensitivity and secretion

The real time aspect refers to neglecting the use of insulin and C-peptide measurements during parameter identification. With the use only of glucose measurements, a diagnostic outcome can be generated within few minutes of test completion. Thus, it could immediately provide an outcome that can be used by the researcher/ clinician to further understand and better control of the participant's health condition.

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