High Resolution Clinical Model-Based Assessment of Insulin Sensitivity

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Nomenclature

ACRONYMS	
ADA	American Diabetes Association
BMI	Body Mass Index (kg/m^2)
BSA	Body Surface Area (m^2)
CIGMA	Continuous Infusion of Glucose and Model Assessment
CLAMP	Euglycaemic/Hyperglycaemic Clamp Test
CV	Coefficient of Variation (SD/mean)
CV_{inter}	CV between different assay runs
CV_{intra}	CV within the same assay run
EGP	Endogenous Glucose Production
FPG	Fasting Plasma Glucose (mmol/l)
FPI	Fasting Plasma Insulin (mU/l)
GDM	Gestational Diabetes Mellitus
GFR	Glomerular Filtration Rate (ml/min)
HbA_{1C}	Glycosylated Hemoglobin
HOMA	Homeostatic Model Assessment
HOMA-IR	HOMA insulin resistance metric
HOMA-%B	HOMA β-cell function metric
ICU	Intensive Care Unit
IGT	Impaired Glucose Tolerance
IFG	Impaired Fasting Glucose
IR	Insulin Resistance
ITT	Insulin Tolerance Test
IV	Intravenous
IVGTT	Intravenous Glucose Tolerance Test
MM	Minimal Model of glucose kinetics
MSD	Multiplicative Standard Deviation
NGT	Normal Glucose Tolerance

OGTT	Oral Glucose Tolerance Test
OGIS	Oral Glucose Insulin Sensitivity
OMM	Oral Minimal Model
PD	Pharmacodynamic
PK	Pharmacokinetic
PID	Proportional-Integral-Derivative Controller
RMSE	Root Mean Square Error
SD	Standard Deviation

MATHEMATICAL VARIABLES

α_I	Michaelis-Menten parameter for liver clearance rate saturation from plasma (l/mU)
α_G	Michaelis-Menten parameter for insulin-stimulated glucose clearance saturation (l/mU)
γ	Steady state concentration gradient Q_{ss}/I_{ss}
$AUC_{10,20,total}$	Integrated insulin secreted above basal secretion over 10, 20 or total minutes (Area Under Curve) (mU)
$C(t)$	C-peptide concentration in plasma (pmol/l)
$C_{est}(t)$	Interpolated C-peptide concentration profile (pmol/l)
D_{1-4}	Points of discontinuity in the C-peptide profile
EGP_b	Total EGP at a fasting state ($= EGP_{GE} + EGP_{Ib}$) (mmol/l)
EGP_{GE}	Fraction of EGP that compensates for constant glucose uptake GU_G (mmol/l)
EGP_{Ib}	Fraction of EGP that compensates for glucose uptake at basal insulin GU_{Ib} (mmol/l)
EGP_{suppr}	Maximal suppression of EGP in Monte Carlo simulation
F_h	Hepatic blood flow rate (l/min)
$G(t)$	Plasma glucose concentration above equilibrium level G_E (mmol/l)
G_E	Equilibrium level of plasma glucose concentration (mmol/l)
G_{clamp}	Target glucose concentration during a CLAMP (mmol/l)
GU_G	Constant insulin independent glucose uptake (mmol/min)
GU_{Ib}	Constant insulin dependent glucose uptake at basal insulin concentration I_b
$i(t)$	Insulin mass in plasma (mU)
$I(t)$	Plasma insulin concentration (mU/l)
ISI	Insulin Sensitivity Index from a CLAMP test

	(mg/kg/min/(mU/l))
ISI_G	ISI normalised by G_{clamp} (mg/kg/min/(mU/l)/(mmol/l))
k	Rate constant controlling ISF insulin half-life (min^{-1})
k_{1-4}	Transport rates (min^{-1})
K_G	Insulin sensitivity metric (exponential decay rate during IVGTT)
K_{ITT}	Insulin sensitivity metric (exponential decay rate during ITT)
M	Steady state glucose infusion rate during CLAMP (mg/kg/min)
n	Insulin clearance rate (min^{-1})
n_C	Irreversible loss rate of insulin from ISF, by binding to cell receptors and subsequent degradation (min^{-1})
n_I	Transport rate of insulin from plasma to interstitium (l/min)
n_K	Kidney clearance rate of insulin from plasma (min^{-1})
n_L	Liver clearance rate of insulin from plasma (min^{-1})
$P(t)$	Exogenous glucose infusion rate (mmol/min)
p_G	Fractional clearance of plasma glucose at basal insulin (min^{-1})
p_{GU}	Glucose clearance attributed to uptake independent of insulin (min^{-1})
p_{GS}	Glucose clearance attributed to glucose dependent suppression of EGP (min^{-1})
$q(t)$	Insulin mass in ISF (mU)
$Q(t)$	Insulin concentration in ISF (mU/l)
Q_b	Basal insulin concentration in ISF (mU/l)
$S(t)$	C-peptide secretion rate, equivalent to insulin secretion rate u_{en} (pmol/min)
S_G^{MM}	Minimal Model parameter for glucose effectiveness (min^{-1})
S_I	Insulin sensitivity (l/mU/min)
S_I^{MM}	Minimal Model parameter for insulin sensitivity (l/mU/min)
S_{max}	First phase peak insulin/C-peptide secretion rate (mU/l, pmol/l)
S_{I-MC}	S_I from Monte Carlo simulation (l/mU/min)
S_{I-SS}	S_I at steady state during a CLAMP test (l/mU/min)
S_{I-TR}	S_I at transient state during a CLAMP test (l/mU/min)
$u_{en}(t)$	Endogenous insulin secretion rate (mU/min)
$u_{ex}(t)$	Exogenous insulin administration rate (mU/min)
V_B	Blood volume (plasma + hematocrit)
V_P	Plasma volume (+fast exchanging tissues) (l)
V_Q	Interstitial fluid (ISF) volume (l)

V_G	Glucose distribution volume (l)
x_L	Fractional first pass hepatic insulin extraction
$X(t)$	Minimal Model remote insulin action (min^{-1})
$Y(t)$	C-peptide concentration in ISF

Abstract

Type 2 diabetes has reached epidemic proportions worldwide. The resulting increase in chronic and costly diabetes related complications has potentially catastrophic implications for healthcare systems, and economies and societies as a whole. One of the key pathological factors leading to type 2 diabetes is insulin resistance (IR), which is the reduced or impaired ability of the body to make use of available insulin to maintain normal blood glucose levels.

Diagnosis of developing IR is possible up to 10 years before the diagnosis of type 2 diabetes, providing an invaluable opportunity to intervene and prevent or delay the onset of the disease. However, an accurate, yet simple, test to provide a widespread clinically feasible early diagnosis of IR is not yet available. Current clinically practicable tests cannot yield more than a crude surrogate metric that allows only a threshold-based assessment of an underlying disorder, and thus delay its diagnosis.

This thesis develops, analyses and pilots a model-based insulin sensitivity test that is simple, short, physiological and cost efficient. It is thus useful in a practical clinical setting for wider clinical screening. The method incorporates physiological knowledge and modelling of glucose, insulin and C-peptide kinetics and their pharmaco-dynamics. The clinical protocol is designed to produce data from a dynamic perturbation of the metabolic system that enables a unique physiologically valid assessment of metabolic status. A combination of a-priori information and a convex integral-based identification method guarantee a unique, robust and automated identification of model parameters.

In addition to a high resolution insulin sensitivity metric, the test also yields a clinically valuable and accurate assessment of pancreatic function, which is also a good indicator of the progression of the metabolic defect. The combination

of these two diagnostic metrics allow a clinical assessment of a more complete picture of the overall metabolic dysfunction. This outcome can assist the clinician in providing an earlier and much improved diagnosis of insulin resistance and metabolic status and thus more optimised treatment options.

Test protocol accuracy is first evaluated in Monte Carlo simulations and subsequently in a clinical pilot study. Both validations yield comparable results in repeatability and robustness. Repeatability and resolution of the test metrics are very high, particularly when compared to current clinical standard surrogate fasting or oral glucose tolerance assessments. Additionally, the model based insulin sensitivity metric is shown to be highly correlated to the highly complex, research focused gold standard euglycaemic clamp test.

Various reduced sample and shortened protocols are also proposed to enable effective application of the test in a wider range of clinical and laboratory settings. Overall, test time can be as short as 30 minutes with no compromise in diagnostic performance. A suite of tests is thus created and made available to match varying clinical and research requirements in terms of accuracy, intensity and cost. Comparison between metrics obtained from all protocols is possible, as they measure the same underlying effects with identical model-based assumptions.

Finally, the proposed insulin sensitivity test in all its forms is well suited for clinical use. The diagnostic value of the test can assist clinical diagnosis, improve treatment, and provide for higher resolution and earlier diagnosis than currently existing clinical and research standards. High risk populations can therefore be diagnosed much earlier and the onset of complications delayed. The net result will thus improve overall healthcare, reduce costs and save lives.

Chapter 1

Introduction

The number of people with diabetes is increasing rapidly due to aging populations, and an increase in the prevalence of obesity and sedentary lifestyles. The resulting increase in diabetes related complications has catastrophic implications on healthcare systems and on entire economies and societies. Understanding the underlying metabolic disorder allows identification of those at risk of developing diabetes up to 10 years earlier, and this would provide an invaluable opportunity to intervene and prevent or delay the onset of the disease. This chapter discusses the overall prevalence of diabetes, its development and underlying problems, and its clinical classification and diagnosis.

1.1 The Diabetes Epidemic

Type 2 Diabetes is a disease that has reached epidemic proportions. An estimated 171 million people were diagnosed worldwide in the year 2000. This number is expected to rise to 366 million by 2030 [Wild et al., 2004]. About the same numbers are estimated to have undiagnosed diabetes or pre-diabetes [Hossain et al., 2007; Wild et al., 2004], effectively doubling those numbers. In New Zealand it is estimated that by 2021, 250,000 people will have diabetes and 500,000 more will have pre-diabetes, resulting in about 15 % of the population directly affected by the disease [PriceWaterhouseCoopers, 2001].

The total diabetes related health expenditure in the USA in 2002 was US$ 132 billion, second only to all cancer types combined [Kleinfield, 2006]. These costs are incurred primarily by treatment of chronic long-term complications, such as

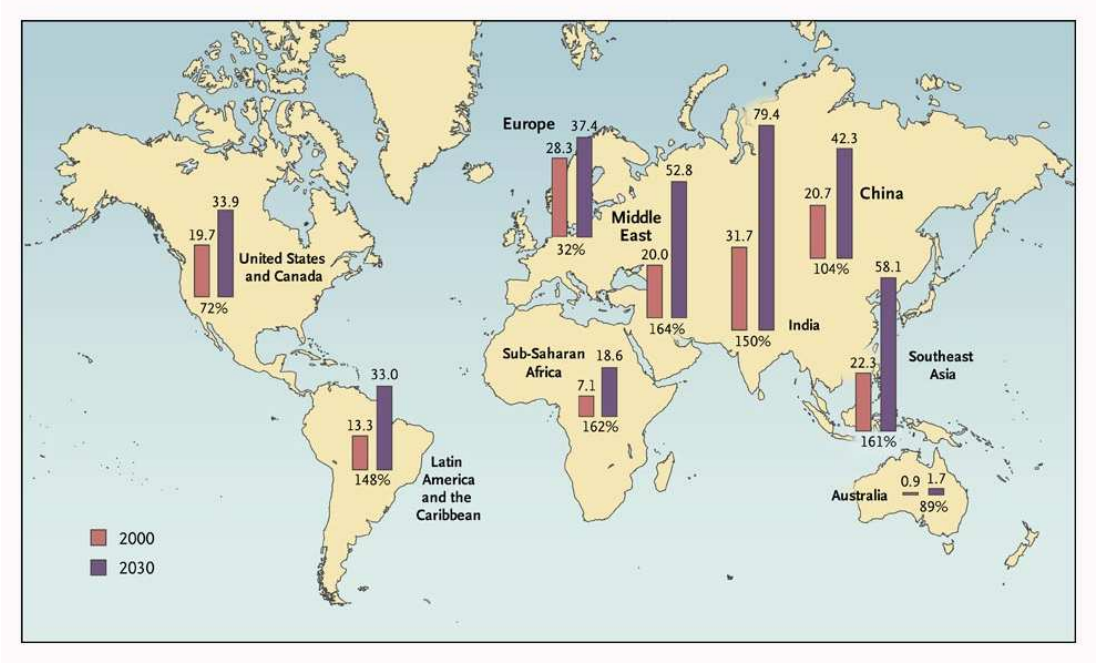


Figure 1.1 Cases of Diabetes in 2000 and estimated numbers for 2030 with the projected percentile increases [Hossain et al., 2007; Wild et al., 2004]. Largest increases are seen in developing countries.

eye damage or blindness, renal failure, nerve damage in limbs leading to amputation, hypertension and cardiovascular disease [ADA, 2006]. As a result, diabetes is the third most common cause of death in the USA.

One of the underlying causes of this epidemic is a worldwide obesity epidemic and increasingly sedentary lifestyles [Hossain et al., 2007]. It is estimated that worldwide 1.1 billion people are overweight, 312 million of those obese (BMI $>$ 30), a number that has tripled in the past 20 years [Hossain et al., 2007]. An estimated 155 million children are overweight or obese. The greatest threat of obesity is on populations in China, the Middle East, Southeast Asia and the Pacific Islands, as is shown in Figure 1.1, mainly due to changing dietary habits [ADA, 2006; Hossain et al., 2007]. Whereas the disease was seen as a problem of developed countries in the past, it is the developing countries that now have the most rapid rise in prevalence [Hossain et al., 2007; Wild et al., 2004].

Typically, type 2 diabetes is not recognised early enough to intervene before permanent damage has begun to occur, and is thus often diagnosed only when treating its symptoms or complications at later stages [ADA, 1998; Gastaldelli et al., 2004; Kleinfield, 2006]. This late diagnosis is due to the nature of the

disease development, where noticeable symptoms do not arise until significant irreversible damage has occurred [ADA, 1998]. With accurate early diagnosis, pre-diabetic states can be identified up to 10 years earlier [Martin et al., 1992], which can significantly reduce the onset of further damage and complications. Unfortunately, the need for preventive diagnosis and treatment is not recognised sufficiently by health expenditures [Hogan et al., 2003; Kleinfield, 2006; PriceWaterhouseCoopers, 2001].

1.2 Development of Diabetes

The complete name of the disease is *Diabetes mellitus*. *Diabetes* is derived from the greek word for "passing through", and *mellitus* from the latin word "honey", referring to the excessive sugar in the urine of the patients [Dobson, 1776]. Diabetes combines a group of different metabolic disorders, which have different origins, but all resulting in hyperglycaemia or high blood glucose levels [ADA, 2006]. Insulin is needed by the cells as a mediator for glucose uptake. High blood glucose levels are mainly caused by a deficiency or a resistance to available insulin.

The three main recognised types of diabetes are type 1, type 2 and gestational diabetes mellitus (GDM), the latter occurring temporarily during pregnancy. As only the first two are lasting disease states, and a persisting GDM after pregnancy is classified as type 2 diabetes, GDM will not be described in detail. Type 1 and type 2 diabetes represent significantly different metabolic conditions with different pathologies.

1.2.1 Type 1

Type 1 diabetes is characterised by a significant, often sudden *deficiency* of insulin. It is caused by an auto-immune disorder destroying the insulin producing β-cells in the pancreas and has a strong genetic pre-disposition. This type of diabetes is commonly known as juvenile-onset diabetes, but can also strike adults and is not linked to obesity [ADA, 2006]. About 10 % of people with diabetes have type 1 [ADA, 2006].

The destruction of the insulin producing β-cells resulting in type 1 diabetes can occur very rapidly over weeks or months. However, it can also take many years, and sometimes a minimal insulin secretory function can remain intact. Treatment is mainly by regular insulin injections, taken multiple times per day for the rest of the patients' lives. If glucose levels are not kept within a tight range, long-term complications can occur. Despite the difficulties, many type 1 diabetic individuals have lived long healthy lives through frequent glucose monitoring and tight glycaemic control.

1.2.2 Type 2

Type 2 diabetes is characterised by a *resistance* to insulin in the majority of individuals. The development of type 2 diabetes is a more gradual process than in type 1 diabetes. It starts with the pre-diabetes stages of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), before a clinical classification of diabetes is made [ADA, 2006]. The progression of the disease is often undiagnosed and untreated for many years, until first health complications start to appear.

The risk of developing type 2 diabetes has a partial genetic pre-disposition, but is strongly affected by increased body weight and obesity, which significantly increase insulin resistance [Ferrannini et al., 1997; Hossain et al., 2007; Kahn et al., 2006b; Petersen and Shulman, 2006]. Weight reduction and lifestyle change to a healthier diet and increased exercise have been shown to greatly decrease insulin resistance and the prevalence of developing type 2 diabetes [Camastra et al., 2005; McAuley et al., 2002; Tuomilehto et al., 2001]. However, these interventions are difficult to implement in some patients, necessitating other forms of treatment.

The development of insulin resistance and reduced β-cell function in the progression to type 2 diabetes is shown in Figure 1.2. A gradual decrease of insulin sensitivity (increase of insulin resistance) is seen. This decrease is initially accompanied by a compensatory increase in pancreatic insulin secretion to maintain normal glucose levels. When the pancreas cannot keep up anymore with the increased demand, it begins to exhaust itself. The result is a further increase in basal plasma glucose levels. It is not fully understood if the primary underlying

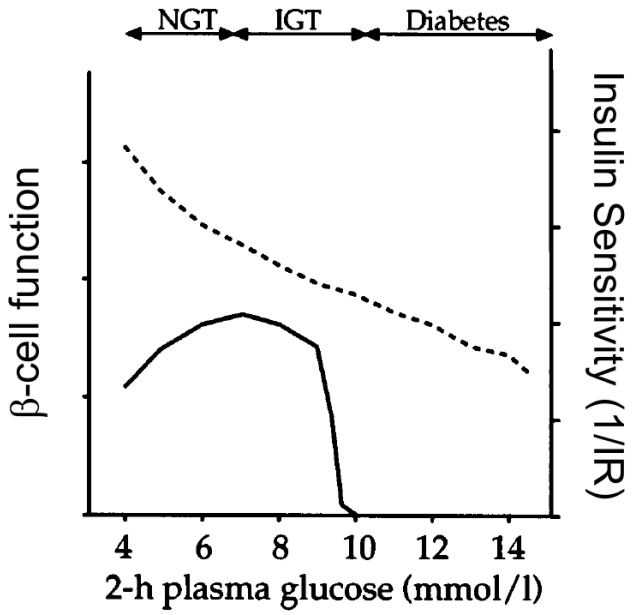


Figure 1.2 Progression of β-cell function (solid) and insulin sensitivity (dashed), opposite of insulin resistance, in the development from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT), resulting in type 2 diabetes [Ferrannini, 1997]. The x-axis indicates blood glucose concentration 2 h post oral glucose challenge, a diagnostic criteria of diabetes.

problem is insulin resistance or a defect in β-cell function [Ferrannini and Mari, 2004]. However, it is well accepted that both factors play an important role in maintaining glucose balance [ADA, 2006; Schinner et al., 2005].

Treatment of type 2 diabetes consists first of lifestyle changes to increase insulin sensitivity, followed by, or combined with, medication to enhance insulin sensitivity or stimulate the pancreas. In later, or more extreme stages, insulin replacement therapy, as in type 1 diabetes, is required to maintain normoglycaemia.

1.2.3 Insulin Sensitivity

Insulin resistance, a decrease in the body's sensitivity to insulin, is the main underlying problem in the pathogenesis of type 2 diabetes. Insulin sensitivity is not a discrete metric that can be assessed with a simple, well defined test. Rather, it is a concept to quantify the body's ability to reduce blood glucose levels with insulin. This definition is very broad and includes many underlying physiological

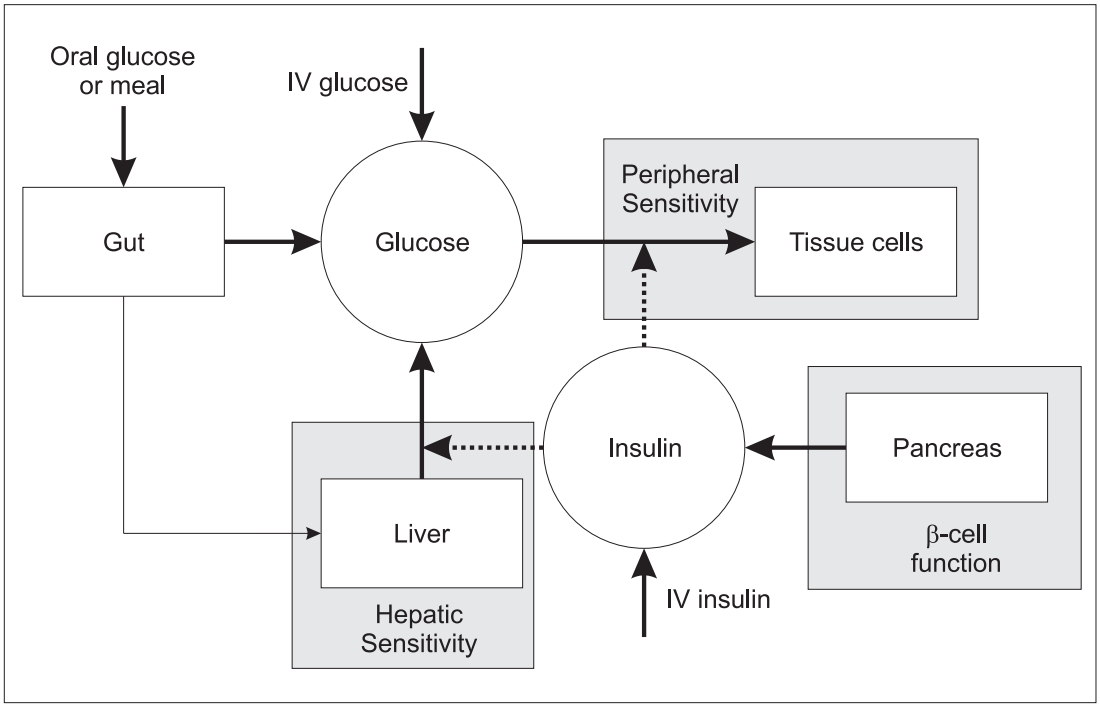


Figure 1.3 Overview of the physiological effects measured by insulin sensitivity tests. Depending on the design of the test, it can measure either one, a combined effect of two, or all three of these effects. The dashed lines indicate a mediated or enhanced effect.

effects that contribute to the whole body response.

The main effects contributing to insulin dependent glucose uptake are shown schematically in Figure 1.3. The three primary effects are the sensitivity of tissue cells to bind insulin (peripheral sensitivity), the effect of insulin on the liver to suppress glucose production (hepatic sensitivity), and the ability of the pancreas to respond with insulin secretion to an increase in glucose concentration (β-cell or pancreatic function). These effects may also be time-varying and are different in fasting or perturbed states [Scheen et al., 1994]. Depending on the structural design of the chosen method to assess insulin sensitivity and its assumptions, one or more of these effects can be combined in the assessment, thus yielding varying results requiring different interpretations [Radziuk, 2000].

The cellular defects of IR are not fully understood, but it is clear that there are genetic and environmental factors influencing them [ADA, 1998; Petersen and Shulman, 2006]. It is assumed that there is a downregulation of the insulin binding receptors, which is caused by an increased intracellular lipid content [Petersen and Shulman, 2006; Schinner et al., 2005], in line with the findings of increased

IR in obesity [Camastra et al., 2005; Kahn et al., 2006b]. Another finding is that IR is caused by inflammation, as the proinflammatory cytokine TNF-α produced by adipose tissue seems to induce IR [Hotamisligil, 2006; Shoelson et al., 2006]. Other inflammatory markers, such as IL-6, Leptin and C-reactive protein (CRP) have been correlated with the incidence of type 2 diabetes [ADA, 1998; Herder et al., 2006; Shoelson et al., 2006]. Overall, systemic inflammatory markers are strongly associated with type 2 diabetes and its complications.

Insulin sensitivity can be treated primarily by a lifestyle change. Increases in exercise, healthier diet [Duncan et al., 2003; McAuley et al., 2002; Nishida et al., 2002; O'Gorman D et al., 2006; Tuomilehto et al., 2001] and weight loss [Camastra et al., 2005; Ferrannini et al., 2005, 1997] are proven to increase sensitivity and thus reduce the prevalence or impact of type 2 diabetes. Other treatments include sensitivity enhancing medication, such as thiazolidinediones (Rosiglitazone), biguanides (Metformin) or sulfonylureas (Glyburide) [Kahn et al., 2006a]. Finally, if β-cell function is strongly diminished, additional insulin therapy can be provided as needed [Kahn et al., 2006a].

1.3 Diagnostic Criteria

The general diagnosis of diabetes, as recommended by the American Diabetes Association [ADA, 2006] is by any of three criteria:

1. **Symptoms of diabetes** (polyuria, polydipsia, unexplained weight loss) plus plasma glucose concentration any time of the day ≥ 11.1 mmol/l (200 mg/dl).
2. **Fasting plasma glucose (FPG)** ≥ 7.0 mmol/l (126 mg/dl).
3. **2-h post OGTT glucose** ≥ 11.1 mmol/l (200 mg/dl) during an oral glucose tolerance test (OGTT) (75 g glucose content dissolved in water).

People with elevated glucose levels that do not meet the criteria for type 2 diabetes are classified with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). These conditions are defined as follows:

- **IFG**: Fasting glucose between 5.6-6.9 mmol/l (100-125 mg/dl).
- **IGT**: 2-h post load OGTT glucose between 7.8-11.1 mmol/l (140-199 mg/dl).

These thresholds do not directly measure the underlying disorders of IR and impaired β-cell function, but are only surrogates that can be easily measured clinically. Many insulin resistant people have normoglycaemic levels due to increased compensatory pancreatic function. Hence, they would not meet these criteria and would not be screened until their β-cell function begins to deteriorate in the later developments, as shown in Figure 1.2 [ADA, 2006; Gastaldelli et al., 2004].

In a long-term follow-up study by Martin et al. [1992], it has been shown that 10 years ahead of the formal diagnosis, people that developed type 2 diabetes had a 60 % lower mean insulin sensitivity than those that did not develop the disease. Additionally, in another study it was found that IR is the strongest predictor of type 2 diabetes and cardiovascular disease risk in obese individuals [McLaughlin et al., 2007]. An early diagnosis of IR could therefore recognise this defect and allow early treatment to delay or prevent the onset of complications. Unfortunately, the existing accurate tests are too complex to be feasible in a wider clinical setting, and the often used clinical surrogate tests are too crude [ADA, 1998]. A simple, accurate test would thus be especially useful to test high risk groups, such as the offspring of type 2 diabetic individuals, or to monitor the direct effect of treatment on IR, as specifically noted by the ADA [ADA, 1998]:

> [...] *lacking a clinically practical test for insulin resistance or a way to follow it longitudinally in a clinical setting, it is impossible for the clinician to know whether a given treatment is specifically alleviating insulin resistance and preventing its associated conditions.*

1.4 Preface

The goal of this research is to develop a test that is able to accurately assess insulin sensitivity without the complexity of existing accurate tests. It should be simple, short and cost efficient to be useful in wider clinical settings, enable early diagnosis of IR, and allow frequent monitoring of treatment. This goal has been

targeted by researchers for several decades, but a satisfactory solution has not yet been found.

This goal is pursued in this thesis by systematically analysing the physiology and previously developed tests and models, to determine the key aspects that need to be captured. With the design restrictions of a short clinical test, an optimal protocol can be engineered to minimise the impact of confounding factors that can affect the test outcome. More specifically, detailed knowledge of metabolic behaviour is combined with smart modelling and algorithms to achieve this goal. A brief overview of the thesis includes:

Chapter 2 reviews current methods of insulin sensitivity testing used in research and clinical settings. Key aspects of each method are discussed and compared. The methods are finally put in perspective and compared to define the design restrictions for a better and simpler protocol.

Chapter 3 presents the insulin modelling and identification methods required for such a test. It systematically addresses the physiology and modelling of insulin and the estimation of insulin secretion rate using C-peptide. The result is a new and optimised insulin system model and fitting method.

Chapter 4 presents the glucose modelling and identification methods required. Physiology and modelling of glucose are described, and an optimised glucose/insulin pharmaco-dynamic model presented. The result is a new and optimised overall metabolic system model and fitting method that is applicable to a short clinical protocol.

Chapter 5 validates the insulin models and methods developed on data from the literature and obtained from other studies. The physiological validity of each model is shown.

Chapter 6 validates the overall model-based metric of insulin sensitivity against gold standard euglycaemic clamp test data.

Chapter 7 presents the proposed protocol for the new test developed and estimates its expected accuracy in a Monte Carlo analysis applying all identified sources of variability.

Chapter 8 presents the clinical pilot study to validate the protocol clinically. The design of the study is described and its results are discussed.

Chapter 9 optimises the protocol in terms of test duration, sample numbers and timing. This task is achieved by testing protocol performance on sample and time-reduced data sets from the pilot study. Shorter and simpler versions of the protocol are proposed with the expected difference in variability.

Chapters 10 and 11 summarise the key aspects of the thesis and present possible future improvements and applications for this research.

Chapter 2

Review of Current Methods

Soon after the discovery of insulin by Banting et al. [1922], it was evident that some people needed more insulin than others to achieve normal blood glucose levels. Thus, a first approach to assess this sensitivity to insulin was proposed by Horgaard and Thayssen [1929], followed by others, such as Andres et al. [1966]; Himsworth [1936] and Shen et al. [1970]. In the past 40 years in particular, a great deal of research has focused on the development and validation of more accurate, simpler and/or more physiological methods [Ader and Bergman, 1987; Ferrannini and Mari, 1998; Scheen et al., 1994].

This chapter gives an overview of the state of the art in insulin sensitivity testing. A variety of methods are included, differing by application. More specifically, there are those used mainly in research to assess the effects of treatment or medication on insulin sensitivity, and there are tests that are clinically focused, designed to screen populations and diagnose diabetes, or the risk of developing the disease. The test methods are compared in terms of their accuracy and practicality for diagnosis, focusing on reported issues or problems that might be improved in a new test. Tables summarising all of the tests described are given in Appendix A.

2.1 Overview

A detailed description of insulin sensitivity is found in the previous chapter. Tests that measure insulin sensitivity can be divided into two main types, based upon their design:

1. **Direct tests**
2. **Surrogate tests**

Direct assessments usually involve the oral or intravenous administration of glucose and/or insulin, and a subsequent sampling of these molecules to determine their pharmaco-dynamic interaction. Another localised direct method is to use the arterio-venous (A-V) difference technique, by comparing these concentrations at the in- and outflow of certain tissues or organs to determine their glucose uptake [Scheen et al., 1994]. Surrogate tests are empirical methods, mostly regression models, that are designed to correlate well with certain gold standard test metrics. They can use data from a dynamic test or just a fasting sample, the latter of which makes them very attractive for screening purposes. When using fasting metrics, it is to be noted that these metrics only quantify the sensitivity during a fasting state, which can be different to that observed during the dynamic or hyperglycaemic state used in other tests [Scheen et al., 1994].

One of the difficulties in assessing insulin sensitivity with a dynamic test is to separate observed results between the different effects influencing them, a task that cannot always be achieved. The contribution of the pancreas, or β-cell function, can be isolated by sampling C-peptide concentrations, which are a good indicator of pancreatic insulin secretion [Pacini and Mari, 2003]. Hepatic sensitivity, defined as insulin's effect in inhibiting endogenous glucose production (EGP), can be measured with the additional use of glucose tracers [Caumo and Cobelli, 1993], or isolated by suppressing EGP completely [DeFronzo et al., 1979]. Additionally, some physiological effects are not always accounted for in certain tests, such as non-insulin dependent glucose uptake [Zierler, 1999] or saturation of glucose uptake [Natali et al., 2000; Prigeon et al., 1996], and are thus lumped into the measured insulin-dependent result, skewing or biasing the result.

Other factors that can affect the measured results are effects that are not identified or hard to measure in vivo. These effects include for example the gut absorption rate, if glucose is ingested orally [Radziuk et al., 1978], or the counter-regulatory response to hypoglycaemic glucose concentrations [Monzillo and Hamdy, 2003].

All of these factors have lead to different approaches to test insulin sensitivity. However, they do not necessarily measure the same effects, even if they might

correlate well. Their respective use is therefore mostly limited to certain applications. The more accurate, mostly intravenous and more intense/invasive tests are only viable in a research setting. In contrast, fasting, or the briefer oral dose tests, are ideal in clinical settings due to their simplicity. However, because they may measure different effects and have different levels of accuracy and resolution, there is no consistent test useful across this range of application requirements.

2.2 Comparative Statistics

Before discussing the different methods to test insulin sensitivity and how they compare, it is important to point out some aspects and limitations of commonly used comparative statistics and performance metrics. In particular, this section defines the comparative and accuracy metrics and terms used in this thesis.

2.2.1 Correlation Coefficient

The similarity between two tests is commonly expressed as a correlation coefficient. The correlation coefficient is a measure of the linearity of the relationship between two data sets [Salkind and Rasmussen, 2007] and is always a value between $r = -1$ and $r = 1$. A value of $r = 1$ or $r = -1$ indicates perfect linearity with a positive or negative slope, respectively. The closer the correlation is to $r = 0$, the less linear the relationship. Note that the lack of a linear relationship does not imply a lack of a relationship, but only that the direct linear association between values is weak.

Different correlation coefficients can be calculated depending on the data distribution. For normally distributed data, the parametric *Pearson product-moment correlation coefficient* can be used. Otherwise, non-parametric coefficients, such as the *Spearman's rank correlation coefficient* are better suited [Salkind and Rasmussen, 2007].

The correlation coefficient is very sensitive to outliers, which is illustrated in the example shown in Figure 2.1. In this case, two different tests were performed on n=10 individuals [Caumo et al., 2000]. The data set includes one highly insulin

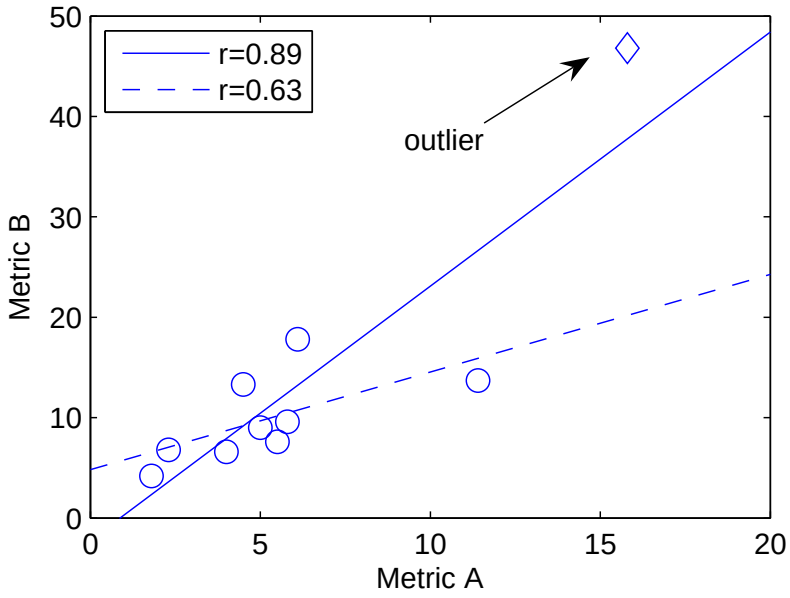


Figure 2.1 Correlation plot between two different test metrics on n=10 individuals from a study by Caumo et al. [2000]. The solid line represents the linear relationship calculated with all samples, resulting in a Spearman's correlation $r = 0.89$. If the high outlier (diamond) is taken out, this relationship is strongly reduced to a Pearson correlation $r = 0.63$ (dashed line).

sensitive outlier. If this subject is included, the distribution of the data set is not normal and the Spearman correlation coefficient has to be calculated, which results in a high correlation of $r = 0.89$. Without this subject, the distribution is normal and the Pearson correlation coefficient is used, resulting in a correlation of $r = 0.63$. This limitation is explicitly mentioned by the authors [Caumo et al., 2000], but that level of detail is usually not provided, making comparisons difficult.

Sample size also has a large impact on calculated correlation coefficients, as visualised in Figure 2.2 on data from a study by Mari et al. [2001]. Correlating the full data set of n=91 individuals results in $r = 0.77$. If the individual subgroups are examined separately, correlations drop to $r = 0.59$ in the lean (circle), $r = 0.73$ in the obese (square) and $r = 0.49$ in the type 2 diabetes (triangle) subgroups. This drop shows that linear relationships might not be transferable if different subgroups are compared separately, indicating that the relationship both changes and grows weaker. Overall, this example shows that a relationship calculated from one subgroup cannot necessarily be extrapolated to another subgroup. Therefore, comparison between studies should be considered critically.

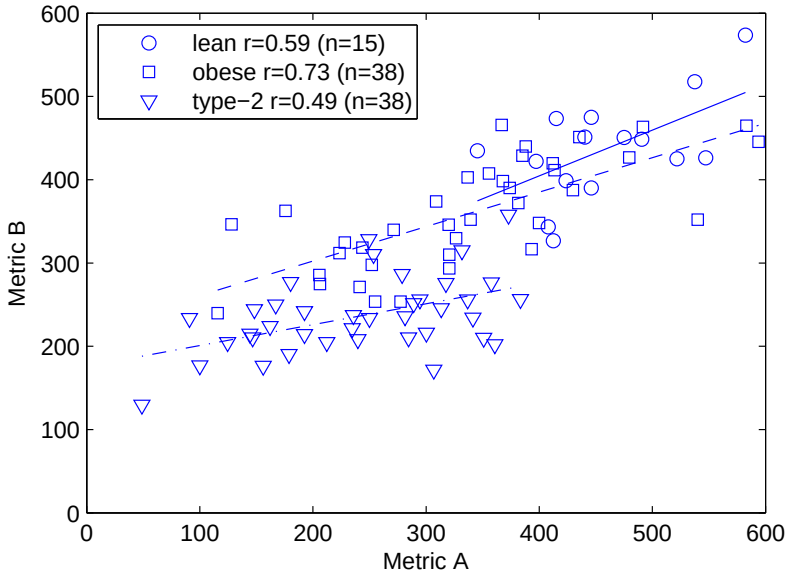


Figure 2.2 Correlation plot between two different test metrics on n=91 individuals from a study by Mari et al. [2001]. The full data set correlates n=0.77, but correlations and slopes of regression lines differ if subgroups are regarded individually. The subgroups are lean (circle, solid line), obese (square, dashed line) and type 2 diabetes (triangle, dash-dotted line).

Correlations in data sets with smaller sample sizes are usually worse than in larger groups because. This is because the ratio between the y-axis data range to the x-axis data range is larger. This effect can be seen in reverse for the obese subgroup (square) in Figure 2.2, in which the regression line is longer than in the other two subgroups, yielding a higher correlation coefficient. Note that the smaller coefficients for the lean and type 2 diabetes subgroups are thus due to their shorter ranges and larger y/x ratios. Keeping these aspects in mind allows the reader to more critically assess and compare reported values.

A correlation coefficient is thus highly dependent on the sample size and distribution. A large sample size is more robust to outliers and yields much more significant correlation coefficient. An equally distributed smaller sample size might result in the same correlation, but any outlier can significantly reduce this correlation coefficient. Hence, it is harder and less reliable to compare results with relatively small sample sizes and/or significant outliers.

Statistical significance of the correlation is usually calculated with hypothesis testing and given as a p-value, with a significance threshold commonly defined as 0.05 [Petrie and Sabin, 2005]. A correlation is thus only considered valid if its

significance level is smaller than the threshold where the null hypothesis is being tested. The range of acceptable correlations in biological and medical research studies differs to that in engineering, due to the more variable nature of biological and physiological system behaviours across subjects and tests. A correlation of $r = 0.5$ is thus normally considered relatively strong, if significant. Correlations between $r = 0.6$ to $r = 0.8$ would be considered very good, with values over $r = 0.9$ being extremely rare.

2.2.2 Test Accuracy

Test accuracy cannot really be determined in absolute terms, as only an effect is measured and the true underlying value is not known. Instead, when assessing the outcome metrics of a test, they are usually correlated to a gold standard test that is known to be highly repeatable. In this case, the accepted gold standard is the euglycaemic-hyperinsulinaemic clamp [DeFronzo et al., 1979]. This comparison is sometimes difficult, as not all tests measure exactly the same effects, but is mostly done nonetheless [Ferrannini and Mari, 1998; Radziuk, 2000].

Accuracy of an insulin sensitivity test is usually defined as its accuracy in repeatability for the same subject. A highly repeatable test can yield consistent and comparable results and allow effective monitoring of interventions. In contrast, natural variability in insulin sensitivity can be captured and interpreted as test inaccuracy or a lack of repeatability. In validation studies, the accuracy of a test is usually given as the coefficient of variation, defined as the standard deviation SD divided by the mean over all subjects in the cohort, CV=SD/mean. This value thus provides a combined measure of the natural variability and systematic or assay variability of a given test. The most repeatable test, the euglycaemic clamp, has been shown to have a CV$= 6\,\% - 10\,\%$ [DeFronzo et al., 1979; Mari et al., 2001; Monzillo and Hamdy, 2003].

2.3 Intravenous Tests

Intravenous tests utilise an injection or infusion of glucose and/or insulin to cause a perturbation of the metabolic system. The accuracy of these tests is generally

better than for oral dosed tests, as the system is more controlled and the unknown gut absorption rate is bypassed, minimising error or variability. These tests are usually more invasive and often take longer to perform. They are thus used primarily in research settings, and are too costly and intense for a wider clinical use.

2.3.1 Euglycaemic/Hyperglycaemic Clamp

The euglycaemic-hyperinsulinaemic clamp method was first presented by DeFronzo et al. [1979]. The idea behind the test is to infuse 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 mmol/l [e.g., McAuley et al., 2001], although any reasonable level may be used. An example glucose and insulin concentration profile for this test with the respective infusion profiles is shown in Figure 2.3.

Due to the high dose infusions of insulin and glucose, hepatic glucose production and pancreatic insulin secretion are almost completely suppressed, and it is assumed that the glucose uptake rate now equals the glucose infusion rate. For this assumption to hold, a steady state needs to be achieved, which can take between 2-3 hours [Ader and Bergman, 1987]. A closed loop system with glucose sampling every 10 minutes, and a continuous adjustment of the glucose infusion rate [DeFronzo et al., 1979; Ferrannini and Mari, 1998] is therefore required to reach and hold this euglycaemic steady state [Bergman et al., 1985]. Measured insulin sensitivity represents mainly peripheral sensitivity, as endogenous glucose production (EGP) is almost completely suppressed.

The insulin sensitivity index, ISI, is then defined as the mean glucose infusion at steady state, M, divided by the mean insulin concentration I at steady state:

$$ISI = \frac{M}{I} \tag{2.1}$$

The physiological hypothesis of Equation 2.1 is thus straightforward. At a steady euglycaemic or fasting level, the infused glucose M over one hour is assumed to be removed by the average plasma insulin present over that hour, since a steady

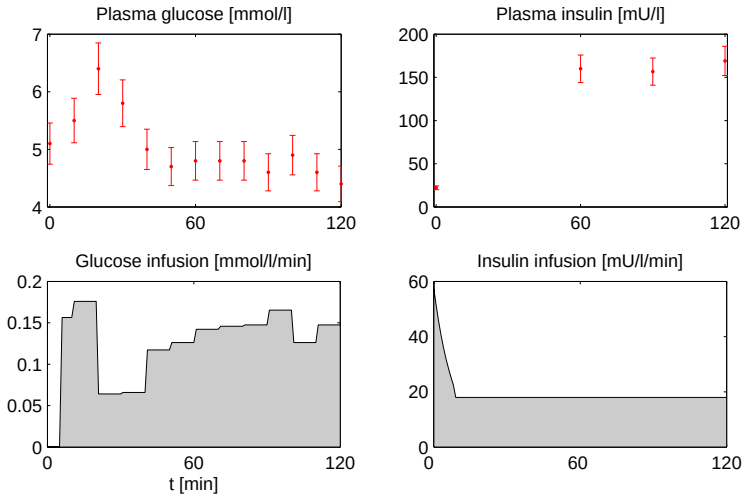


Figure 2.3 Example of a two hour euglycaemic-hyperinsulinaemic clamp from a study by McAuley et al. [2002]. Shown are glucose concentration (upper left), insulin concentration (upper right), glucose infusion rate (bottom left) and insulin infusion rate (lower right).

fasting glucose level was maintained. This ratio is assumed to hold since EGP is suppressed at the supra-physiological insulin infusion rates used.

Due to the clamping of the system and the relatively high infusions, the metabolic system is well controlled and the assessment highly repeatable, which make this method the current gold standard. The test is also very flexible, as it can be performed at different glycaemic or insulinaemic levels to study changes in metabolic effects at these states, or in combination with tracers or other drugs to assess their effect on glucose uptake.

Information about β-cell function can be obtained with the hyperglycaemic clamp, in which a step increase in clamped glucose concentration is introduced to trigger first- and second-phase insulin secretion. Indices commonly used are, for the first-phase secretion, the incremental insulin concentration area under the first 8-10 minutes after the step increase, and for the second-phase the mean insulin levels after the first secretory peak has faded [DeFronzo et al., 1979; Pacini and Mari, 2003]. Unfortunately, insulin sensitivity and β-cell function cannot be obtained simultaneously and require a longer or separate test protocol.

The main drawbacks of the clamp method are the intensity and length of the test. It takes between 2-6 hours to perform, with considerable safety wind-down times. In addition, highly trained medical personnel must be involved full-time,

increasing cost and making widespread use infeasible. Special equipment, such as calibrated pumps, a heating box to arterialise venous blood and a glucose meter are also required. The infusion rates are also supra-physiological, with steady state insulin concentrations of around 100 mU/l, where normal fasting levels are only ∼5-10 mU/l. Overall the test is very complex and intense for both the subject and clinical personnel.

Regarding the test's physiological accuracy, the assumption that all glucose uptake is mediated by plasma insulin is not completely correct. In particular, tissue uptake is dependent on insulin in the interstitium, which is considerably lower than the concentration in plasma at steady state [Yang et al., 1989]. Additionally, non-insulin dependent uptake, such as the constant uptake by the brain and the central nervous system [Zierler, 1999], is not separately accounted for and included in the measured uptake. The test also yields different results if performed at different infusion rates, which suggests a saturable peripheral glucose uptake [Natali et al., 2000; Prigeon et al., 1996] that will lead to an underestimation of the actual sensitivity. Dose dependency of the result also means that ISI values cannot be directly compared if different insulin infusion rates are used. Nonetheless, these effects most likely only introduce a shift in the result and do not affect the overall accuracy in repeatability at those inputs.

Overall, the test is highly repeatable with a reported CV$= 6\,\% - 10\,\%$ [DeFronzo et al., 1979; Mari et al., 2001; Monzillo and Hamdy, 2003]. It also offers a steady state assessment of a wide range of possible metabolic states, as the glycaemic level can be clamped at any reasonable value. Other tests are thus always judged in their performance by their correlation to the clamp metric ISI.

2.3.2 Insulin Tolerance Test (ITT)

The Insulin Tolerance Test (ITT) is one of the first proposed tests to measure insulin sensitivity in vivo [Horgaard and Thayssen, 1929]. A bolus of insulin is injected (0.1 U/kg) and the decay rate of glucose measured at 10-40 minutes after the input. The insulin input is relatively large, translating to 8 U for an 80 kg individual. Insulin sensitivity is defined as the decay rate constant of a single exponential decay. It can be calculated from the time needed to reduce glucose

concentrations by half ($t_{1/2}$):

$$K_{ITT} = \frac{\ln(0.5)}{t_{1/2}} \tag{2.2}$$

The test is relatively simple and short and has been shown to perform well, with good accuracy in repeatability (CV= $6\,\% - 9\,\%$) [Gelding et al., 1994; Monzillo and Hamdy, 2003] and comparability to the clamp ($r = 0.81$ [Bonora et al., 1989; Gelding et al., 1994]) and the IVGTT ($r = 0.76$) [Lindheim et al., 1994], but not to the fasting surrogate HOMA-IR ($r = 0.44$) [Inchiostro, 2005].

Disadvantages include a high risk of hypoglycaemia due to the sudden drop in glucose induced by the relatively large insulin input [Ferrannini and Mari, 1998]. In particular, inaccuracies may be introduced by the counter-regulatory response triggered by hypoglycaemia. This effect can be reduced by applying a shortened version, which takes samples up to 15 minutes post injection [Inchiostro, 2005], avoiding the counter-regulatory response that starts at ∼20 minutes [Monzillo and Hamdy, 2003]. Due to concerns over safety and the relatively large insulin dose, it is primarily a research tool.

2.3.3 Intravenous Glucose Tolerance Test (IVGTT)

Direct Assessment

Assessing glucose tolerance by an intravenous injection of glucose is a practice that has been used for a long time. In particular, a large number of studies discussed this approach in the 1920s-40s [Greville, 1943; Lozner et al., 1941; Orr-Ewing, 1931]. Before the invention of the insulin assay, this decay could only be measured, but not separated into insulin-dependent and independent components. A common practice was to inject a bolus of glucose, or use a fast infusion, and assess the decay rate, defined as the slope K_G of the logarithm of the glucose concentration curve as measured over 1-3 hours [Greville, 1943; Lozner et al., 1941]. This method is comparable to that used in the insulin tolerance test (ITT) and can be applied with no further computer analysis of the data. Measured sensitivity is the combined effect of peripheral and hepatic sensitivity.

An improvement can be made by relating K_G to the increase in insulin concentration, as described by Galvin et al. [1992]. This approach was validated in its 40-minute version against the clamp with good correlation of $r = 0.85$ and a CV=21 % in the overall range of subjects studied (n=30, wide range of glucose tolerance). The performance and resolution was not satisfactory in subjects with low insulin sensitivity, which can be attributed to the small relative increase in insulin concentration in these subjects. It also does not allow any assessment of β-cell function.

Minimal Model Based Assessment (Semi-Direct)

The Minimal Model (MM) of glucose kinetics was presented by Bergman et al. [1979] as a simplified description of the glucose kinetics observed during an IVGTT protocol. By fitting the model parameters to match IVGTT data, metabolic information about the glucose metabolism can be obtained [Bergman et al., 1981; Pacini and Bergman, 1986]. The model differentiates between glucose uptake at basal insulin (S_G^{MM}) and glucose uptake at increased insulin (S_I^{MM}). Additionally two metrics of first- and second-phase insulin secretion can be derived. Insulin dependent uptake is mediated by insulin action in a site remote of plasma, and represents both peripheral and hepatic sensitivities. The model thus incorporates a minimal description of the most important physiological aspects. It was also the first use of mathematical models and computers for testing insulin sensitivity.

The original protocol includes a bolus injection of glucose (0.3 g/kg body weight) and frequent blood sampling for 180 minutes, with a total of about 22 samples assayed for glucose and insulin concentrations. Both arms are cannulated, one for injection and the other for sampling. A heated box is used to arterialise venous blood at the site of sampling [Bergman et al., 1985]. The model is fitted to the data with a non-linear least squares algorithm, estimating three model parameters.

The method has been used very widely in research studies, and is recognised as a reasonably accurate alternative to the slightly more intense clamp procedure. Reported accuracies vary between CV= $14\,\% - 30\,\%$ [Ferrannini and Mari, 1998; Monzillo and Hamdy, 2003; Scheen et al., 1994]. Correlation between Minimal

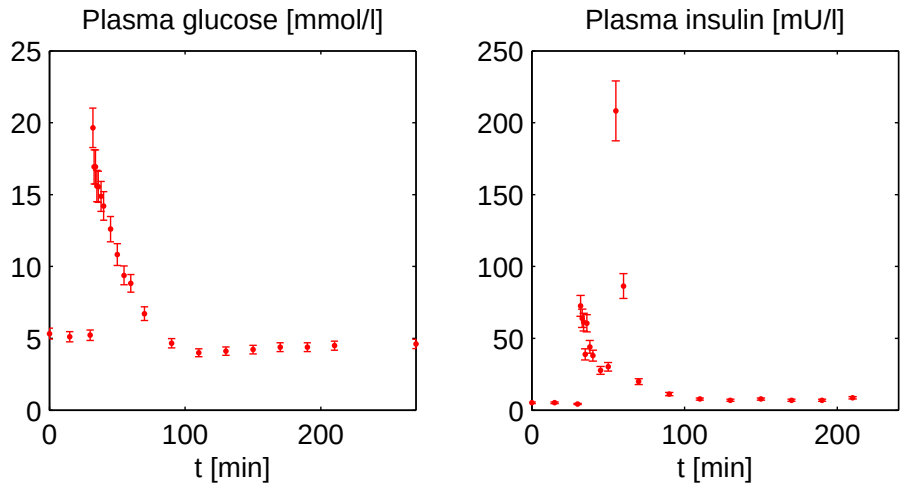


Figure 2.4 Example of an insulin-modified IVGTT on a healthy individual, from Mari [1998]. Shown are the resulting glucose concentrations (left) and insulin concentrations (right). Glucose is administered at $t = 30$ min and insulin between $t = 50 - 55$ min.

Model S_I^{MM} and clamp ISI varies greatly between $r = 0.44 - 0.92$ [Bergman et al., 1987; Coates et al., 1995; Finegood et al., 1984; Foley et al., 1985; Katz et al., 2000] depending on the subgroups and populations studied, and potentially the specific protocols used. Note that this wide range of correlation casts some doubt on the tests accuracy and/or robustness.

Accuracy has also been found to be significantly diminished in subjects with low insulin sensitivities, such as obese and type 2 diabetes individuals, leading to frequent identifiability problems [Cobelli et al., 1986; Donner et al., 1985; Finegood et al., 1984]. To overcome some of these problems, protocol variations were proposed to increase the otherwise weak insulin signal. These variations include infusing tolbutamide to trigger a pancreatic response [Beard et al., 1986; Yang et al., 1987] or injecting insulin 20 minutes after glucose input [Finegood et al., 1990; Quon et al., 1994a]. These modifications improved accuracy, but problems in accurately and repeatedly identifying the parameters still persist. Numerous studies have been performed since to find better fitting approaches to this non-convex problem [Erichsen et al., 2004; Krudys et al., 2006; Pillonetto et al., 2002; Vicini and Cobelli, 2001]. An example of the data from an insulin-modified IVGTT on a healthy individual is shown in Figure 2.4.

More complex modifications were proposed to improve test performance. These enhancements include the addition of glucose tracers to separate the ef-

fects of glucose production and utilisation (Hot MM) [Cobelli et al., 1986], an enhanced two-compartment minimal model (2CMM) [Caumo and Cobelli, 1993], or a circulatory model [Mari, 1998]. All of these approaches yielded mostly better fits, but at a cost of considerably increasing complexity. While this increased complexity is not a big problem in physiological research studies, it renders the test impractical for use in wider clinical settings [ADA, 1998].

When directly compared with clamp derived measurements, the MM-method seems to overestimate S_G^{MM} and consequently underestimate S_I^{MM} [Cobelli et al., 1998; Mari, 1997]. This issue was identified as an undermodelling problem. A proposed solution was to use a two-compartment description [Cobelli et al., 1999], as glucose kinetics during the first 10 minutes are strongly affected by systemic mixing rather than uptake. In addition, the estimation of S_G^{MM} is not very accurate and its estimation is only possible from the portions of the data with low insulin concentrations. Unfortunately, these portions are either in the early 20 minutes, which are mostly affected by mixing, and after 100+ minutes, which is primarily affected or contaminated by counter-regulatory triggered EGP [Callegari et al., 2003; Ferrannini and Mari, 1998]. Shortening the test to 90 minutes was shown to improve parameter estimation in the face of these issues [Callegari et al., 2003].

Overall, the test is still very accurate and allows the assessment of different effects through the addition of tracers and different model variations. Unfortunately, well known modelling and identification problems make the results less reliable. Finally, its length and intensity limit it to a research-only application.

2.3.4 Continuous Infusion of Glucose with Model Assessment (CIGMA)

This test consists of a relatively low dose infusion of glucose over 60 minutes (5 mg/kg/min) to mimic a postprandial state. Glucose and insulin are then sampled during the final 15 minutes, over which a near steady state is attained [Hosker et al., 1985]. The test data are then compared to known physiologic data using a model of whole body glucose homeostasis that accounts for all key inputs and clearances. Accuracy in repeatability is within CV$= 17\,\% - 21\,\%$ [Hosker

et al., 1985; Nijpels et al., 1994] and reported correlations to the clamp vary between $r = 0.66$ [Nijpels et al., 1994] and $r = 0.87$ [Hosker et al., 1985].

As the glycaemic state attained during the test is very physiological, the estimated sensitivity is likely a very accurate match to the true combined peripheral and hepatic sensitivity. Due to the administration of only glucose, the test is not applicable to type 1 individuals, nor is it very accurate in individuals with a weak pancreatic insulin response [Ferrannini and Mari, 1998]. These limitations make it less useful or reliable in clinical situations, although the relative simplicity, safety and potential physiological accuracy make it appealing in research and some limited clinical settings.

2.4 Oral Tests

2.4.1 Surrogate Assessments

The protocol of an oral glucose tolerance test (OGTT) has a clear advantage over intravenous tests. In particular, it is less invasive and the administration of glucose through the gut is very simple, does not require a venous administration, and is more physiological. An example of the resulting concentrations is shown in Figure 2.5.

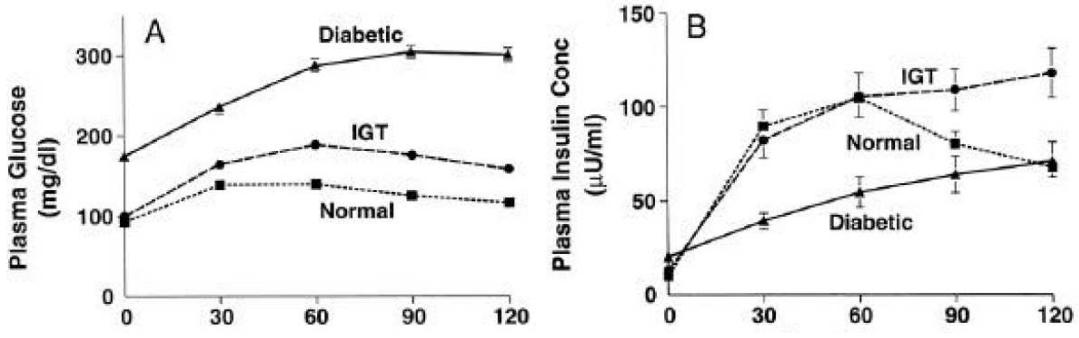


Figure 2.5 Example of a 75 g oral glucose tolerance test (OGTT) [Matsuda and DeFronzo, 1999]. Shown are the resulting glucose concentrations (left) and insulin concentrations (right) for normal, impaired glucose tolerance (IGT) and type 2 diabetes individuals.

A standard OGTT consists of a rapid ingestion of a 75 g or 100 g solution of glucose, followed by timed blood samples for 2-3 hours [Pacini and Mari, 2003].

Blood glucose levels, and sometimes insulin or C-peptide concentrations, are sampled at least hourly, including a fasting sample. The glucose concentration drop during the following hours is an indicator of the body's ability to take up glucose. This indicator of insulin sensitivity usually combines all three effects, peripheral sensitivity, hepatic sensitivity and β-cell function.

Due to its simplicity, it is the current method of choice for a clinical diagnosis of diabetes and recommended by the American Diabetes Association [ADA, 2006]. It is important to note that any of these surrogate measures do not strictly measure a physiological effect, but only give surrogate indications of the actual effect. In particular, the OGTT measures the body's ability to remove a glucose load, which is dependent on insulin sensitivity, but does not measure insulin sensitivity directly. The interpretation of the sampled data varies between tests, sometimes including insulin values, but not always. Some commonly used calculations are described here.

2-hour Glucose

The glucose concentration sampled two hours after glucose ingestion is the most often used indicator of impaired glucose tolerance, as it does not involve any further calculations. It is one of the metrics used as a criterion for the diagnosis of type 2 diabetes (11.1 mmol/l), recommended by the American Diabetes Association (ADA) [ADA, 2006]. Its correlation against the clamp has been found to be good at $r = 0.74$ in a large population (n=188) covering all levels of insulin resistance [Ferrannini et al., 2005]. The repeatability is debatable, with various studies reporting changes in diagnosis in $30\,\% - 65\,\%$ of subjects after a repeat test [Ganda et al., 1978; Ko et al., 1998; Levy et al., 1999; Riccardi et al., 1985]. Its simplicity still makes it the test of choice in clinical practice [Monzillo and Hamdy, 2003].

Matsuda

In the study by Matsuda and DeFronzo [1999] the authors propose an index of insulin sensitivity derived from an OGTT on n=153 subjects with a wide range of glucose tolerance, and validate it against the clamp. Their idea is to incorporate

fasting levels (G_0, I_0), as well as the mean glucose and insulin concentrations (G_{30-120}, I_{30-120}) during the final 90 minutes of a 120-min OGTT. The formula they derived is defined:

$$ISI_{Matsuda} = \frac{10000}{\sqrt{I_0 \times G_0 \times G_{30-120} \times I_{30-120}}} \tag{2.3}$$

Their reported comparison to the clamp is good at $r = 0.73$ in the whole group, but diminishes to $r = 0.66$ for impaired glucose tolerance (IGT) cohorts and $r = 0.54$ in diagnosed type 2 diabetes individuals. Good correlation was found in some studies, ranging from $r = 0.66 - 0.86$ [Chiu et al., 2001; Kirwan et al., 2001; Soonthornpun et al., 2003; Stumvoll et al., 2000], but not all, with $r = 0.21$ found by Kanauchi [2002] in n=113 Japanese subjects covering a wide range of glucose tolerance.

Stumvoll

In a similar study by Stumvoll et al. [2000], the authors propose a more empirical linear regression model, including glucose concentration at 90 minutes (G_{90}) and insulin concentration at 120 minutes (I_{120}), as well as the subject's BMI. The formula they derived on n=104 nondiabetic subjects is defined:

$$ISI_{Stumvoll} = 0.226 - 0.0032 \times \text{BMI} - 0.0000645 \times I_{120} - 0.00375 \times G_{90} \tag{2.4}$$

In their validation against the clamp, they achieve a high correlation of $r = 0.79$ across all subjects. They also derive formulae to estimate β-cell function that are well correlated to the clamp derived metrics. The correlations found in other studies were not as high, with a range of $r = 0.51 - 0.69$ [Chiu et al., 2001; Kanauchi, 2002; Mari et al., 2005; Soonthornpun et al., 2003]. An advantage of this index is that it does not require all the samples taken during a standard OGTT, but only two timed values. It thus reduces time and personnel requirements, making wider use somewhat easier.

Others

Other indices have been derived with similar performances to those described above, including different combinations of glucose and insulin samples taken from an OGTT. Common ones are those by Gutt et al. [2000], Cederholm and Wibell [1990], Kanauchi [2002], Soonthornpun et al. [2003], Avignon et al. [1999] and Belfiore et al. [2001]. Indices of β-cell function have also been derived, usually included some ratio of incremental insulin over incremental glucose concentrations [Pacini and Mari, 2003].

2.4.2 Model-Based Assessments

In spite of the reasonable performance reported for the surrogate metrics discussed in Section 2.4.1, they are not based on a physiological explanation. To measure physiological effects with an OGTT, some studies have proposed models with varying complexity to directly measure insulin sensitivity from OGTT data.

OGIS

The Oral Glucose Insulin Sensitivity index (OGIS) was presented by Mari et al. [2001]. It was derived by assuming a physiological representation of the glucose-insulin interaction during an OGTT. The model parameters were then estimated in that study on a population of n=104 subjects with different degrees of glucose tolerance, from which a mean set was chosen to provide a universally applicable formula. Two different versions are proposed, for use with a 120 or 180 minute test. Correlation to the clamp was good at $r = 0.77$ in the whole group, but worse in the individual subgroups, moving down to $r = 0.49$ in type 2 diabetes individuals. Even if the direct correlation to the clamp is not much better than in other tests, the approach does have a physiological basis that allows a more comparable assessment of metabolic status.

Oral Minimal Model

The Oral Minimal Model (OMM) was proposed by Caumo et al. [2000] for use with meals and Breda et al. [2001] for an OGTT. It combines the original MM by Bergman et al. [1979] with a description of the rate of glucose appearance in plasma, R_a, to estimate the same MM parameters from an orally administered glucose load. This approach allows a direct measurement of IR during a more physiological state, and has been followed up with considerable research and validation efforts. Validation using a meal input against the IVGTT was performed in healthy individuals with good correlations of $r = 0.89$ [Caumo et al., 2000] and $r = 0.75$ [Dalla Man et al., 2002], an example of which is shown in Figure 2.6. The OGTT version was validated against the clamp, resulting in a correlation of $r = 0.81$ [Dalla Man et al., 2005b] and yielding accuracies in repeatability of CV$= 12\,\% - 15\,\%$ in another study [Breda et al., 2001]. The results were also validated with tracer studies and resulted in good linear comparisons with correlations of $r = 0.96$ [Dalla Man et al., 2005a] and $r = 0.86$ [Dalla Man et al., 2004] for the meal and OGTT comparisons, respectively. With the addition of C-peptide sampling and the C-peptide MM, this method also yields pancreatic secretion metrics [Breda et al., 2001].

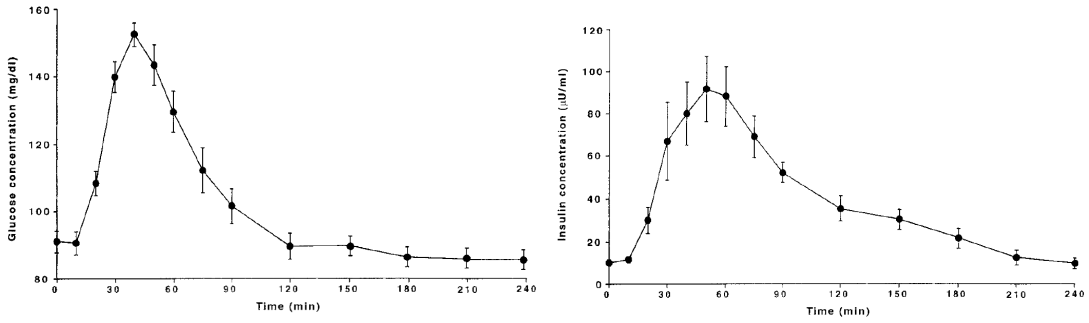


Figure 2.6 Example of a meal tolerance test from Caumo et al. [2000]. Shown are the resulting glucose (left) and insulin (right) concentrations after ingestion of a standardised meal with approximately 75 g carbohydrate content.

2.5 Fasting Tests

Fasting tests rely solely on fasting blood samples to obtain information about the state of insulin resistance. They are very attractive, as no input of glucose or insulin is required and the time and stress involved for the individual and the

medical personnel are minimal. The derivation of the different calculations is sometimes purely empirical, while others are based on linear approximations of physiological model descriptions. The accuracy of many of these fasting surrogate tests can be improved by sampling two or three times and using the mean value [Wallace et al., 2004b]. Some of the most commonly used fasting metrics are described here.

Fasting Plasma Insulin (FPI)

Fasting plasma insulin concentrations are often used to diagnose insulin resistance [Ferrannini and Mari, 1998; Monzillo and Hamdy, 2003]. High concentrations are usually a good diagnostic indicator of insulin resistance in normoglycaemic individuals, and correlations in the range of $r = -0.37$ to $r = -0.59$ have been found when comparing to the clamp test ISI [Ferrannini and Mari, 1998; Kang et al., 2005; McAuley et al., 2001; Ruige et al., 2006]. Only some of the variability in IR is seen in the insulin concentration, as this value also depends on other factors, such as insulin secretion, clearance and distribution [Monzillo and Hamdy, 2003]. In subjects with type 2 diabetes, fasting insulin levels tend to become very high, but then decrease over years as the pancreas exhausts. This behaviour results in inaccurate assessments of IR with this test value [Kang et al., 2005; Monzillo and Hamdy, 2003].

Fasting Plasma Glucose (FPG)

High FPG levels are often used as a first screening indicator of impaired glucose tolerance [ADA, 2006]. The problem is that IR is apparent many years before glucose levels rise, and compensated by higher endogenous insulin secretion. FPG is thus only a valid indicator if the state of impaired glucose tolerance is significantly progressed. Nonetheless, when compared to the clamp, reasonable correlations of $r = 0.50 - 0.59$ are achieved [Kang et al., 2005; Lewanczuk et al., 2004]. FPG is an ADA recommended criterion for screening IGT and type 2 diabetes [ADA, 2006].

Fasting Plasma I/G ratio

A logical conclusion from just using FPG or FPI is to use the ratio of both. This ratio is commonly used in large screening studies, but does not necessarily perform better than the individual metrics, when compared to the clamp, with correlations reported in the range of $r = -0.41$ to $r = -0.68$ [Hanley et al., 2003; McAuley et al., 2001]. Note that the index assesses insulin resistance, which is equivalent to the reciprocal of sensitivity, thus resulting in a negative correlation.

Homeostatic Model Assessment (HOMA-IR)

The HOMA-IR index (also called just HOMA) was derived from a physiological whole body glucose-insulin model, developed on known physiological data [Matthews et al., 1985]. It is the same model used in the assessment of CIGMA. The authors proposed a simplified linear equation to approximate the computer model:

$$\text{HOMA} - \text{IR} = \frac{G_0 \times I_0}{22.5} \tag{2.5}$$

A new, more accurate solution of the original computer model was presented as HOMA2 [Levy et al., 1998], but the approximated formula of Equation 2.5 is still widely used, due to its simplicity. The index assesses IR, which is equivalent to the reciprocal of sensitivity. Comparison to the clamp and IVGTT are thus negative. Many studies have used HOMA-IR so far to assess IR, and correlations against the clamp vary widely between $r = -0.19$ to $r = -0.94$ [Bonora et al., 2000; Mari et al., 2001; Mather et al., 2001; McAuley et al., 2001; Ruige et al., 2006]. However, most results reside within $r = -0.4$ to $r = -0.6$ depending on the subgroups used. One example of this variability is seen in the study by Mari et al. [2001], in which the overall population achieved $r = -0.75$, but the type 2 diabetes subgroup only $r = -0.19$. Even so, the index is considered a reasonable indicator of IR, and has been widely used to date due to its simplicity and comparability across studies.

A separate HOMA index of β-cell function, HOMA-%B, can be calculated similar to above:

$$\text{HOMA} - \%\text{B} = \frac{20 \times I_0}{G_0 - 3.5} \tag{2.6}$$

McAuley Index ($ISI_{McAuley}$)

The McAuley index was developed as an empirical regression model on data from n=178 clamp tests on normoglycaemic individuals [McAuley et al., 2001]. Different surrogate indicators of IR were used to find the most useful predictors. The equation derived by the authors includes fasting insulin and triglyceride (TG) concentrations, the latter of which is often increased in type 2 diabetes:

$$ISI_{McAuley} = e^{(2.63 - 0.28 \times \ln(I_0) - 0.31 \times \ln(\text{TG}))} \tag{2.7}$$

The correlations against the clamp are not significantly different to other fasting metrics, as assessed in various studies with $r = 0.48 - 0.61$ [McAuley et al., 2002; Oterdoom et al., 2005; Ruige et al., 2006].

$\mathbf{HbA_{1C}}$

The use of glycosylated hemoglobin levels, $\text{HbA}_{1\text{C}}$, has been proposed in some studies as a marker to screen and diagnose diabetes [Bennett et al., 2007; Droumaguet et al., 2006; Peters et al., 1996]. $\text{HbA}_{1\text{C}}$ levels represent a 2-3 month average of blood glucose levels and are thus not affected by short term changes or incomplete fasting states, which is a significant advantage in screening applications. The performance of this approach was compared to the ADA-recommended guidelines, the 2-h OGTT [Bennett et al., 2007; Peters et al., 1996] and a fasting glucose value [Bennett et al., 2007; Droumaguet et al., 2006]. Performance was found to be similar, with comparable sensitivities and specificities as these other metrics. However, it also offered the advantage of a higher intra-individual repeatability, with assay accuracy within CV$= 2\,\% - 4\,\%$ [Barr et al., 2002]. It

could thus be the most reliable of the fasting metrics for screening purposes.

Others

Further fasting indices include QUICKI [Katz et al., 2000], which is essentially a log-transformed HOMA-IR, and similar metrics proposed by Hanson et al. [2000] and Belfiore et al. [1998] that do a different calculation with fasting glucose and insulin values. All of these indices perform well in some studies and not so well in others. They are mostly good indicators if used for broad screening purposes, but do not achieve the necessary resolution to monitor frequent changes in IR, or to provide significant early warning or diagnosis.

2.6 Method Comparisons

Comparing the different methods is a difficult task as they do not always measure the same physiological effects or have the same units. As discussed, the three effects, peripheral sensitivity, hepatic sensitivity and β-cell function all contribute to the overall whole body sensitivity and response to glucose. Some of the more complex tests, as the clamp, IVGTT, or the OMM, can differentiate between all three effects if performed with tracer labelled glucose and C-peptide sampling. This flexibility is an advantage over simpler tests for physiological research studies in which the effects of drugs on each individual aspect can be assessed. In contrast, the high intensity, duration and cost involved, make them infeasible for wider clinical studies or any reasonable form of population screening.

In a clinical setting, factors such as simplicity, safety and time are much more critical. In addition, the amount of training necessary for the performing medical personnel is important, as it cannot be expected that every general practitioner will undergo lengthy and costly specialised training to be able to offer such a test. In this typical clinical setting, much simpler, and less intense and costly surrogate tests, such as an OGTT or fasting assessments are desirable. The loss in repeatability and reliability is often acknowledged as a necessary compromise, or simply not acknowledged and used nonetheless. The consequence is that a state of resistance can often only be diagnosed when the state is significantly

progressed and irreversible damage has already begun to occur.

Another aspect to be considered is the different settings each test is performed in. Insulin sensitivity can be different in a fasting state, in which the system is in equilibrium, than in an intravenous perturbation [Ferrannini and Mari, 1998]. It can then be different again for a more physiological oral perturbation [Ferrannini and Mari, 1998]. In fact, oral glucose triggers insulin-stimulating gastrointestinal hormones, that are not present in an IV input [Breda et al., 2001]. The supra-physiological concentrations achieved during a clamp test have been shown to result in different results, as saturation effects become evident and significant [Natali et al., 2000]. Other differing effects are seen in test performance if insulin is injected or stimulated using drugs such as tolbutamide, which is necessary in insulin dependent diabetes subjects to increase the insulin signal [Quon et al., 1994a].

All the tests described in this chapter, except the HbA_{1C}, require a fasted state to yield reliable results. This aspect can potentially confound results if not adhered to, which probably makes the HbA_{1C} value the most robust analysis. An early diagnosis of IR is still not possible with this assessment, as an elevated HbA_{1C} is only seen after significant β-cell damage has occurred.

As a general observation it can be said that intravenous tests have the highest repeatability, as they are the most controlled tests. The highest repeatability is seen in the clamp, due to the suppression of all endogenous glucose and insulin output, thus reducing any unknown dynamics. The IVGTT triggers unmodelled regulatory responses that negatively affect model fitting [Cobelli et al., 1998; Mari, 1997]. The source of these problems is evident and can be addressed in improved fitting algorithms and fine-tuning of the protocol, rather than via more complex modelling or clinical exercises [Lotz et al., 2006a].

Oral tests are more variable, due to the additional transport path involved in the administration of glucose. The gastric absorption rate cannot be measured without tracers and can be very variable. Any estimation of this rate involves simplified assumptions and thus results in a larger introduced variability in the overall test result. A larger number of samples used in the calculation of the metric can reduce variability by capturing the dynamic response more completely. Hence, the composite indices perform better than using just an OGTT 2-hour

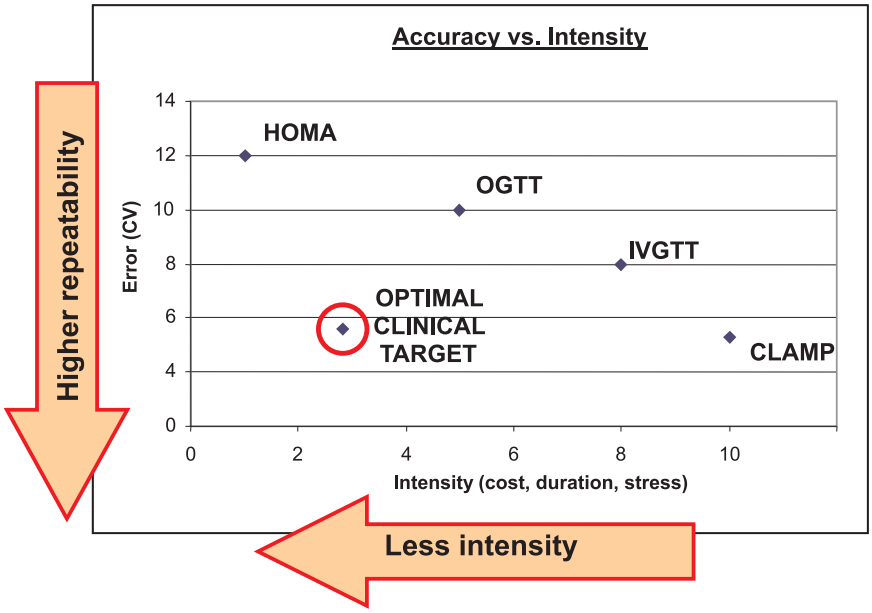


Figure 2.7 Comparison of different insulin sensitivity tests and the target area for a better clinical test. The points are plotted according to the test's intensity and accuracy in terms of repeatability (CV in percent). Intensity is a composite symbolic measure of cost, duration and overall stress involved.

post load glucose sample. The fasting tests also show reduced performance for similar reasons, as only one sample is taken. Note that the repeatability of a fasting test can be improved by using the mean of 2-3 samples, instead of only one [Katz et al., 2000; Wallace et al., 2004b], but at a cost of increased intensity. Nonetheless, even with a perfect test, varying results would be seen as insulin sensitivity has been shown to vary with times of day and night [Bolli, 1988; Van Cauter et al., 1997].

When designing a new test to measure insulin sensitivity that is repeatable, accurate and feasible in a clinical setting, the positive aspects of the different methods described can be exploited. In particular, improvements in the protocol and algorithms can be made to prevent sources of error identified in previous approaches. In Figure 2.7 the accuracy in repeatability of insulin sensitivity tests is plotted against the test intensity, with intensity being a composite measure of cost, duration and overall stress involved. Thus, this figure clearly highlights the design space and goal region for any clinically useful, yet accurate, test.

The overall goal of this research is to develop a new clinical insulin sensitivity test to match or exceed the target defined in Figure 2.7. Important test aspects

that contribute to meeting this goal include:

- **Duration:** Duration can be minimised by developing a fasting test or by using an intravenous administration. Orally administered glucose takes 30-60 minutes to peak, rendering a short accurate test impossible.
- **Simplicity:** Special equipment (pumps, heated box, realtime test adjustments) or tracers should be minimised and preferably eliminated. The test should also ideally require no specialised procedures and need a minimal amount of samples.
- **Cost:** Cost is a logical consequence of duration, simplicity and number of samples.
- **Accuracy:** By incorporating physiological knowledge about the kinetics of glucose and insulin, the test protocol can be optimised to minimise known sources of error, such as systemic mixing, counter-regulatory responses, EGP, or saturation effects. By also sampling C-peptide, β-cell function can be estimated in addition to insulin sensitivity.
- **Safety:** To maximise safety, any dosing should be as low as possible, while still providing a clear signal. This approach also improves physiological accuracy by reducing saturation effects.
- **Robustness:** The robustness of the protocol should be such that possible sources of experimental error, both computational and ergonomic/systemic, are minimised. The data analysis algorithm should not require manual analysis, input, or fitting.

2.7 Summary

Many different approaches to measure insulin sensitivity have been proposed to date, varying in complexity and accuracy. Tests that measure sensitivity directly use intravenous or oral administration of glucose and include sampling of glucose, insulin and C-peptide to capture the dynamic metabolic response to that perturbation. The more repeatable tests, such as the clamp or the IVGTT are only

used in research settings, as they are too complex, intense and costly for regular or widespread clinical application.

Simpler, but less accurate, surrogate metrics vary from calculations derived from OGTT data to using only one fasting sample. These assessments are primarily derived from empirical regression models and do not have the resolution to give more than a high/low result. Nonetheless, they are the only clinically feasible tests to date to gain widespread clinical usage and acceptance.

A clinically useful test that is repeatable and that provides a good resolution to monitor small changes in sensitivity would enable earlier and more accurate diagnosis of insulin resistance. Such a test could be engineered by considering the good aspects of available accurate research tests, while reducing their intensity, and also avoiding errors identified in their methods. This engineering task is the primary goal of this research.

Chapter 3

Insulin Modelling and Identification

To assess insulin sensitivity to glucose uptake, physiological models of the key metabolic pharmaco-dynamics (PD) are required. These dynamics can be categorised as the kinetics of insulin, glucose and their pharmaco-dynamic interaction. Endogenous insulin secretion can be estimated indirectly in this process by modelling C-peptide kinetics.

The modelling goal of this chapter is to attain pharmaco-kinetic (PK) models of insulin, that are physiologically valid, yet simple enough to be identifiable with a short, simple test and limited blood sampling. The physiology and modelling of insulin and C-peptide are discussed. Appropriate identification methods that can be applied within the proposed test protocol are presented for each model. The identification methods are presented with each specific model as they are inter-related and specific.

3.1 Insulin Kinetics

3.1.1 Physiology

Insulin is a hormone secreted by the pancreas, that plays a very important role in regulating carbohydrate metabolism. It enables glucose uptake by muscle and adipose tissue cells, regulates storage and release of glucose in the liver, and promotes fat synthesis and storage [Guyton and Hall, 2000; Jefferson and Cherrington, 2001]. It also has several other effects, including anti-inflammatory effects [Dandona et al., 2006; Hansen et al., 2003], that are not the focus of this

thesis and are well discussed in the relevant literature.

The insulin hormone is a polypeptide, composed of 51 amino acids with a molecular weight of 5808 Daltons (Da). It is produced by the β-cells within the islets of Langerhans of the pancreas [Guyton and Hall, 2000]. The pancreas secretes insulin into the portal vein, where it first passes through the liver and subsequently enters circulation. From there it is distributed to interstitial fluid, where it binds to cell-membrane receptors to activate glucose uptake [Jefferson and Cherrington, 2001], as shown in Figure 3.1.

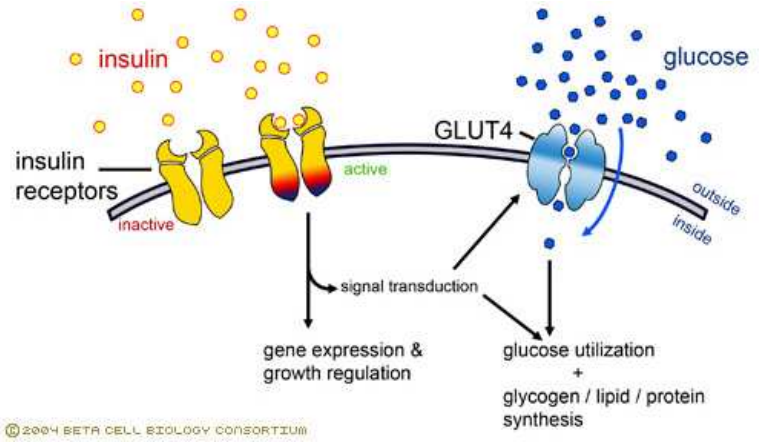


Figure 3.1 Schematic of insulin binding to receptors on tissue cells to activate glucose uptake (taken from www.betacell.org [2004]).

Secretion of insulin by the pancreas is bi-phasic in healthy individuals [Guyton and Hall, 2000; Jefferson and Cherrington, 2001]. The first phase consists of pre-produced and stored insulin and is secreted as a burst immediately after plasma glucose concentrations rise significantly. After approximately 10 minutes, a second phase secretion starts, which is a rise in the steady production rate to meet the body's metabolic need [Guyton and Hall, 2000; Jefferson and Cherrington, 2001]. Note that this response to glucose matches the behaviour of a PID controller, which has consequently also been used as an approach in closed loop control of diabetes [Chase et al., 2006; Chee et al., 2003; Steil et al., 2006].

Clearance of insulin by the body is mainly accomplished by the liver, accounting for up to 80 % of total clearance [Duckworth et al., 1988; Ferrannini and Cobelli, 1987b]. After secretion into the portal vein, insulin passes through the liver, where approximately 50 % is extracted and stored or degraded (first pass extraction), before insulin even reaches the systemic circulation [Duckworth

et al., 1988; Ferrannini and Cobelli, 1987b]. This mechanism allows for a fast response and control of circulating insulin. Further clearance is taken over by the kidneys, as a function of glomerular filtration rate (GFR) [Duckworth et al., 1998], and through cellular degradation after binding to enable glucose uptake in the periphery [Guyton and Hall, 2000; Jefferson and Cherrington, 2001].

Insulin was first isolated from a dog pancreas by Banting et al. [1922], and was soon produced commercially to treat diabetes [Jefferson and Cherrington, 2001], mainly by extracting pork, beef or fish insulin, which are very similar to the human molecule. The first genetically engineered insulin (Humulin) appeared on the market in 1982. Presently, most insulins sold are genetically engineered human insulins or analogs, that provide a wide range of half-times in the blood plasma following subcutaneous injection. The goal is to better mimic first or second phase pancreatic insulin response.

In this study, insulin is assumed to be human insulin, or equivalent, as administration is intravenous and no delay in action is required. The remaining kinetics can thus be modelled physiologically. A physiologically accurate model should thus include the main volumes of distribution, the main mechanisms of transport between these volumes, and the dominant irreversible losses to the liver and kidneys.

To summarise the physiology to be modelled: Insulin is initially secreted into the bloodstream by the pancreas and cleared by the liver, and to a lesser extent, the kidneys. Through transcapillary transport, insulin is diluted into interstitial fluid, reaching tissue cells where it binds to activate glucose uptake. In this final process, insulin is internalised and degraded by the cell.

3.1.2 Modelling

The fundamental goal in modelling insulin kinetics is to develop a physiological insulin kinetics model that includes all major physiological pathways. Only a model that is physiologically valid can provide a useful diagnostic outcome, where physiological validity in this modelling work is achieved when all structural components are derived from actual physiological mechanisms, and the identified parameter values lie in a physiologically justifiable range.

Equally important, it must also be both identifiable and simple enough to be useful in clinical applications proposed with limited measurements. These requirements imply a model to which simple, but accurate, parameter estimation techniques can be applied. Identified parameters can then provide a diagnostically relevant outcome.

3.1.2.1 Background

Many different insulin modelling approaches have been pursued since the late 1960s, analysing insulin kinetics with one to three compartment models with different losses and physiological explanations [Frost et al., 1973; Hovorka et al., 1993; Jones et al., 1984; McGuire et al., 1979; Sherwin et al., 1974; Silvers et al., 1969; Tranberg and Dencker, 1978]. Physiological explanations for the compartments and their parameters differed depending on the parameter values identified using clinical data.

In the pioneering work in this field by Sherwin et al., an IV bolus of insulin and a constant infusion is fit by different models ranging from one to four compartments [Sherwin et al., 1974], as shown in Figure 3.2. The authors concluded that a three compartment model is necessary to accurately reflect the kinetics of the decay curve, and they propose a model with compartments representing plasma, hepatic plasma and extravascular fluids. Sherwin et al.'s model also contains inputs to the hepatic and plasma compartments, and irreversible losses from the plasma compartment. Due to the large number of parameters and the limited sampling resolution available, this model is difficult to identify uniquely.

Other studies have examined simplifying this model to two compartments as two exponentials can describe the observed plasma insulin decay sufficiently well within measurement error [Ferrannini and Cobelli, 1987a; Turnheim and Waldhausl, 1988]. These studies typically unify the plasma and hepatic compartments, as shown in Figure 3.3, approximating them as fast exchanging relative to other dynamics [Frost et al., 1973; Polonsky et al., 1986a; Tranberg and Dencker, 1978]. These simplifications allow easier identification, but the assumed transport paths are not always physiologically accurate and thus do not give an accurate representation of the complete observed kinetics. Ferrannini and Cobelli [1987a] concluded, after a detailed review of modelling efforts to that date, that a two

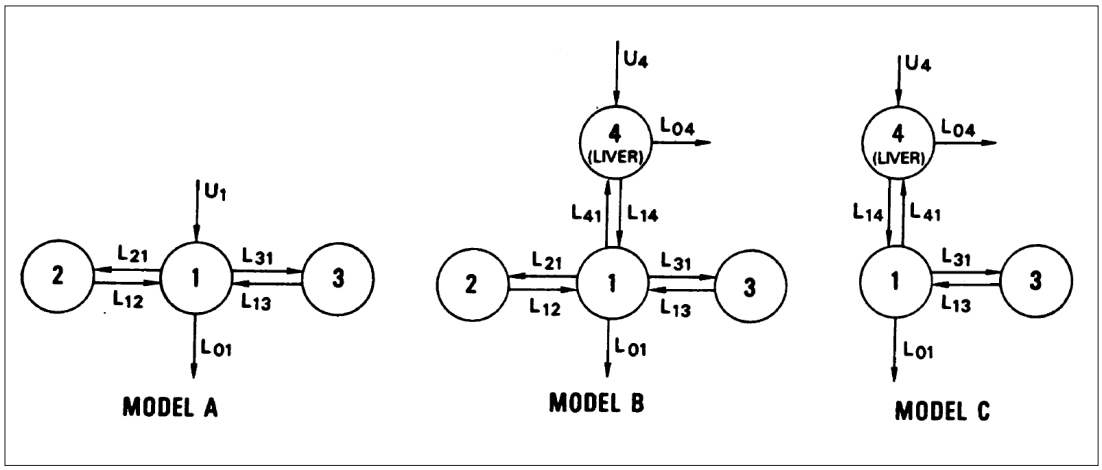


Figure 3.2 The three models analysed in the pioneering work by Sherwin et al. [1974].

compartment model with irreversible losses to both compartments would provide the best compromise in physiological accuracy and identifiability. However, a model would only be useful if combined with an identification method capable of providing a unique solution [Ferrannini and Cobelli, 1987a]. To date, such a method of uniquely identifying all the parameters has not been presented.

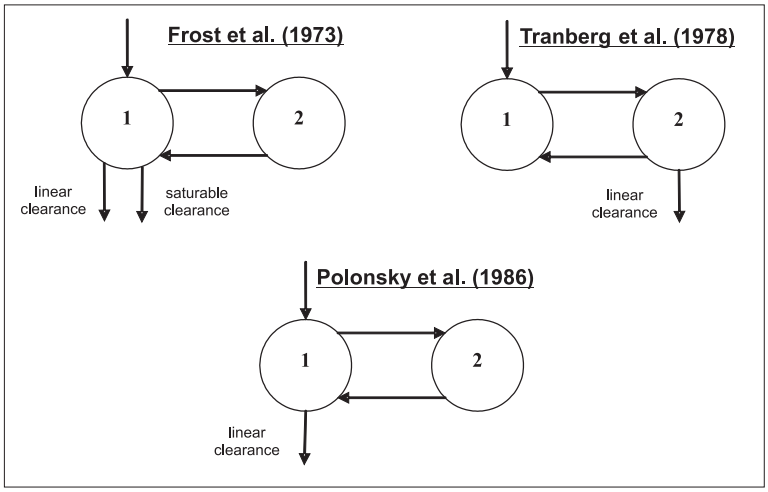


Figure 3.3 Three two compartment models with varying locations and interpretations of irreversible losses [Frost et al., 1973; Polonsky et al., 1986a; Tranberg and Dencker, 1978].

Due to these identification problems, most recent studies have employed a mono-compartmental description, with a single linear loss and an input term [Bergman et al., 1985; Carson and Cobelli, 2001; Toffolo et al., 2006, 1995]. Combined with the physiology of a delayed, remote site of insulin action, this description results in a basically two compartment description, without a back-

flow into the plasma compartment. A form of this simplified kinetics model has been successfully used in glycaemic control studies in the ICU [Chase et al., 2006, 2005a; Wong et al., 2006a]:

$$\frac{d}{dt}I(t) = \frac{-nI(t)}{1+\alpha_I I(t)} + \frac{u(t)}{V_P} \tag{3.1}$$

$$\frac{d}{dt}Q(t) = -kQ + kI \tag{3.2}$$

This model is a reasonably accurate representation of insulin kinetics. It has been particularly useful in control applications with limited knowledge of the actual insulin concentrations. However, it does not necessarily adhere to mass conservation laws and thus lacks important physiological knowledge that is absolutely required for a detailed physiological test.

3.1.2.2 Model Structure

The proposed insulin kinetics model shown in Figure 3.4 is derived from Sherwin et al.'s three compartment model [Sherwin et al., 1974]. However, it is reduced to two compartments by integrating the hepatic and plasma compartments, as the transport between these compartments is very fast [Ferrannini and Cobelli, 1987a; Sherwin et al., 1974]. The decay of an IV injection of insulin has been shown to follow a double exponential decay curve sufficiently well in several studies [Carson and Cobelli, 2001; Ferrannini and Cobelli, 1987a; Turnheim and Waldhausl, 1988], further justifying this reduction.

The model in Figure 3.4 consists of a central or accessible compartment representing the plasma space and fast exchanging tissues, such as the splanchnic bed, and a peripheral compartment representing the interstitial fluid. These compartments are further described as plasma and interstitial spaces, with distribution volumes V_P and V_Q, respectively. Transport between them is bi-directional and each compartment has a clearance pathway. The plasma input, $u(t)$, represents the appearance of insulin into the model.

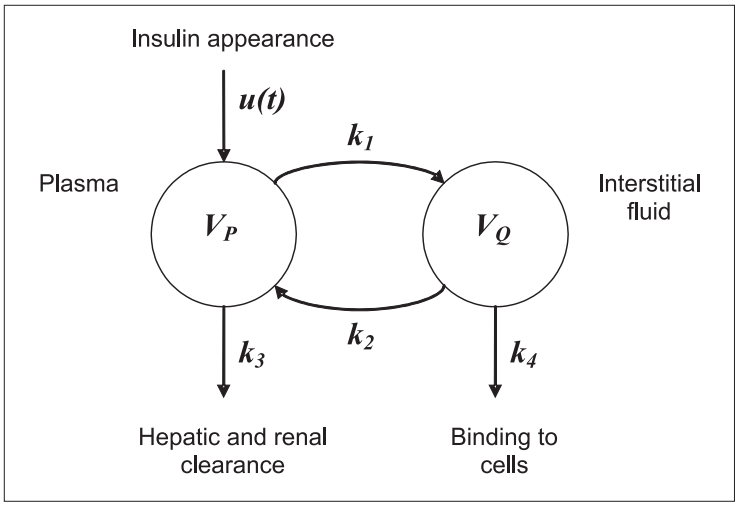


Figure 3.4 Initial structure of two compartment insulin kinetics model.

The differential equations describing the amounts of insulin in the two compartments are dependent on the fractional turnover rates $k_1 - k_4$ (min^{-1}), the amount of insulin in both compartments, $i(t)$ and $q(t)$ in mU and the model input $u(t)$ in mU/min:

$$\frac{d}{dt}i(t) = -(k_1 + k_3)i(t) + k_2 q(t) + u(t) \tag{3.3}$$

$$\frac{d}{dt}q(t) = -(k_2 + k_4)q(t) + k_1 i(t) \tag{3.4}$$

Reformulating Equations 3.3-3.4 in terms of concentrations, with $I(t) = i(t)/V_P$ and $Q(t) = q(t)/V_Q$ yields:

$$\frac{d}{dt}I(t) = -(k_1 + k_3)I(t) + k_2\frac{V_Q}{V_P}Q(t) + \frac{u(t)}{V_P} \tag{3.5}$$

$$\frac{d}{dt}Q(t) = -(k_2 + k_4)Q(t) + k_1\frac{V_P}{V_Q}I(t) \tag{3.6}$$

where V_P and V_Q are given in litres and the concentrations $I(t)$ and $Q(t)$ in mU/l.

This formulation includes the main pathways of insulin, but still combines some separate physiological processes into single parameters. Specifically, the hepatic and renal clearances are combined in k_3. It also allows for differing transports between the compartments by allowing an independent estimation of k_1 and k_2. The model can still achieve a good fit to clinical data, but estimated parameters could be non-physiological and therefore may not be accurate for prediction or model-based clinical testing.

To make the model description more physiological, the following equivalent model formulation is suggested, with explicit choices of transport and mass conservation fully enforced:

$$\frac{d}{dt}I(t) = -n_K I(t) - n_L \frac{I(t)}{1+\alpha_I I(t)} - \frac{n_I}{V_P}(I(t) - Q(t)) + \frac{u_{ex}(t)}{V_P} + (1-x_L)\frac{u_{en}(t)}{V_P} \quad (3.7)$$

$$\frac{d}{dt}Q(t) = -n_C Q(t) + \frac{n_I}{V_Q}(I(t) - Q(t)) \quad (3.8)$$

where the parameters n_K, n_L, n_C are in min^{-1}, n_I in l/min, x_L as a fraction and α_I in l/mU.

The irreversible clearance of insulin from plasma is mainly taken care of by the liver and the kidneys. The liver clears as much as 80 % of the total losses [Ferrannini and Cobelli, 1987b] and can vary widely depending on concentrations and individual state. Studies have also shown a saturation of liver clearance at higher concentrations [Ferrannini et al., 1983; Thorsteinsson, 1990], due to the mechanism by which insulin is cleared, namely binding to liver cells and its resulting degradation. The liver clearance can thus be described by the parameter n_L, which includes a Michaelis-Menten saturation term with the saturation parameter α_I [Thorsteinsson, 1990].

At low insulin concentrations the kidneys clear insulin mainly by glomerular filtration (GFR), a constant rate dependent on blood flow [Despopoulos and Silbernagl, 2003]. At higher plasma insulin concentrations, mainly postprandial or during exogenous input, kidney clearance has been measured to be about twice

GFR [Samnegard and Brundin, 2001; Zavaroni et al., 1987]. This additional clearance appears to be by peritubular excretion [Rabkin et al., 1984]. Clearance by the kidney can thus be described in this model by a separate parameter n_K, and is assumed to be linear. Although it could be time-varying between 1-2 times GFR, it is assumed constant in this study, allowing any variation to be captured by n_L. The parameter k_3 in Figure 3.4 is thus split into the renal and hepatic clearances n_K and n_L.

Transcapillary transport from plasma to interstitium has been studied by others and found to occur mainly via diffusion in vivo in dogs and humans [Castillo et al., 1994; Gudbjornsdottir et al., 2003; Rasio et al., 1967; Sjostrand et al., 1999; Steil et al., 1996; Yang et al., 1989]. In this model a passive transport by diffusion alone is assumed, due to a large amount of evidence that it is the primary and/or dominant mechanism. Transcapillary diffusion is concentration driven and bi-directional, and is described in this model by the diffusion constant n_I, defined by the volumes and k_1 and k_2 from Equations 3.3-3.4:

$$n_I = k_2 V_Q = k_1 V_P \tag{3.9}$$

This relationship implies the fixed ratio $k_1/k_2 = V_Q/V_P$, a relation that will hold in general for any pair of fractional turnover rates between two compartments, provided the transport is passive. For example, this passive transport could be in the form of diffusion or convective exchange of fluid between the compartments. Finally, Equation 3.9 also enables equal transport and thus enforces mass conservation, where independent identification of k_1 and k_2 might not.

The irreversible loss from the peripheral interstitial compartment is believed to occur mainly due to binding of insulin to the cells and its subsequent degradation [Conn and Goodman, 1998; Jefferson and Cherrington, 2001]. The rate at which insulin is degraded at the cells is described by n_C, which is equivalent to k_4 from Equation 3.4.

The input $u(t)$ to the plasma compartment is split into separate exogenous and endogenous inputs, described with $u_{ex}(t)$ and $u_{en}(t)$, respectively. Exogenous inputs are typically known. In contrast, endogenous secretion is less well

known, but can be estimated using the population model of C-peptide kinetics by Van Cauter et al. [1992].

The final formulation of the model is shown in Figure 3.5. The model now has 6 parameters and cannot be uniquely identified by a double exponential, unless constraints are applied to reduce the degrees of freedom. This process could include physiological a-priori identification of less critical parameters and fitting of more dominant parameters. The identification approach could also vary depending on the application and given data density.

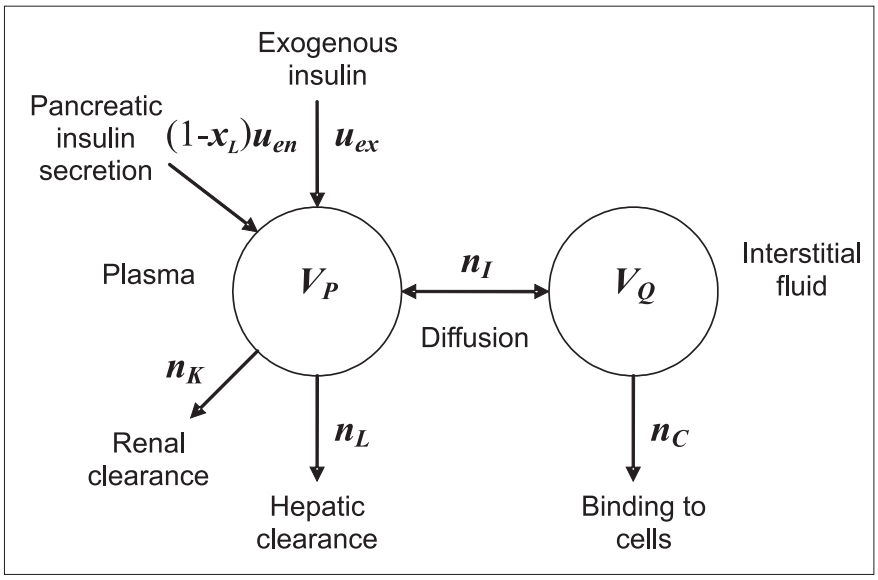


Figure 3.5 Final structure and formulation of the two compartment insulin kinetics model.

The key differences between this model and previously proposed models are the physiological plausibility of all parameters and the diffusion constant between the compartments, that fixes the gearing ratio of the transport between the compartments and enforces strict mass conservation. This aspect has not always been strictly maintained in previous models [Eaton et al., 1984; Sherwin et al., 1974]. One simplification made from the more complex three compartment structure is the combination of the hepatic and plasma compartments into one, as a separate identification of both would not be possible in a clinical setting. Overall, the model has physiological validity and is simple enough for use in clinical applications.

3.1.3 Model Identification

Unique identification of all the model parameters in Figure 3.5 is difficult if all parameters are variable. Commonly used non-linear recursive least squares (NRLS) approaches are starting point dependent and computationally intense [Carson and Cobelli, 2001]. Used correctly, a wide range of starting values should be employed to enable an identification of the global minimum ([e.g. Thorsteinsson et al., 1987]). To overcome this issue, other methods have been proposed, such as Bayesian approaches employing a-priori known parameter distributions to bind parameter estimation to a physiological range [Carson and Cobelli, 2001; Pillonetto et al., 2002]. Nonetheless, with a rising number of parameters, unique identification becomes difficult and often a wide range of parameter combinations can result in a comparable quality of fit to clinical data, even though the parameter values are not necessarily physiological.

3.1.3.1 A-priori Information

A commonly used strategy to overcome this limitation is to reduce the number of parameters by exploiting known a-priori physiological information to fix parameters to constant values or introduce relationships between them [Carson and Cobelli, 2001; Hann et al., 2005b; Hovorka et al., 1993]. As the insulin sensitivity test is meant to only require plasma samples, parameters governing interstitial concentrations, such as n_I and n_C are difficult to identify directly. To improve identifiability, cellular clearance n_C is thus linked to n_I to achieve a fixed steady state concentration gradient γ between both compartments:

$$\gamma = \frac{Q_{ss}}{I_{ss}} \tag{3.10}$$

This concentration gradient has been studied by a variety of researchers by measuring lymph concentrations of insulin and comparing them to plasma concentrations [Castillo et al., 1994; Steil et al., 1996; Yang et al., 1989]. These studies have reported mean values of $\gamma = 0.34 - 0.6$. In recent studies, measuring concentrations directly at muscle tissue with a new microdialysis catheter technique [Gudbjornsdottir et al., 2003; Sjostrand et al., 1999, 2000], Q_{ss}/I_{ss}

gradients of $0.4 - 0.6$ have been measured during euglycaemic clamps. The ratio $\gamma = 0.5$ is chosen as a consistent mean value of the above mentioned studies. The choice of this value has an effect on estimated insulin sensitivity, as described in more detail in Chapter 7. By fixing this constant subject-independent value across individuals, the bias introduced by this effect is only systematic and equal in magnitude across all individuals, thus not introducing added variability.

Cellular clearance n_C can thus be calculated as a function of n_I and V_Q using steady state assumptions of Equation 3.8:

$$0 = -n_C I_{ss}\gamma + \frac{n_I}{V_Q} I_{ss}(1-\gamma) \qquad \Rightarrow \qquad n_C = \frac{n_I}{V_Q}\left(\frac{1}{\gamma} - 1\right) \tag{3.11}$$

Saturation of hepatic insulin clearance, described by parameter α_I, is difficult to estimate and would require specialised multi-dose tests to estimate correctly. A mean value found in studies investigating this effect is $\alpha_I = 0.0017$ [Ellemann et al., 1987; Thorsteinsson, 1990]. As the insulin sensitivity test proposed here is designed for low, physiological concentrations after a fasting state, hepatic saturation is not very likely and this value can potentially be set to $\alpha_I = 0$.

A further option to reduce the number of parameters to be identified is to exploit kinetic similarities with other substances, in which the kinetic parameters are uniquely identifiable. Two examples of substances with similar molecular weights to insulin are inulin and C-peptide [Clark, 1999; Rasio et al., 1967]. Due to similar molecular sizes, it can be assumed that these substances share similar distribution volumes and passive kinetic transport rates. As both substances are only cleared by the kidneys, their kinetics can be described by an identifiable two compartment model with four parameters [Eaton et al., 1980; Sturgeon et al., 1998]. A C-peptide kinetics model that is identifiable with a population approach has been presented by Van Cauter et al. [1992] and is used in this study to identify insulin parameters with similar physiological characteristics. After these steps, the few remaining unknown parameters can be identified with a convex integral-based fitting approach [Hann et al., 2005b; Lotz et al., 2006a], or alternatively using only fasting information and steady state model analysis.

3.1.3.2 A-priori ID with C-peptide Information

C-peptide is secreted by the pancreas in equimolar amounts to endogenous insulin [Rubenstein et al., 1969]. The kinetics of C-peptide were initially studied by Faber et al. [1978] and Eaton et al. [1980], analysing decay curves of IV injections of C-peptide. Eaton et al. presented a two compartment model of C-peptide kinetics that accurately described the observed data. Unlike insulin, C-peptide is not cleared by the liver or degraded by the cells, thus simplifying the kinetics to be modelled.

Van Cauter et al. [1992] developed population parameters for Eaton et al.'s model, enabling identification without the need of analysing the C-peptide decay curve in every individual [Van Cauter et al., 1992]. This population model has been successfully validated in a variety of studies [Hovorka et al., 1998; Jones et al., 1997; Toffolo et al., 1995]. The error in estimated C-peptide secretion from the population model compared to individually estimated parameters is in the range of $10\,\% - 20\,\%$ [Hovorka et al., 1998; Toffolo et al., 1995; Van Cauter et al., 1992], showing a very narrow range across broad ranges of individuals and groups.

Given the knowledge of the respective clearance sites of the two peptides and a similar molecular weight of 5808 Da (insulin) [Guyton and Hall, 2000] and 3021 Da (C-peptide) [Clark, 1999], the assumption can be made that insulin and C-peptide share certain passive characteristics. Transcapillary transport rate and distribution volumes of inulin (5500 Da [Rasio et al., 1967]) and insulin have also been shown to be comparable [Rasio et al., 1967; Steil et al., 1996; Yang et al., 1989], leading to the assumption that they could be similar in C-peptide. This assumption enables the use of the validated C-peptide parameters to describe shared portions of the insulin kinetics model. This approach thus reduces the number of parameters to be estimated, making the overall proposed model more readily identifiable.

Insulin kinetic model parameters that can adopt values of similar C-peptide parameters are the distribution volumes V_P and V_Q, the transcapillary diffusion rate n_I, and the renal clearance n_K. All of these parameters are assumed to be similar in insulin and C-peptide, given their shared molecular properties and the common mechanisms of renal clearance [Despopoulos and Silbernagl, 2003].

Hepatic and cellular insulin clearance rates, n_L and n_C, remain to be estimated, as C-peptide is not cleared by the liver and does not bind to the cells.

The insulin model parameters V_P, V_Q, n_I and n_K are thus calculated from the method proposed by Van Cauter et al. [1992], in which the amplitudes and time constants of a double-exponential decay are estimated patient specific as functions of the individual's age, sex, weight, BSA, BMI and diagnosis of type 2 diabetes. The formulae derived in that study to calculate these parameters are shown in Table 3.1. From the four decay parameters, representing the system response to an IV injection, the kinetic parameters of the insulin model used in this research can be calculated as shown in the table.

Table 3.1 Steps to calculate kinetic population parameters of C-peptide model, as proposed by Van Cauter et al. [1992]. Step 6 relates these parameters to insulin model parameters used in this thesis.

Steps	**Normal**	**Obese**	**NIDDM**
1. Short half life $t_{1/2}$-short [min]	4.95	4.55	4.52
2. Fraction $F = A/(A+B)$	0.76	0.78	0.78
3. Long half life $t_{1/2}$-long	$0.14 \times (age[yr]) + 29.2$		
4. Plasma volume V_P	Female: $V_P = 1.11 \times \text{BSA} + 2.04$ Male: $V_P = 1.92 \times \text{BSA} + 0.64$ $(\text{BSA} = \sqrt{height[\text{cm}] \times weight[\text{kg}]/3600})$		
5. C-peptide kinetic parameters	$k_2 = F \times (b-a) + a$ $k_3 = a \times b/k_2$ $k_1 = a + b - k_2 - k_3$ with: $a = log_{10}(2)/t_{1/2} - short$ $b = log_{10}(2)/t_{1/2} - long$		
6. Insulin kinetic parameters	$V_Q = V_P \times k_1/k_2$ $n_I = V_Q \times k_2$ $n_K = k_3$		

3.1.3.3 Integral-Based Fitting

Parameters left to be identified from data are the hepatic clearance related parameters n_L and x_L. They are identified using measured data and an integral-based fitting method. The fitting method uses the integrals of the differential equations to reduce the nonlinear estimation problem to a set of linear equations that

can be easily solved by minimising the L_2-Norm between the measured and estimated values. The method has the dual advantages of being convex and not starting point dependent. Equally important, parameters can be defined as stepwise constants for different time segments to enable identification of time varying parameters if required [Hann et al., 2005b; Lotz et al., 2006a].

Equation 3.7 is integrated in a chosen time interval $t \in [t_0, t_1]$, obtaining the following expression:

$$\begin{aligned} I(t_1) - I(t_0) = &-n_L \int_{t_0}^{t_1} \frac{I(t)}{1+\alpha_I I(t)} dt - x_L \int_{t_0}^{t_1} \frac{u_{en}(t)}{V_P} dt \\ &- \int_{t_0}^{t_1} \left[(n_K + \frac{n_I}{V_P}) I(t) + \frac{n_I}{V_P} Q(t) - \frac{u_{ex}(t)}{V_P} - \frac{u_{en}(t)}{V_P} \right] dt \end{aligned} \tag{3.12}$$

where the analytical solution of Equation 3.8 can be used to express $Q(t)$ as a function of the measurable plasma insulin $I(t)$ and the kinetic parameters of Equation 3.8.

$$Q(t) = \frac{n_I}{V_Q} \int_0^t I(\tau) e^{-(n_C + \frac{n_I}{V_Q})(t-\tau)} d\tau \tag{3.13}$$

Thus, given n_I, V_Q and n_C from other steps, a single linear equation for n_L and x_L can be obtained for each numerically evaluated integral in Equation 3.12. Integrating over several time steps provides several such equations, and the problem is reduced to solving the following set of linear equations:

$$\bar{A} \left\{ \begin{array}{c} n_L \\ x_L \end{array} \right\} = \bar{b} \tag{3.14}$$

where constraints can be added to the least squares solution to keep the parameter within known physiological ranges.

To best compute the integrals in all time intervals, the profile of $I(t)$ needs to be approximated by interpolating between discrete measurements. Linear

interpolation is sufficient in longer term data [Hann et al., 2005b] or control trials [Wong et al., 2005]. However, the dynamic nature of the impulse response to a bolus insulin injection requires an exponential decay curve to be fit. This task can be done by fitting a double exponential curve to the data. In a situation with limited sampling, this can be done by first estimating the slow exponential with later samples in the data and then back-calculating the fast exponential using the earlier samples, as shown schematically in Figure 3.6.

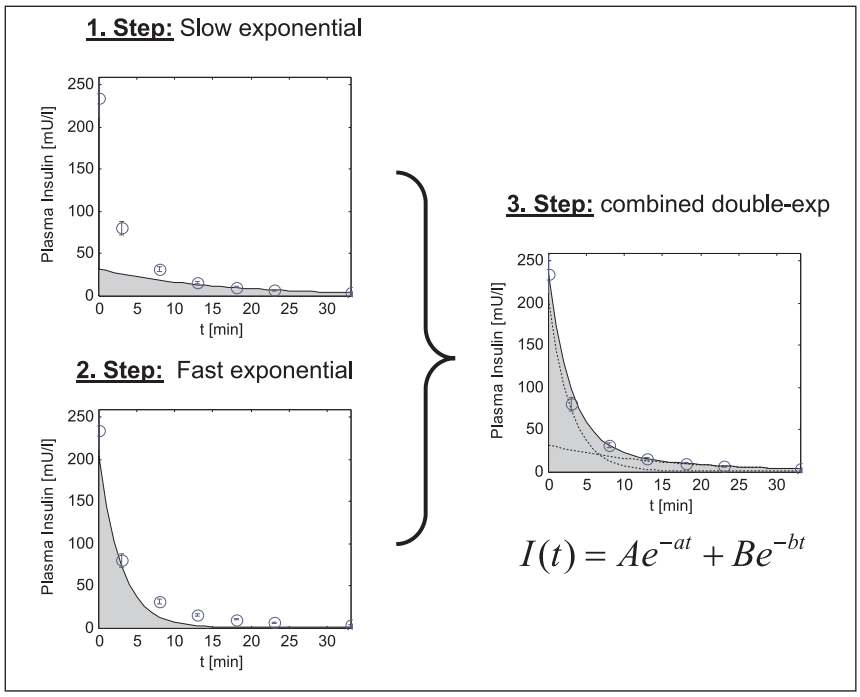


Figure 3.6 Schematic of the stepwise approximation of the discrete data samples with a double-exponential decay. Step 1 approximates the slow exponential from all samples >10 minutes after insulin input. Step 2 approximates the fast decay using samples <10 minutes after insulin input. The combined curve is obtained in Step 3 by adding both exponentials. The estimated curve is a good match of the experimentally sampled data.

The resulting approximation errors of any reasonable approximation to the true curve can be shown to be very small due to the integrations over several time intervals [Hann et al., 2005b]. More specifically, integral functions have the advantage of being robust to noise in the measured data, by effectively providing a low-pass filter in the summations involved in numerical integration. Hence, the dominant sources of bias will be due to model, rather than computational or methodological error.

This integral-based approach effectively matches the area under the measured response curves for each interval considered. This approach is in contrast to stan-

dard, well accepted methods that typically use gradients to directly match the response trajectory. Given the multiplication and summation operations used in the numerical integration, there are several analogies to a digital filtering identification process that could possibly be made. More importantly, this approach converts a computationally intense, non-convex problem into a much simpler convex problem, offering several advantages in speed and the quality of the results [Hann et al., 2006, 2005b].

3.1.4 Summary - Insulin Kinetics

Insulin kinetics have been discussed and a physiological model proposed that includes all main kinetics. The model is physiologically valid, yet simple enough to be applicable in a clinical setting. An identification method is proposed, that in a first step identifies most parameters a-priori with information from a C-peptide population model, and then identifies the hepatic clearance with an integral-based fitting approach. The overall method is robust on typically seen clinical sampling profiles. These identification steps are summarised in the schematic in Figure 3.7.

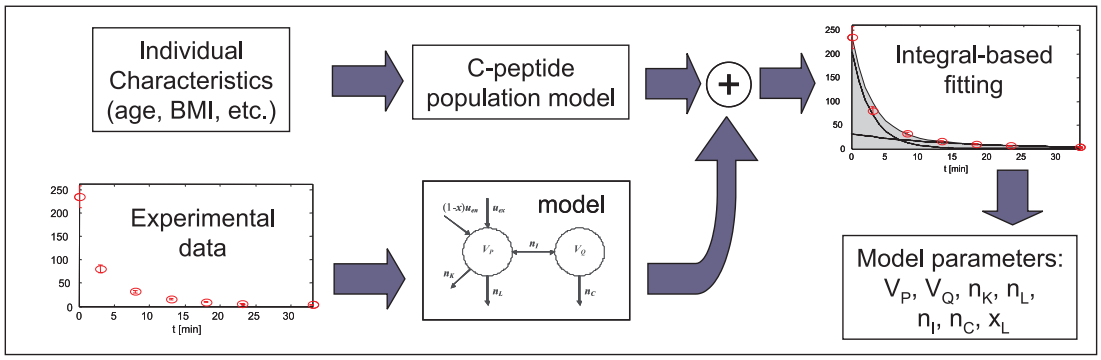


Figure 3.7 The overall method of identifying the model parameters from clinical experimental data and a-priori C-peptide kinetics information, as described in Section 3.1.3.

3.2 Insulin/C-peptide Secretion

3.2.1 Physiology

C-peptide (connecting peptide), just like insulin, is produced by the β-cells located in the islets of Langerhans in the pancreas [Despopoulos and Silbernagl, 2003; Guyton and Hall, 2000]. In fact, C-peptide is seen as a by-product of insulin production, as both peptides originate from the precursor Proinsulin, which splits into insulin and C-peptide, thus producing equimolar amounts of both [Rubenstein et al., 1969]. More specifically, C-peptide is a single chain of 31 amino acids, connecting the A and B chains of insulin in the Proinsulin molecule, as shown in Figure 3.8.

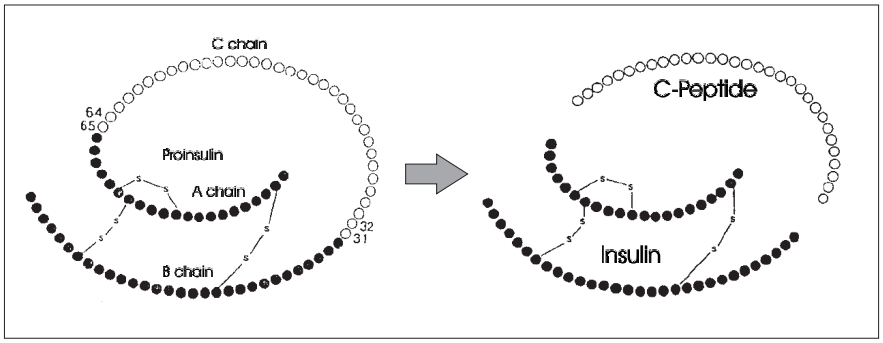


Figure 3.8 Splitting of Proinsulin into C-peptide and insulin [Chevenne et al., 1999].

For a long time the physiological role of C-peptide was unknown and it was merely seen as a waste product of insulin production [Wahren, 2004]. More recent research has indicated a biologically active role, in binding to cell membranes and activating intra-cellular signalling pathways, resulting in improved renal and nerve functions [Wahren, 2004]. These effects are not critical for this research and do not affect the modelling approach, as its function does not affect its appearance or degradation significantly.

As C-peptide is only cleared by the kidneys, it has a longer half-life than insulin ($\sim$2-5 times longer). This difference results in higher concentrations of C-peptide in the peripheral circulation and less fluctuations in its concentration than insulin. Hence, the kinetics of C-peptide are easier to capture in both measurement and model.

Due to its equimolar secretion and longer half-life, plasma C-peptide concentrations independently reflect pancreatic insulin secretion. In fact, the slower, less variable clearance by a single route (rather than three) makes it a more reliable measure of endogenous insulin secretion than the plasma concentration of insulin itself. Therefore, models independently linking C-peptide kinetics and insulin kinetics can be used to determine and capture endogenous insulin secretion [Eaton et al., 1980; Faber et al., 1978; Hovorka et al., 1996; Polonsky et al., 1986b; Van Cauter et al., 1992].

3.2.2 Model Structure

The kinetics of C-peptide were first analysed by Faber et al. [1978] who administered synthetic human C-peptide and measured the dose-response plasma decay curve over time. They concluded that a three component exponential equation is necessary to describe its kinetics. Eaton et al. [1980] later described the kinetics of C-peptide with a two compartment model and calculated kinetic parameters assuming a double-exponential decay. Eaton et al. [1980] also show that a three compartment model does not significantly improve accuracy of the data fit, as the fast decay rate is too fast to estimate accurately with practical clinical sampling limitations.

A smaller two compartment model was thus proposed that describes C-peptide distribution and degradation. The kinetic parameters of C-peptide clearance were derived for each subject using this model from the decay curve observed after a bolus injection of biosynthetic human C-peptide. The model is shown in Figure 3.9.

The primary compartment is described as the accessible, central (intravascular) compartment, which represents blood plasma and fast exchanging tissues. The peripheral (extravascular) compartment represents interstitial fluid. The concentrations $C(t)$ and $Y(t)$ are the C-peptide concentrations at time t, in the intravascular and extravascular compartments respectively. Parameters k_1, k_2 and k_3 are fractional transport rates, where k_3 represents an irreversible loss from the central compartment via the kidneys. The input rate to the intravascular compartment $S(t)$ consists of either endogenous C-peptide secreted by the pancreas or C-peptide administered exogenously, depending on the specific clini-

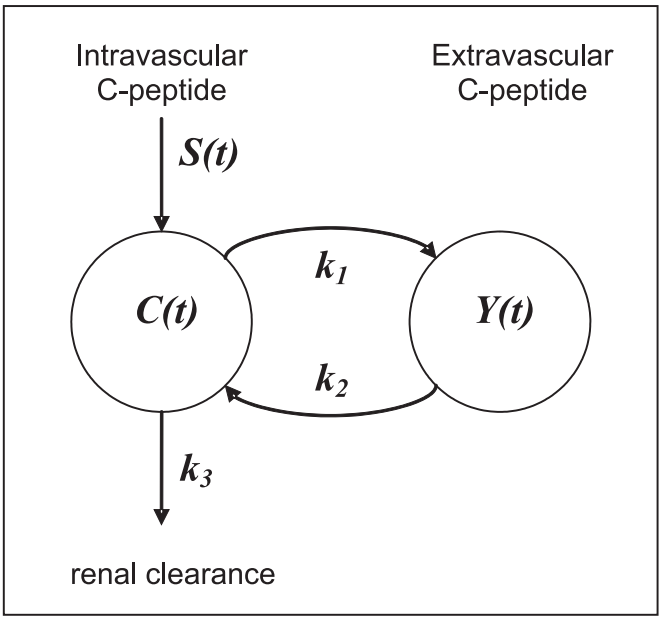


Figure 3.9 Two-compartment C-peptide model by Eaton et al. [1980].

cal test. Note that the endogenous component of $S(t)$ equals endogenous insulin secretion $u_{en}(t)$.

The differential equations describing the dynamic system in Figure 3.9 are defined:

$$\frac{d}{dt}C(t) = -(k_1 + k_3)C(t) + k_2 Y(t) + S(t) \tag{3.15}$$

$$\frac{d}{dt}Y(t) = k_1 C(t) - k_2 Y(t) \tag{3.16}$$

where the parameters and variables are as defined above.

3.2.3 Model Identification

To estimate the C-peptide, or insulin, secretion rate $S(t)$, the C-peptide model kinetic parameters for a given individual have to be known. These parameters can be estimated by fitting the model to the individually measured data from a

bolus intravenous injection of C-peptide. The identified parameters and model can then be used to deconvolute the input rate of endogenous secreted C-peptide given clinical data [Eaton et al., 1980; Hovorka et al., 1996; Polonsky et al., 1986a; Van Cauter et al., 1992].

3.2.3.1 Population Parameters

As an individual estimation of kinetic parameters is not feasible in a short clinical test, an alternative method to estimate the kinetics has to be applied. A population regression model was presented by Van Cauter et al. [1992], in which they analysed the decay curves of 200 subjects including 111 normal subjects (71 males, 40 females), 53 obese subjects (19 males, 34 females), and 36 individuals diagnosed with type 2 diabetes (20 males, 16 females). Population based kinetic parameters are defined from this study as a function of gender, height, weight, age and diagnosis of diabetes. This method thus allows a-priori identification of kinetic parameters. The formulas to calculate the model parameters as proposed by Van Cauter et al. [1992] are shown in Table 3.1 on Page 50.

3.2.3.2 Estimation of Secretion Rate

Estimation of the C-peptide secretion rate $S(t)$ is performed with an integral-based method, previously employed in real-time parameter identification in glycaemic control trials in the critically ill [Chase et al., 2005a; Wong et al., 2006a] and in fitting long-term retrospective insulin and glucose profiles [Hann et al., 2005b; Lotz et al., 2006a, 2005a, 2006b]. To best compute the integrals in all time steps, the profile of C-peptide is approximated using linear interpolation between data points, which introduces no additional error over model error [Hann et al., 2005b].

C-peptide secretion rate $S(t)$ is thus estimated as a time-varying step function, with stepsize of 1 min. Therefore, during any given 1 min time interval $t \in [t_0, t_1 = t_0 + 1]$, $S(t)$ is assumed constant. Integrating Equation 3.15 in the

interval $[t_0, t_1]$ yields:

$$\int_{t_0}^{t_1} \dot{C}(t)dt = -(k_1 + k_3) \int_{t_0}^{t_1} C(t)dt + k_2 \int_{t_0}^{t_1} Y(t)dt + \int_{t_0}^{t_1} S(t)dt \qquad (3.17)$$

Solving Equation 3.16 analytically for $Y(t)$ yields:

$$Y(t) = k_1 \int_0^t C_{est}(\tau)e^{-k_2(t-\tau)}d\tau \qquad (3.18)$$

where C_{est} represents the interpolated C-peptide values estimated from the discrete measurements. Combining Equations 3.17 and 3.18, and solving for the assumed constant secretion rate $S_{0,1}$ in this time interval yields:

$$\begin{aligned} S_{0,1} \cdot (t_1 - t_0) &= C_{est}(t_1) - C_{est}(t_0) + (k_1 + k_3) \int_{t_0}^{t_1} C_{est}(t)dt \\ &\quad -k_2 k_1 \int_{t_0}^{t_1} \int_0^t C_{est}(\tau)e^{-k_2(t-\tau)}d\tau dt \end{aligned} \qquad (3.19)$$

where $S_{0,1}$ is the only unknown given the population model values for $k_1 - k_3$.

Repeating this process for the intervals $[t_1, t_2]$, $[t_2, t_3]$, $\ldots$ $[t_{n-1}, t_n]$, results in a 1-min stepwise constant secretion profile $S(t)$. This estimated $S(t)$ is constrained to be non-negative. Smoothing the estimated stepwise constant profile with a zero-phase 3-point moving average is done to avoid overfitting to noisy data and interpolated measurements [Hann et al., 2005b]. This last step is not required in frequently sampled data, but results in a more physiological profile between more sparsely sampled data.

Using the same model and Van Cauter et al.'s parameter estimation method, estimation of secretion rate has previously been proposed by deconvolution [Eaton et al., 1980] and a more elaborate constrained regularisation method [Hovorka et al., 1996]. The main drawbacks of these methods, in comparison to the meth-

ods presented here, are the individualised method adjustments required for each subject, including knot placements for cubic spline interpolations [Eaton et al., 1980], and a separate step to find the optimal proportionality constant in each subject [Hovorka et al., 1996]. All of these extra steps introduce time, computational complexity and intensity, and human variability into the results.

In contrast, the integral-based method described is a single step, computationally convex and fast method that only requires linearly interpolated data. By constraining the linear least squares estimation to physiologically valid non-zero values and smoothing the estimated secretion rate to remove the effects of noise, the resulting profile is physiologically accurate and the effects caused by noisy data are reduced [Hann et al., 2005b]. The secretion rate of insulin, which equals that of C-peptide, can thus be estimated robustly from C-peptide concentration samples during a short clinical test.

3.2.4 Summary - C-peptide

C-peptide is secreted in equimolar amounts to insulin and its kinetics can be identified uniquely with more certainty than those of insulin. The C-peptide, and consequently also insulin, secretion rate can be estimated from a well validated population model of C-peptide kinetics, applying a novel integral-based estimation method. The estimation method enables robust and physiologically valid estimation of pre-hepatic insulin secretion rate from sampled C-peptide concentrations. The steps involved in the method described in this section are summarised and shown schematically in Figure 3.10.

3.3 Summary

The insulin model derived in this chapter enables a physiological and accurate description of the relevant metabolic dynamics of the hormone. The model is physiologically valid, meaning that all its structural elements and identified parameter values are derived and explainable from physiological mechanisms. Additionally, it is useful for application in a clinical setting as the parameter values can provide information about the metabolism. The estimation methods proposed,

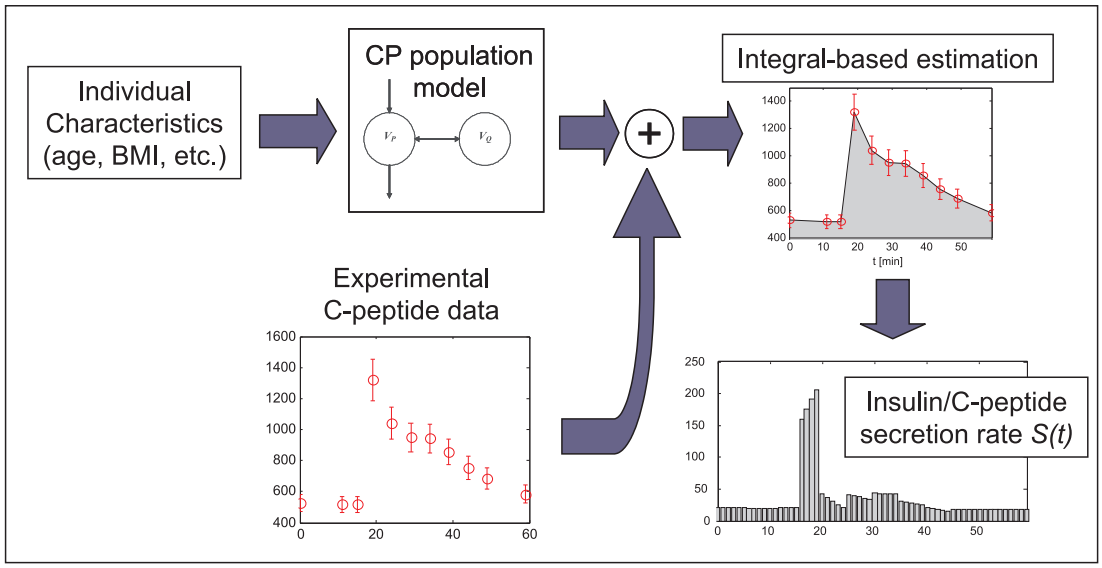


Figure 3.10 The overall method of estimating pre-hepatic insulin secretion, which is equivalent to C-peptide secretion. Sampled C-peptide concentration data is approximated linearly, and in conjunction with a population model of C-peptide kinetics, the secretion rate is estimated with an integral-based method, as described in Section 3.2.3.

employing a-priori information and parallels with C-peptide kinetics, combined with a robust and convex integral-based estimation of hepatic clearance rate, enable a fast and physiological identification of all parameters.

The estimation of endogenous insulin secretion through C-peptide kinetics is a well validated accurate approach [Hovorka et al., 1998; Van Cauter et al., 1992]. The population model of C-peptide kinetics [Van Cauter et al., 1992] enables an a-priori identification of C-peptide kinetic parameters without additional tests required. The integral-based estimation of a stepwise constant secretion of insulin is robust and simple, as it does not require manual data analysis and intervention. Overall, the models and methods presented are well suited for application in a clinical insulin sensitivity test.

Chapter 4

Glucose Modelling and Identification

To derive a model-based metric of insulin sensitivity, a pharmaco-dynamic model of the interaction of glucose and insulin is required. The model must include all other, non-insulin dependent glucose clearance and uptake mechanisms to improve its physiological validity.

The modelling goal in this chapter is to obtain a glucose PK and PD model, that includes the key glucose uptake mechanisms, and is physiologically valid, yet simple enough to be identifiable with a short, simple test and limited blood sampling. Previous model-based approaches are discussed to improve overall method performance and robustness, and reduce possible sources of methodological error. An appropriate identification method that can be applied within the proposed test protocol is presented.

4.1 Physiology

Glucose is a monosaccharide used as the main, and thus most important, source of energy in the body. It is oxidised in the cells to provide ATP, which in turn provides energy to the cell [Guyton and Hall, 2000]. The body uptake of glucose is through carbohydrates in food, which are broken up in the alimentary tract and released into plasma mainly in the form of glucose. Glucose in plasma is transported to the cells for use as energy, and if available in abundance, stored by the liver and the cells for future use.

The molecular weight of glucose is 180 Da, which is small enough to diffuse

rapidly within plasma and body fluids, its main site of action [Guyton and Hall, 2000]. The uptake by cells in the brain and the central nervous system is by diffusion alone, as they are highly permeable to glucose. In contrast, muscle and adipose tissue cells control a majority of the total uptake and require insulin binding to cell receptors to activate or mediate glucose uptake [Despopoulos and Silbernagl, 2003; Guyton and Hall, 2000]. Hence, glucose uptake in this form is referred to as "insulin-mediated" versus "non-insulin-mediated" uptake in other organs.

Excess circulating glucose is stored in the liver and cells in the form of *glycogen*, a large polymer of glucose, which is created by a process called *glycogenesis* [Guyton and Hall, 2000; Zierler, 1999]. If glycogen stores are saturated, further glucose is converted into fat and stored in the liver and in fat cells in the adipose tissue. These processes can be reversed in times of energy demand. Glucose can be rapidly released from glycogen by a process called *glycogenolysis*, and if the glycogen stores are used up, fat is metabolised with amino acids to form glucose in a process called *gluconeogenesis* [Guyton and Hall, 2000; Zierler, 1999].

The combined processes of glycogenolysis and gluconeogenesis are commonly also described as endogenous glucose production (EGP) [Zierler, 1999]. EGP is tightly regulated by the body to keep plasma glucose levels as constant as possible. External appearance or input of glucose, through meals or intravenous injection, immediately results in a rapid inhibition of EGP [Caumo and Cobelli, 1993; Jefferson and Cherrington, 2001]. Low plasma glucose has the contrary effect, stimulating glucagon secretion by the pancreatic α-cells, which activates glycogenolysis and thus rapidly increases glucose concentrations in plasma. Overall, these processes operate in a balance with insulin-mediated glucose removal to maintain normal blood glucose levels or glucose homeostasis. In type 2 diabetes this balance in glucose homeostasis does not function optimally anymore, resulting in high blood glucose levels.

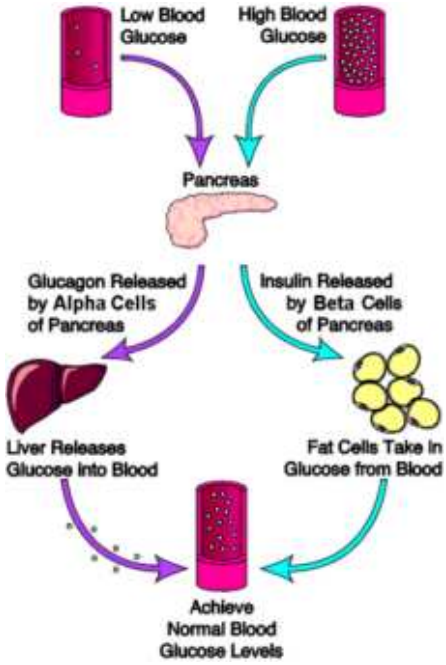


Figure 4.1 Hormonal control of glucose metabolism to store or release glucose on demand (taken from www.endocrine.com).

4.2 Modelling

4.2.1 Background

The kinetics of glucose have been described in similar ways as insulin, with one to three compartment models [e.g., Bergman et al., 1979; Carson and Cobelli, 2001; Cobelli et al., 1984; Insel et al., 1974]. As glucose is a smaller molecule than insulin, with a molecular weight of 180 Da (compared to insulin with 5808 Da) [Guyton and Hall, 2000], it distributes in the body much more freely and rapidly. Well perfused organs in the splanchnic area, primarily the liver, are known to take up or store glucose very rapidly, further adding to the difficulty in measuring these kinetics to create accurate models.

A three compartment model was used to fit glucose kinetics in an early study by Insel et al. [1974]. The model incorporated insulin-dependent and insulin-independent glucose losses, and was identified using data from various dose-response and glycaemic clamp tests with the help of glucose tracers. They concluded that the fast compartment was impossible to identify from sampled data,

as equilibration between this compartment and plasma was too fast. The losses were assumed to occur from the fast and medium exchanging compartments, not accounting for peripheral losses, thus limiting its validity.

Later attempts by Cobelli et al. [1984], Jacquez [1992] and Overkamp et al. [1997] resulted in more physiological modelled losses and explanations for the model parameters. However, identification of these models still required complicated and costly multi-tracer experiments and further user imposed parameter constraints. These aspects limit their use to very specialised research studies and render them impractical for control or clinical use. Some of these modelling attempts are shown schematically in Figure 4.2.

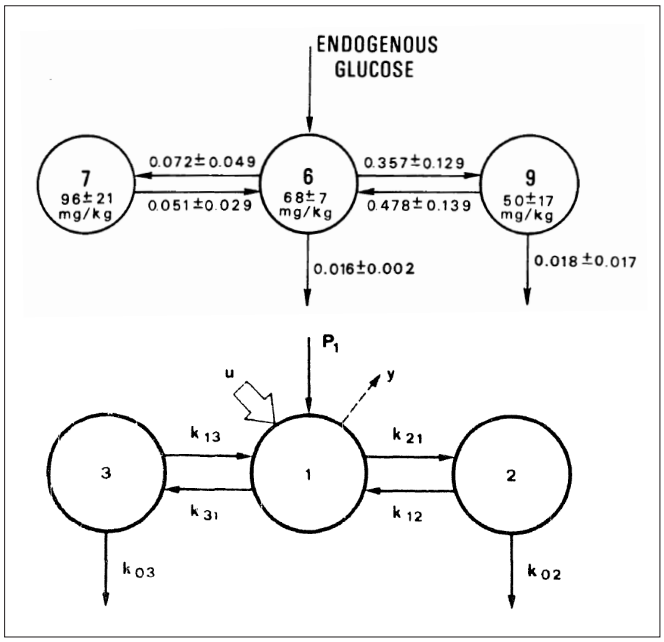


Figure 4.2 Two three compartment glucose kinetics models developed by Insel et al. [1974] (above) and Cobelli et al. [1984] (below).

As the fast equilibrating compartment was found to be too fast to identify accurately (time constant 0.6 min [Cobelli et al., 1984]), it was proposed to combine the fast and the medium compartment [Cobelli et al., 1984]. This merger is similar to the assumption made in modelling insulin, resulting in an accessible compartment representing plasma and fast exchanging tissues and a slow compartment representing interstitial fluid. Similar two compartment models had been proposed earlier by Radziuk et al. [1978], in which the losses of both compartments were equalised to enable identifiability, and later by Caumo and Cobelli [1993] and Hovorka et al. [2002]. These latter models contain constant,

as well as glucose dependent and insulin dependent losses, and more complicated dynamics, allowing an estimation of endogenous glucose production by deconvolution [Carson and Cobelli, 2001; Caumo and Cobelli, 1993]. Again, these models require the use of glucose tracers to be uniquely identifiable and are thus impractical for clinical or widespread screening use. For reference, the model presented by Caumo and Cobelli [1993] is shown in Figure 4.3.

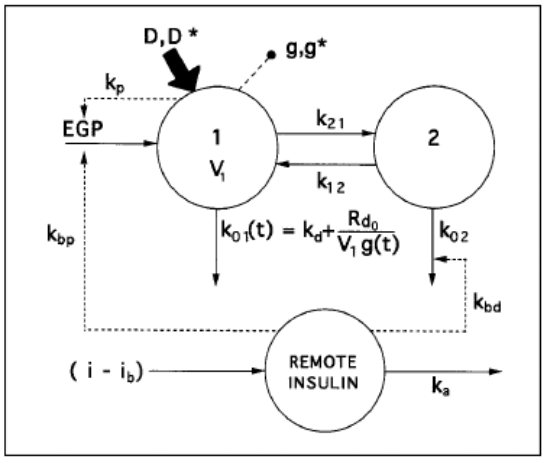


Figure 4.3 Two compartment model of glucose kinetics proposed by Caumo and Cobelli [1993].

The simplest description of glucose kinetics is by using only one compartment, with the best known model being the 'Minimal Model' of glucose kinetics proposed by Bergman et al. [1979]. The model is described by the following equations:

$$\frac{dG(t)}{dt} = -S_G^{MM}(G(t) - G_b) - G(t)X(t) \qquad G(0) = \frac{D}{V_G} \tag{4.1}$$

$$\frac{dX(t)}{dt} = -p_2X(t) + p_3(I(t) - I_b) \qquad S_I^{MM} = \frac{p_3}{p_2} \tag{4.2}$$

where $G(t)$ is plasma glucose concentration, G_b fasting glucose, D the glucose dose, V_G the volume of distribution, $X(t)$ remote insulin effectiveness, $I(t)$ plasma insulin concentration, I_b fasting insulin, S_G^{MM} glucose effectiveness at basal insulin, S_I^{MM} insulin sensitivity, p_2, p_3 transport rates defining delay in insulin effect.

This approach assumes fast equilibration between the compartments and thus equivalent concentrations throughout the body. Losses are possible by insulin-independent pathways (brain, liver, kidneys) via the parameter S_G^{MM} (min^{-1}), denoted as *glucose effectiveness* at basal insulin, and by insulin-dependent (mainly muscle and adipose tissue cells), as mediated by remote *insulin effectiveness* $X(t)$ (min^{-1}). The variable $X(t)$ in this model accounts for the combined delay in insulin transport to the periphery, as well as the insulin sensitivity of the cells, thus combining transport kinetics and action dynamics.

The Minimal Model is widely used, mostly combined with an IVGTT to assess insulin sensitivity in research studies [Bergman et al., 1985, 1981]. Its main advantages are simplicity and thus practicality. However, numerous studies have questioned the validity of its derived parameters, and the question was postulated as to whether it is "too minimal" [Caumo and Cobelli, 1993; Caumo et al., 1996, 1999; Quon et al., 1994b; Regittnig et al., 1999]. In particular, studies have shown that the estimation of S_G^{MM} is imprecise and usually results in a significant overestimation of its contribution, with the consequent result being a significant underestimation of S_I^{MM} [Caumo et al., 1999].

The reason identified for this problem by Caumo et al. [1999] is that glucose kinetics should be described by two compartments to describe the fast decay during the initial 30 minutes after a glucose dose and the slower decay thereafter. When fitting the Minimal Model to IVGTT data using accepted methods, the model tries to match the initial fast decay with a single exponential, resulting in an overestimation of the slow decay that follows [Caumo et al., 1999; Quon et al., 1994b]. Despite this problem, the model is able to capture the dominant dynamics and has been somewhat successfully used in a slightly modified form in glycaemic control trials in the critically ill [Chase et al., 2005a; Wong et al., 2006a].

Further models of glucose kinetics usually combine more complex kinetics, such as a circulatory model by Mari [1998], accounting for mixing of injected glucose in the circulation. More complex simulation models include those presented by Lehmann and Deutsch [1992] or Arleth et al. [2000]. These models include many more physiological effects, such as gastric glucose uptake through meals, liver feedback, and renal clearance thresholds. In particular, the glucose surface by Arleth et al. [2000] is built on knowledge and assumptions on the behaviour

of the GLUT glucose transporters [Guyton and Hall, 2000], incorporating saturation of glucose clearance. Its parameters are identified by fitting the model to values gathered from a wide range of clinical studies of glucose and insulin metabolism, resulting in a 'glucose surface' that allows prediction of metabolic behaviour in a population sense.

To summarise, modelling approaches exist to capture most metabolic characteristics of glucose. However, they are usually limited to tailored experimental situations, overly simplified, or both. For a model to be useful in clinical settings and regular screening, it should be readily identifiable with limited data, but compromising only slightly on physiological accuracy to ensure the relevance of the results.

4.2.2 Model Structure

The model structure chosen for this application is a mono-compartmental description, similar to the Minimal Model and the model used in previous glycaemic control research at the University of Canterbury [Chase et al., 2005b]:

$$\frac{dG}{dt} = -p_G(G - G_E) - S_I G \frac{Q}{1 + \alpha_G Q} + \frac{P}{V_G} \tag{4.3}$$

The main advantage over a multi-compartmental description is its identifiability using limited plasma samples, while still accounting for the dominant dynamics. This model has also performed well in a variety of insulin and nutrition based glycaemic control trials, as well as in retrospective data fitting of critically ill patients [Chase et al., 2005b; Hann et al., 2005b]. As the intended test is much more dynamic, and frequently sampled, errors could be introduced by undermodelling, such as assuming a mono-compartmental structure. The trade-offs between the improved identifiability of a simplified model and potential errors introduced by an over-simplification of the model need to be analysed carefully.

Recent studies sampling interstitial fluid (ISF) concentrations of glucose directly from the muscle tissue using a microperfusion technique [Regittnig et al., 2003, 1999], found a mean delay of 22 min (SD 3 min) between a bolus injection

of 20 g glucose and the equilibration of concentrations in plasma and ISF. This delay explains the fast decay seen in IVGTT plasma glucose data during the first 30 minutes, which causes an overestimation of Minimal Model S_G^{MM} when it is used to fit a single exponential, as discussed in Section 4.2.1. An additional loss at these high glucose concentrations ($\sim$ 14 mmol/l), not identified by the researchers, could be a small glucose clearance by the kidneys, that have a threshold of $\sim$ 10 mmol/l [Windhager, 1992].

Errors introduced by not accounting for the second mixing compartment can be avoided if its causes and effects on the fitting algorithm are known. In the proposed test, a lower glucose dose is intended, reducing the effect of renal clearance and mixing during the first 10 minutes. By disregarding the first few minutes after glucose administration in the fitting approach, any error introduced by mixing can further be minimised. Any remaining error should thus be very small, justifying the use of the simplified mono-compartmental description in this case.

The saturation of insulin dependent glucose clearance, evident in long-term hyperglycaemic individuals [Chase et al., 2004], is likely not evident in a fasted state after a low dose injection of glucose [Prigeon et al., 1996], and is thus set to $\alpha_G = 0$. Note that at higher insulin dosing saturation effects could affect the estimation of S_I, as both parameters trade off [Chase et al., 2004; Prigeon et al., 1996].

Further enhancements are made to Equation 4.3 to include a more physiological and complete description of the uptake and production mechanisms of glucose. The resulting formulation is shown in Equation 4.4 and shown schematically in Figure 4.4. The additions to the model and their physiological justification are explained in more detail in the following sections.

$$\frac{dG}{dt} = -(p_{GU} + p_{GS})(G - G_E) - S_I G Q - \frac{GU_G}{V_G} + \frac{EGP_{Ib}}{V_G} + \frac{EGP_{GE}}{V_G} + \frac{P}{V_G} \quad (4.4)$$

where p_{GU} (min^{-1}) is the insulin independent rate of glucose uptake, p_{GS} (min^{-1}) the insulin independent rate of suppression of EGP, GU_G (mmol/min) the constant insulin independent glucose uptake by the brain and central nervous system,

EGP_{Ib} (mmol/min) the fraction of EGP compensating glucose uptake at basal insulin concentration I_b, EGP_{GE} (mmol/min) the fraction of EGP accounting for the constant glucose uptake by the brain (equal to GU_G), and P (mmol/min) the exogenous glucose administration.

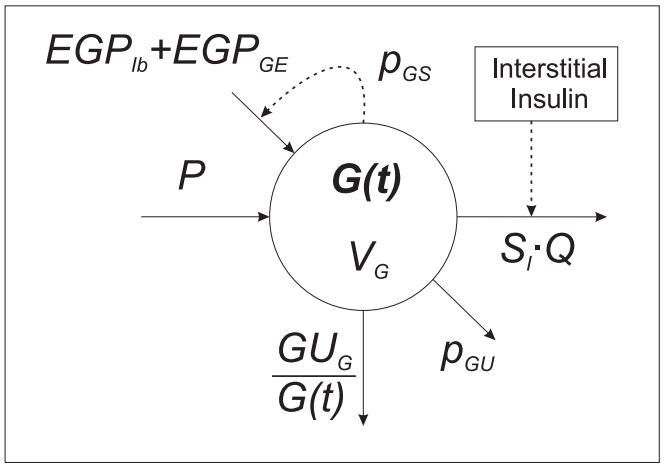


Figure 4.4 Schematic of full glucose PK and PD model. Shown are all exogenous (P) and endogenous (EGP) inputs, the constant loss GU_G, the insulin independent losses p_{GU} and p_{GS}, and the insulin dependent loss mediated by S_I.

4.2.2.1 Insulin-Dependent Uptake

Insulin-dependent glucose uptake, mostly by muscle and adipose tissue cells, is dependent on the product of peripheral insulin Q, total glucose concentration G and insulin sensitivity S_I, as seen in the second term of Equation 4.3. This assumption is physiologically valid and widely accepted. It has also been identified and observed in many studies [e.g., Bergman et al., 1979; Jefferson and Cherrington, 2001; Yang et al., 1989].

As the modelled insulin concentrations are absolute values and not those above basal, as in the Minimal Model, the parameter S_I includes a dynamic that is included in S_G^{MM} in the Minimal Model, namely glucose uptake at basal insulin I_b. This basal glucose uptake can be calculated from a steady state analysis of Equation 4.3 at a fasting state (without saturation):

$$GU_{Ib} = -S_I G_E Q_b \tag{4.5}$$

where Q_b is basal steady state interstitial insulin and can be estimated using Equation 3.10. GU_{Ib} accounts for about 25 % [Best et al., 1981; Cobelli et al., 1984; Zierler, 1999] of basal glucose uptake. As this loss is included in the insulin dependent term of the glucose model, the corresponding fraction of basal EGP, $EGP_{Ib} = -GU_{Ib}$ needs to be included in Equation 4.3 to keep a basal steady state glucose balance.

4.2.2.2 Insulin-Independent Uptake

Insulin independent uptake in a basal state is primarily due to the brain and central nervous system [Zierler, 1999], and to a lesser extent by some splanchnic, well perfused organs. Most of this uptake is independent of glucose concentration and can thus be seen as a constant loss, that is compensated by endogenous production EGP to keep steady state levels. This uptake accounts for about 75 % of basal glucose uptake and is in the magnitude of $\sim$ 1 mg/kg/min [Best et al., 1981; Zierler, 1999]. Converted to mmol, this value results in the constant irreversible loss rate:

$$GU_G = 5.6 \times 10^{-3} \text{ mmol/kg/min} \tag{4.6}$$

This term needs to be compensated by the corresponding fraction of EGP, EGP_{GE}. Total EGP, insulin dependent and independent at basal state is thus defined:

$$EGP_b = EGP_{Ib} + EGP_{GE} \tag{4.7}$$

In addition to insulin independent glucose uptake in the fasting basal state, glucose can enhance its own uptake at hyperglycaemic levels and inhibit EGP [Ader et al., 1997; Best et al., 1996; Jefferson and Cherrington, 2001]. These two effects, uptake rate p_{GU} and suppression rate p_{GS}, are lumped into the parameter p_G of Equation 4.3 and in the Minimal Model parameter S_G^{MM} (along with GU_{Ib}). Del Prato et al. [1997] studied the quantitative effects of glucose uptake and inhibition of EGP during hyperglycaemic clamps at basal insulin and different levels of glycaemia. Their results, interpreted in terms of p_{GU} and p_{GS} were

$p_{GU} = 0.004$ and $p_{GU} = 0.001$ min^{-1} for uptake in healthy, and diabetes subjects, respectively, with a value of $p_{GS} = 0.005$ min^{-1} for suppression of EGP. These rates are in good accordance with results from Best et al. [1996], who suggests that all three effects $(p_{GU}, p_{GS}, GU_{Ib})$ are equally strong.

In similar studies employing an IVGTT at basal insulin on type 1 diabetes subjects [Quon et al., 1994b; Regittnig et al., 1999], the slow decay of glucose at basal insulin can be fit with values of $p_G = 0.0011$ and $p_G = 0.0012$ min^{-1}, respectively, as shown in Figure 4.5. The same values were found for p_{GU} in type 1 diabetes subjects in the study by Del Prato et al. [1997]. In the former study by Regittnig et al. [1999], a tracer was employed, enabling the estimation of endogenous glucose concentration during the test. Interestingly, this concentration does not change significantly during the test, suggesting that the endogenous balance is left unchanged, and only the additional injected tracer is taken up. With this assumption, the above mentioned transport rates would equal to p_{GU} alone, without the effects of p_{GS} and GU_{Ib}, which explains the relatively low values found for p_G, and matching the values found in Del Prato et al. [1997].

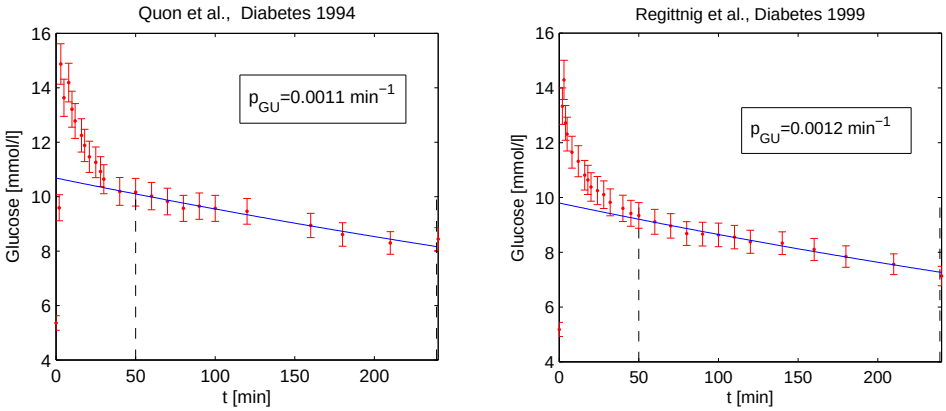


Figure 4.5 IVGTT glucose data at basal insulin concentrations on type 1 Diabetes subjects. Mean plots shown from Quon et al. [1994b] (left) and Regittnig et al. [1999] (right). Shown are sampled data and single exponential fits between $50 - 240$ minutes.

Overall, these results using different clinical protocols, indicate that suppression of EGP due to elevated glucose alone is only apparent during prolonged infusion of glucose, but not during a briefer, bolus-based IVGTT. Suppression of EGP appears to be dependent mainly on elevated insulin levels. The insulin-independent uptake at hyperglycaemia, represented by p_G in Equation 4.3 is thus much smaller than commonly found in Minimal Model fits, in line with the claims of overestimation [Caumo et al., 1999].

GU_G and EGP_{GE} cannot be measured directly without extensive clinical testing. However, in fasting homeostasis or balance, they also cancel each other and can be eliminated. EGP_{Ib} is dependent on S_I and can be combined with the insulin dependent term for easier identification. Finally, p_{GS} can be taken out as well, as its effect does not seem to be very strong during an IVGTT-type test, especially as this test aims at a lower glucose dose. The model shown in Equation 4.4 can thus be simplified, resulting in the final, shortened form of the glucose pharmaco-kinetics:

$$\frac{dG}{dt} = -p_{GU}(G - G_E) - S_I(GQ - G_E Q_b) + \frac{P}{V_G} \tag{4.8}$$

where Figure 4.6 shows the models of Equations 3.7-3.8 and Equation 4.8.

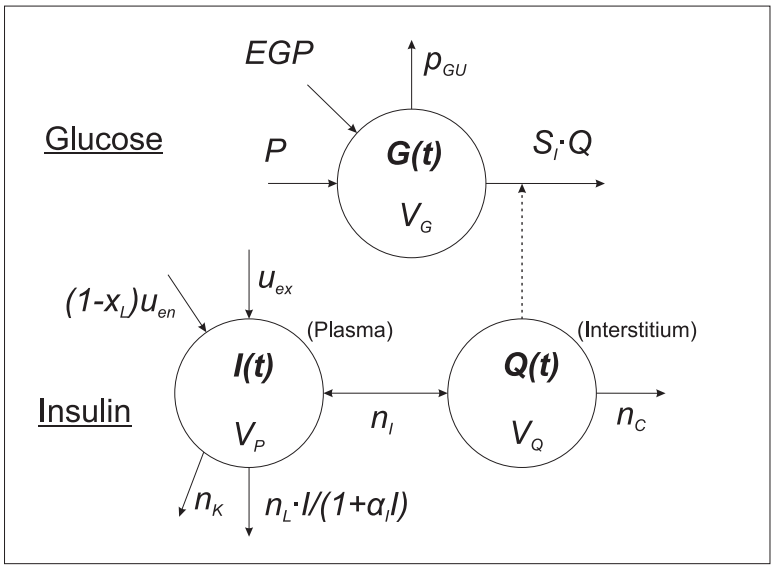


Figure 4.6 Schematic of final glucose and insulin models and their pharmaco-dynamic interaction.

4.3 Model Identification

Identification of model parameters is done using the integral-based fitting method described in Section 3.1.3.3. Equation 4.8 is integrated in the interval $[t_0, t_1]$, and if the data resolution is good enough to usefully identify all parameters [Hann et al., 2005a], p_{GU}, S_I and V_G can be estimated:

$$G(t_1) - G(t_0) =$$

$$- p_{GU} \int_{t_0}^{t_1} (G - G_E)dt - S_I \int_{t_0}^{t_1} (GQ - G_E Q_b)dt + \frac{1}{V_G} \int_{t_0}^{t_1} P dt \quad (4.9)$$

In a dose response test, the volume of distribution V_G can be estimated from the distinct jump in concentration after a bolus input, as seen in the example of Figure 4.7. In a clamp test or an infusion experiment, V_G trades off with loss parameters and can thus not uniquely be identified. A fixed parameter has to be chosen, typically using an estimate based on body weight [DeFronzo et al., 1979; Lotz et al., 2006a].

With less frequent data sampling than an IVGTT, for example using a first post-input sample at +5 min, an integration error can be introduced if the samples are interpolated linearly between 0-5 min. Figure 4.7 shows this potential error schematically. To overcome this limitation, V_G needs to be estimated a-priori by extrapolating back to $t = 0$ min from samples at $\sim 5 - 10$ minutes. This approximation also helps overcome any underestimation of the volume due to mixing effects and is done similarly in the Minimal Model assessment of an IVGTT [Bergman et al., 1981].

Insulin independent clearance p_{GU} is difficult to estimate accurately without specialised experimental protocols to suppress endogenous insulin response [e.g., Best et al., 1996; Del Prato et al., 1997; Quon et al., 1994b]. As explained in Section 4.2.2, its effects are small in a short low dose bolus response test, and it can thus be fixed a-priori to a mean value from the literature. Here a value of $p_{GU} = 0.004$ min^{-1} is chosen in accordance with several studies [Del Prato et al., 1997; Quon et al., 1994b; Regittnig et al., 1999].

Insulin dependent clearance, determined by S_I, can be identified well, as the test design is rich in information in this respect. By allowing most variability to be captured by S_I, the test also predisposes itself by design to capture the same effects as a euglycaemic clamp. As a result, there is an increasing similarity and correlation between them.

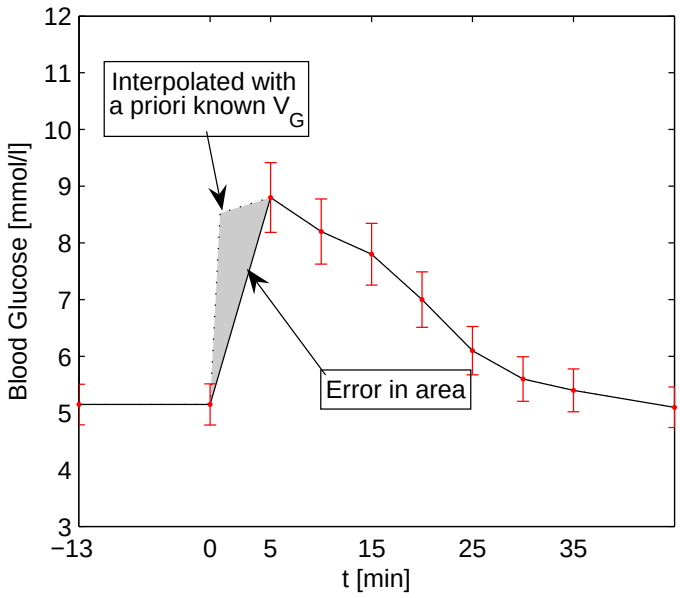


Figure 4.7 Error introduced by interpolating between 0 and 5 min sample, when V_G is not known. Grey area shows underestimated area.

4.4 Summary

Many glucose models have been presented in previous research, ranging from one to three compartments. Identification of glucose model parameters is difficult, as endogenous glucose production (EGP) is difficult to measure in a clinical setting. A single compartment description with minimal parameters can be used with good performance if identified correctly.

The derived glucose model contains insulin-independent and insulin-dependent glucose losses and accounts for endogenous and exogenous glucose input. By analysing the physiology and problems encountered in previous similar approaches, it is evident that some systematic sources of error can be eliminated by a better understanding of the underlying assumptions in modelling and fitting errors. This knowledge, combined with the modelled peripheral insulin from Chapter 3 and the customised integral-based fitting method, allows for a robust and fast estimation of insulin sensitivity S_I, as shown schematically in Figure 4.8. The model and method is simple, requires minimal data and is thus well suited for use in a clinical setting.

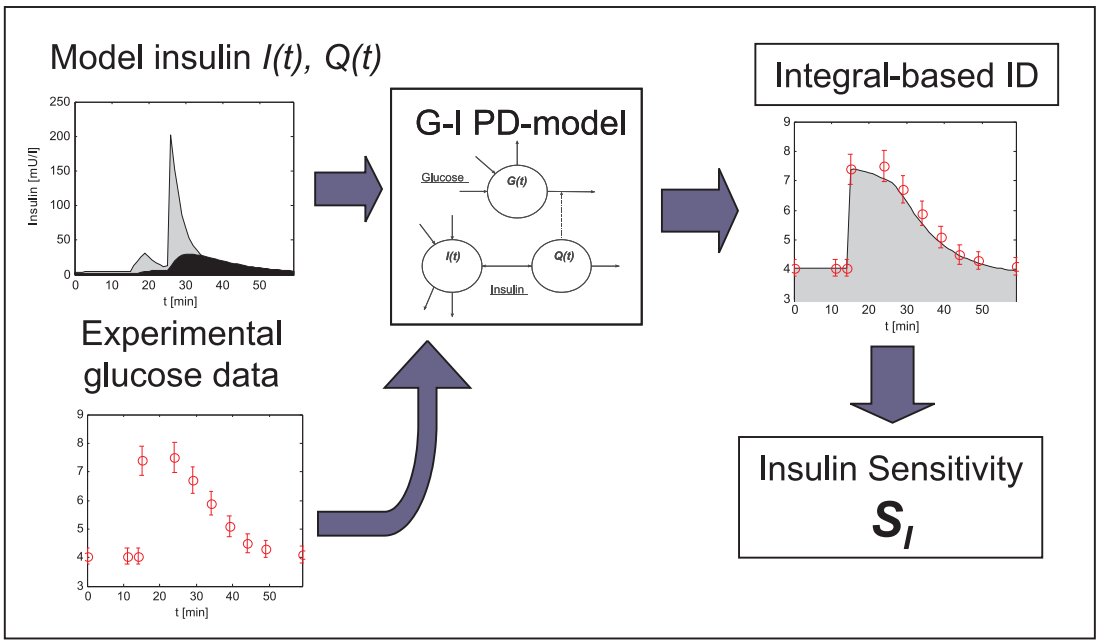


Figure 4.8 The overall method of estimating the model-based insulin sensitivity S_I. Modelled peripheral insulin $Q(t)$ and sampled glucose concentration data are combined in the glucose/insulin pharmaco-dynamic model. Insulin sensitivity is estimated by fitting the model with the integral-based fitting approach, as described in Section 4.3.

Chapter 5

Validation of Insulin Models

The insulin and C-peptide models and identification methods described in Chapter 3 require validation on experimental data. Suitable data to validate all aspects of the models are obtained from the published literature. The two main aspects, endogenous insulin secretion (β-cell function) and insulin kinetics are validated in separate steps to avoid tradeoffs between effects and parameters. The validation of each model starts with details about the experimental data used and the performance metrics chosen to assess model performance, followed by the validation results and discussions.

5.1 Insulin/C-peptide Secretion

Model-based estimation of insulin secretion is validated on intravenous glucose tolerance test (IVGTT) data. The IVGTT is ideal for validation, as it includes the highly dynamic endogenous insulin secretion impulse response of the system to a glucose bolus. Briefly, a glucose bolus is injected intravenously, triggering the bi-phasic endogenous insulin response [Ferrannini and Mari, 2004]. The IVGTT thus provides data from which both phases of β-cell function can be distinctively identified, therefore providing the opportunity to measure and assess pancreatic performance.

5.1.1 Experimental Data

The C-peptide data from IVGTT studies used in this validation have been generously provided by Dr. Andrea Mari (Institute of Biomedical Engineering, National Research Council, Padova, Italy) and Dr. Angelo Avogaro (Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy). The data have been previously published [Mari, 1998], with a full description of the subjects and experimental protocol. Key aspects relevant to this study are briefly reproduced here for clarity.

The study was performed on 12 subjects, 5 with normal glucose tolerance (NGT) (mean±SEM: age 24±2, weight 73±6 kg, fasting glucose 5.2±0.1 mmol/l, fasting insulin 50 ± 5 pmol/l) and 7 with type 2 diabetes (type 2) (mean±SEM: age 49 ± 5, weight 81 ± 3 kg, fasting glucose 8.6 ± 0.8 mmol/l, fasting insulin 125 ± 27 pmol/l). Pharmacological treatment in type 2 diabetic individuals was stopped 3 days before the study to eliminate a confounding affect, and all subjects received a 2000 kcal/day diet (50 % carbohydrate, 35 % fat, 15 % protein) for at least 30 days prior to the study, to standardise this aspect.

An insulin-modified IVGTT was performed on all subjects in the morning after an overnight fast. After three fasting samples at -30, -15 and 0 min, a 0.3 g/kg glucose bolus was injected intravenously. At 20 minutes, insulin was infused intravenously for 5 minutes, totalling 0.03 U/kg (NGT) and 0.05 U/kg (type 2). For an 80 kg individual, these doses are 24 g glucose and 2.4-4 U of insulin. Blood samples were collected at 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 40, 60, 80, 100, 120, 140, 160, 180, 210, and 240 min, and analysed for C-peptide, glucose and insulin concentrations.

5.1.2 Performance Metrics

Pancreatic secretion characteristics are compared, where available, to data estimated in the original study by Mari [1998]. The performance metrics defined try to capture all possible secretory characteristics of interest and include:

1. **First phase peak secretion rate (S_{max})**: Missing samples in the first minutes after the glucose input can lead to large errors in estimated peak secretion rate, due to the slower observed increase in C-peptide concentration that results.

2. **Total insulin/C-peptide secreted in first phase, 0-10 min (AUC_{10})**: The area under curve (AUC), or also described as acute insulin response (AIR), is a common metric to describe the total insulin secreted during first phase response [Ferrannini and Mari, 2004; Pacini and Mari, 2003]. It is calculated by integrating the estimated secretion rate over 0-10 minutes.

3. **Total insulin/C-peptide secreted between glucose and insulin inputs, 0-20 min (AUC_{20})**: As the exogenous insulin administered at $t = 20$ min inhibits pancreatic insulin secretion, it could be of interest to assess the total amount of endogenously secreted insulin until it is inhibited.

4. **Total insulin/C-peptide secreted during the IVGTT (AUC_{total})**: Calculated by integrating the insulin secretion rate over the complete test.

Expected error ranges introduced by the assay were assessed by Monte Carlo analysis of the estimated secretion rate over 10^4 runs. The analysis utilised data that was normally distributed, zero-mean random noise with a coefficient of variation (CV) of 3 %, which is the error reported for current state of the art assays [Roche, 2005]. This value is a conservative choice for this analysis, as older radio immunoassays have CVs up to twice this value [Clark, 1999]. These larger CVs would result in even larger allowable errors from the reduced sampling protocol. Hence, the smallest assay errors were utilised for comparison.

Statistical Analysis

Normality of results was assessed by the single sample Kolmogorov-Smirnov (KS) hypothesis test with a significance level of 0.05. Where results were log-normally distributed, the geometric mean (log-normal mean) and multiplicative standard deviation [Limpert et al., 2001] are used, as noted in the respective results.

5.1.3 Reduced Sampling Intensity Approach

The very frequent sampling performed in this experiment immediately after glucose administration allows an accurate assessment of the first phase secretory peak. In clinical practice, sampling with such a high frequency is not feasible, but a significant error could be introduced in S_{max} and AUC_{10} by not sampling the concentrations in the first 5 minutes after the glucose bolus is administered. To assess this error, these performance metrics are calculated for the case in which the first two samples after glucose administration are at 6 and 10 minutes, as glucose is administered between 0-1 minutes.

The error is introduced by an underestimated area under the concentration curve in minutes 1-6, similar to the potential interpolation error in the glucose curve shown in Figure 4.7. A proposed solution to this lack of data in a clinical test is the introduction of an estimated peak concentration, placed 1 minute after the administration of glucose, in this case at 2 minutes. This introduces a faster rise in concentration in the interpolated profile, and thus a sharper peak in estimated secretion rate.

The concentration profiles in this data set increase immediately after the glucose bolus is administered and peak at approximately 2-3 minutes. The peak concentration is slightly higher than the concentration sampled at 6 minutes. A 'correction' sample is thus introduced at 2 minutes, with a value 10 % larger than the sample taken at 6 minutes. This approach is shown for one subject in Figure 5.1.

5.1.4 Results

Pre-hepatic insulin secretion rate was estimated well with the full data set using the proposed integral-based method of Section 3.2. The overall result was the stepwise constant endogenous insulin and C-peptide secretion profiles shown in Figure 5.2. The qualitative shape of the secretory curves compare well to the clinical data in the original publication [Mari, 1998].

Mean peak secretion rate is slightly higher in this study in both subgroups. This difference may be due to the smaller stepsize (1 minute vs. 2 minutes)

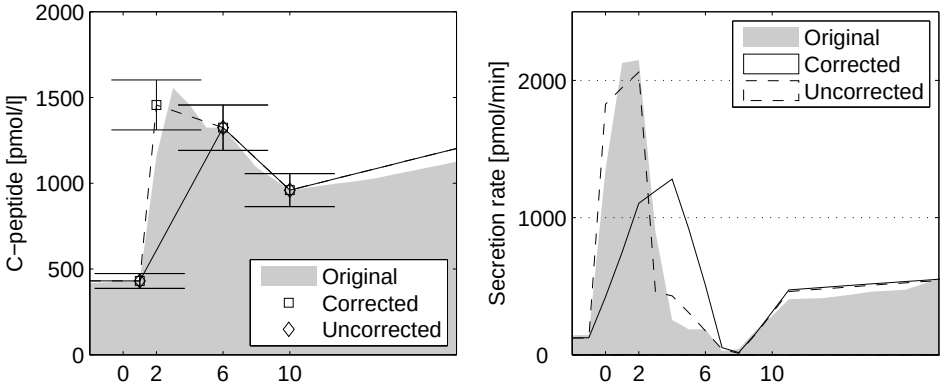


Figure 5.1 Errors potentially introduced by reduced sampling during the first 5-10 minutes after glucose administration. Shown are the C-peptide profiles (left) and the Estimated secretion rate (right). The grey area shows the original profile, the solid line (and diamonds) the profile with only sampling at 6 and 10 minutes, and the dashed line (and squares) the results using a corrected peak.

chosen for fitting in this study. Total mean amount secreted in the first 6 minutes after glucose administration (ISR_1 in Mari study) is virtually identical in NGT (1659 pmol vs. 1667 pmol in Mari's study), but larger in type 2 diabetes (655 pmol vs. 430 pmol in Mari's study). Further performance metric results are given in Table 5.1.

Table 5.1 Performance metrics given as geometric mean and multiplicative standard deviation.

	NGT	**Type 2**
$\mathbf{S_{max}}$ [pmol/min]	2628.1 (1.8)	871.4 (2.5)
$\mathbf{AUC_{10}}$ [pmol]	10456.4 (1.8)	4799.8 (2.5)
$\mathbf{AUC_{20}}$ [pmol]	15303.8 (1.7)	9791.3 (2.4)
$\mathbf{AUC_{total}}$ [pmol]	42804.3 (1.4)	82536.1 (2.2)

Errors in the measured performance metrics due strictly to assay errors were assessed by Monte Carlo analysis and are given as a CV for each metric, with the median and 100 % range over all 12 subjects:

- S_{max}: CV=5.47 %, range $2.97 - 11.01$ %
- AUC_{10}: CV=4.10 %, range $1.92 - 9.39$ %
- AUC_{20}: CV=3.13 %, range $1.90 - 4.26$ %

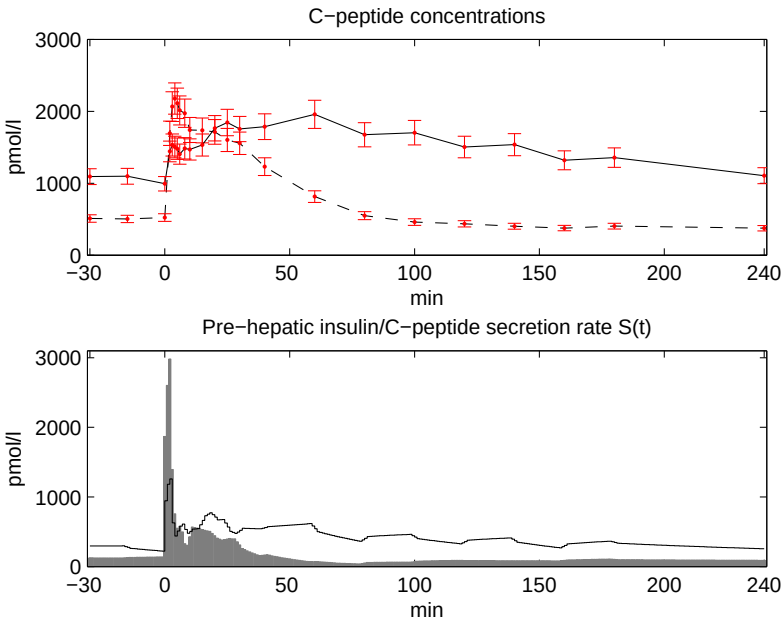


Figure 5.2 Above: Mean C-peptide concentration in NGT (dashed) and type 2 diabetes (solid) subjects. Samples from Mari [1998] are shown with error bars of ±2 SD. Below: Mean estimated C-peptide secretion rate (ISR) in NGT (grey area) and type 2 diabetes (solid line) subjects.

- AUC_{total}: CV=1.11 %, range $0.97 - 1.25$ %

Within these CV ranges it is effectively impossible to determine whether a difference is due to models and methods or to simple assay error. Thus, in comparing results to those in Mari's study, these values are important.

Reconstruction of C-peptide concentrations from the identified secretion profiles resulted in the residuals shown in Figure 5.3. Residuals are given as relative values (decimal percentages). Deviations from the original sample set are caused by smoothing of the estimated secretion profile or by errors introduced through the linear interpolation used between samples in the integral-based fitting method. The ideal goal is to have all variation within the dashed lines due to assay error.

Estimated C-peptide secretion rate with only the 6 and 10 minute samples after glucose administration, and with the correction peak introduced, are shown in Table 5.2, as a relative difference to the original full sample set.

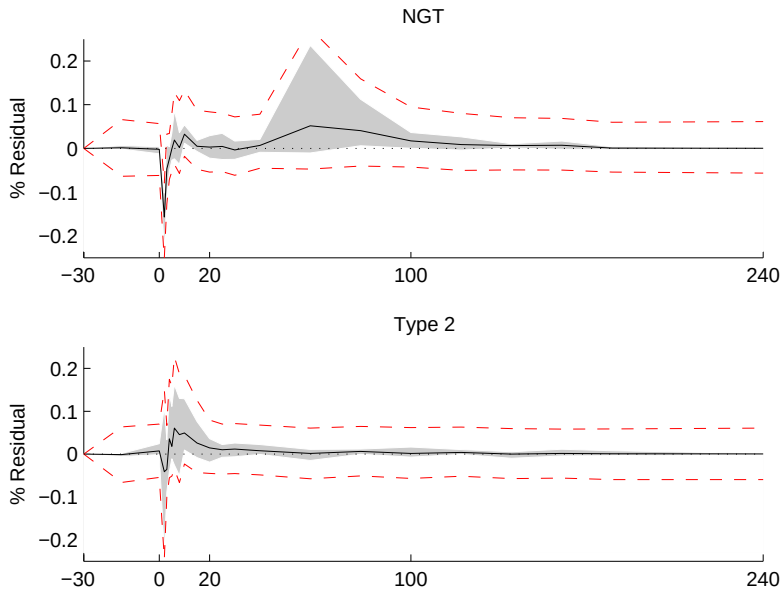


Figure 5.3 Residuals introduced when reproducing the full C-peptide sample profile. The solid line shows the mean residuals and the grey area the 100 % range of residuals. The dashed lines show the 95 % range of residuals introduced by assay error, as estimated by Monte Carlo analysis.

5.1.5 Discussion and Conclusions

Estimating pre-hepatic insulin secretion through modelling of C-peptide kinetics has been a common methodology and it is relatively easy to perform in research settings [Hovorka et al., 1996; Polonsky et al., 1986b; Van Cauter et al., 1992; Watanabe et al., 1989]. In particular, the population method proposed by Van Cauter et al. [1992] enables the estimation of insulin or C-peptide secretion rate with a single experiment. By employing this method, model parameters are consistent across studies, enabling a better comparison, as tradeoffs between estimated parameters and secretion rates are reduced. Nonetheless, the estimation of peak secretion rate and insulin secreted during first phase is still highly dependent on assay errors and sampling frequency during the initial minutes. Ideally, sampling should be performed every minute to assess an accurate profile. However, such frequent sampling introduces significant labour and cost, and reduces robustness of the method.

As the highly frequent sampling performed in this study is not feasible in a clinical setting, the effects on estimated secretion rate of a reduced sampling

Table 5.2 Deviations in performance metrics in the case of lacking samples immediately after glucose administration. Shown are the case of sampling only at 6 and 10 minutes, and the introduction of a correction peak at 2 minutes. Differences are given as percent, relative to the full sample set.

	NGT	**Type 2**
Only sample at 6 and 10 minutes		
$\mathbf{\Delta S_{max}}$	-38.9 % (SD 2.9 %)	-35.8 % (SD 13.8 %)
$\mathbf{\Delta AUC_{10}}$	-12.4 % (SD 1.2 %)	-10.6 % (SD 8.8 %)
Correction peak at 2 minutes		
$\mathbf{\Delta S_{max}}$	-1.8 % (SD 5.3 %)	-0.6 % (SD 21.5 %)
$\mathbf{\Delta AUC_{10}}$	-0.7 % (SD 2.2 %)	-2.4 % (SD 10.9 %)

during the first phase secretion were assessed. Instead of using the full sampling set (2, 3, 4, 5, 6, 8, 10 minutes), only samples at 6 and 10 minutes were used. Due to the slower increase of the interpolated profile between 0 and 6 minutes, the estimation of S_{max} was greatly reduced by ∼36 %-39 % in the NGT and type 2 diabetes subgroups, respectively. The effect on total secretion rate was not as large, only underestimating AUC_{10} by ∼10 %, which is still in the range of errors attributable to assay errors. This minimal effect is due to the longer duration of first phase secretion, compensating for the reduced peak, as seen in Figure 5.1.

By introducing a corrected peak at 2 minutes, the initial slope of the interpolated profile is matched to the initial slope seen in the full data set, and the errors are thus minimised. This effect can be seen clearly in Figure 5.1, in which the shape of the corrected secretion rate (dashed) closely matches the original secretion rate (grey area). This approach can enable a more accurate estimation of peak secretion rate, whereas the errors in AUC_{10} are not as large.

It is important to keep in mind that significant errors are also introduced due to assay inaccuracy. For example, peak estimated secretion rate, S_{max}, has a median CV=5.47 % and can thus vary between ±11 % (±2 SD), even with a 1-minute step sampling protocol. Most of the performance metrics are within, or slightly outside of ±2 SD of assay error, meaning that they are in fact just within the natural variability that can be identified [Clark, 1999].

Using the same model and the parameter estimation method from Van Cauter et al. [1992], estimation of secretion rate has previously been proposed by deconvolution [Eaton et al., 1980] and a more elaborate constrained regularisation method

[Hovorka et al., 1996]. The main drawbacks of these methods are the individualised method adjustments required for each subject, including knot placements for cubic spline interpolations [Eaton et al., 1980], or a separate step to find the optimal proportionality constant in each subject [Hovorka et al., 1996]. All of these extra steps introduce time, computation and human variability into the results, thus obviating a single consistent method.

In contrast, the integral-based method described in this study is a single step, computationally convex and fast method that only requires linearly interpolated data. By constraining the linear least squares estimation to non-zero values and smoothing the estimated secretion rate, the resulting profile is physiologically accurate and the effects caused by noisy data are reduced [Hann et al., 2005b]. First and second phase secretion characteristics were clearly identified, with slight quantitative, but not qualitative deviations from the profiles reported originally with this data. In addition, these smaller deviations can be readily explained by the longer stepsize used in that study. Finally, the integral-based method is consistently applied across all subjects and both subgroups.

From Figure 5.3 it can be seen that the C-peptide concentration profile is reconstructed with the estimated and smoothed secretion rate within the expected assay errors. The highest errors are apparent during 0-20 minutes, which is attributable to the high rate dynamics that occur in this stage following glucose administration. Measurement errors during this stage also have a relatively larger effect on the estimation of secretion rate, thus magnifying the underlying errors.

Finally, the performance metrics show the typical characteristics that clinically and physiologically differentiate NGT and type 2 diabetes subjects, further validating the method. While in the NGT subgroup first phase secretion accounts for about 25 % of total secreted insulin, it is only about 5 % in the type 2 diabetes group, with the secretion rate only peaking at about a third of the value reached by NGT subjects. Total secreted insulin is twice as high in type 2 diabetes as in NGT. These metrics provide further important metabolic information about the subject, which can be used clinically to supply a more complete picture of their metabolic state of health.

Overall, the method to assess insulin secretion could be validated with very good consistency on the IVGTT data. In addition, the IVGTT uses essentially

the same perturbation of the pancreas as the intended test being developed. Further metrics can also be derived from this method, in addition to insulin sensitivity. Hence, the metabolic system and its status can be more completely and accurately defined.

5.2 Insulin Kinetics

The primary goal of the insulin kinetics model validation is to assess the validity of the general fitting approach described in Chapter 3. It is not to perform a full parameter estimation. The objective is thus not a perfect model fit, but a generic and simple approach that yields a good fit even on sparsely sampled data, similar to the data density and requirements intended for a practical clinical test. The fitting approach is considered appropriate if sampled data is matched within measurement noise and parameter values obtained lie within a physiologically valid range.

5.2.1 Experimental Data

To fully validate the insulin model, frequently sampled plasma and interstitial insulin concentrations are required. However, interstitial fluid measurements are difficult to perform and rarely available in most clinical studies. Therefore, separate validation of these two insulin compartments is performed.

To validate the fast and slow components of the decay curve, frequently sampled insulin concentrations are required. This requirement is especially true in the first 10 minutes after a sudden change in input. In addition, endogenous insulin secretion should be captured either through C-peptide sampling or suppressed through somatostatin infusion [Jefferson and Cherrington, 2001; Wahren et al., 1977]. This highly frequently sampled insulin data with complete knowledge of endogenous insulin input are difficult to obtain and have been rarely performed in the past.

In this study, validation is performed using published data from a study investigating the systemic delivery rate of insulin that was performed by Polonsky

et al. [1986a]. In Polonsky et al. [1986a], three different insulin administration profiles were applied to the same group of subjects. These tests allowed all the insulin kinetics to be accurately delineated.

Interstitial insulin kinetics are validated with published data from two different studies. In these studies, the insulin concentration was sampled from muscle interstitial fluid during an OGTT [Sjostrand et al., 2005a] and during a two step euglycaemic clamp [Gudbjornsdottir et al., 2003]. These latter studies allow the interstitial fluid kinetics of the insulin model to be validated.

Plasma Insulin Data

Data used in this validation were taken from a published study by Polonsky et al. [1986a]. Full experimental data could not be obtained from the authors and the three mean concentration plots from page 115-117 of [Polonsky et al., 1986a] were utilised instead. The high frequency sampling in this study make the data ideal to validate the fast component of insulin kinetics.

The study was performed on 8 normal males within 10 % of their ideal body weight. More detailed characteristics are not given in the study description, so a mean weight=75 kg, height=1.80 m and age=30 years were chosen to estimate kinetic parameters. These characteristics approximately match a normal male in the cultural setting in which the study was performed. During all three protocols, a background infusion of somatostatin was administered to suppress endogenous insulin secretion. The three studies reported were performed as follows:

1. **Bolus injection** of insulin (1.5 U) and subsequent sampling for 120 minutes (1 min frequency during the first 10 minutes).
2. **Constant infusion** of insulin for 60 minutes (1 mU/kg/min) and frequent sampling, after which the infusion is stopped and insulin sampled for another 60 minutes (1 min frequency during the first 10 minutes).
3. **Variable rate infusion** of insulin, starting at 0.28 mU/kg/min and progressively increased in 5 min intervals up to 2.14 mU/kg/min by 35 minutes. After 15 minutes at this rate, the infusion was reduced in 10 min steps to reach the initial rate after 60 minutes.

Interstitial Fluid Insulin Data

The first data set used in this validation were taken from a study published by Sjostrand et al. [2005a]. Full experimental data could not be obtained from the authors and the mean concentration plots from page 154 of [Sjostrand et al., 2005a] were utilised instead. Brief study details are reproduced here for clarity. Further details are described in [Sjostrand et al., 2005a]. Note that this publication was later retracted [Sjostrand et al., 2005b] due to a data handling mistake, which is accounted for in this validation, thus not affecting the results.

Oral glucose tolerance tests were performed on 10 lean (5 male, 5 female, BMI=23 (SD 0.6) kg/m^2, age=39 (SD 4) years) and 10 obese (5 male, 5 female, BMI=33 (SD 1.2) kg/m^2, age=41 (SD 3) years) individuals. After an overnight fast, an oral glucose load (75 g) was ingested and plasma and interstitial insulin sampled every 15 minutes for a total of 120 minutes.

The second data set used were taken from a study employing the same ISF sampling technique during a euglycaemic clamp test [Gudbjornsdottir et al., 2003]. A two-step clamp was performed, the first step at an insulin infusion of 120 mU/min/m^2, and the second step at an insulin infusion of 240 mU/min/m^2. Both clamp steps were held for 120 minutes. The study population consisted of 10 lean male subjects with BMI=23 (SD 2.8) kg/m^2 and age=26 (SD 5) years.

Interstitial fluid measurements were performed by means of a microdialysis technique [Sjostrand et al., 1999]. More specifically, interstitial fluid is sampled directly at muscle tissue. This approach is more accurate than earlier interstitial insulin studies sampling lymph concentrations [i.e. Steil et al., 1996; Yang et al., 1989].

5.2.2 Methods

Plasma Insulin Kinetics

Plasma insulin concentrations from the three protocols in Polonsky et al. [1986a] are fitted as described in Chapter 3, using the reported infusion profiles as input

u_{ex}. As no C-peptide data are available, only the parameter n_L is estimated from the data. To account for an incomplete suppression of endogenous insulin by somatostatin [Toffolo et al., 1980; Wahren et al., 1977], a low constant infusion of 4 mU/min (∼25 % of fasting basal secretion) is assumed and included as u_{en}.

Interstitial Fluid Insulin Kinetics

To validate interstitial insulin kinetics, Equation 3.8 is solved with the measured and interpolated plasma insulin concentration $I(t)$ used as input to the equation. In the OGTT study, $I(t)$ cannot be modelled independently, as insulin appearance in these tests is from pancreatic secretion, which cannot be estimated from the data sampled in this study. In the clamp data, the insulin infusion protocol is known, and plasma insulin can be modelled along with ISF insulin $Q(t)$.

The parameters to be validated are n_I and n_C. This task is done with a sensitivity analysis on $\gamma = Q_{SS}/I_{SS}$, which defines the concentration gradient to be reached, and n_I, which defines the speed at which this gradient is reached. Note also that n_I also appears in Equation 3.7 that defines plasma insulin kinetics. This analysis therefore yields information on the robustness of the use of population parameters.

5.2.3 Results

Plasma Insulin Kinetics

Just estimating hepatic clearance n_L from data, while holding V_P, V_Q, n_K, n_I and n_C at population parameter values from Table 3.1, resulted in a very good fit in all three cases. This result can also be seen in Figure 5.4. Estimated cohort values for n_L were 0.19, 0.17 and 0.16 min^{-1} for protocols 1-3 respectively. This limited variation is within expected natural variability [Duckworth et al., 1988; Ferrannini and Cobelli, 1987b] and also within the variability that might be seen from assay errors. The fit could potentially always be improved by fitting more parameters, but that would require an unacceptable compromise in computation, robustness and simplicity. The goal was to validate the described identification

method, not to achieve a perfect fit. A fit was considered good if most data points were matched within measurement noise and the key dynamic aspects of the data were reproduced reasonably well.

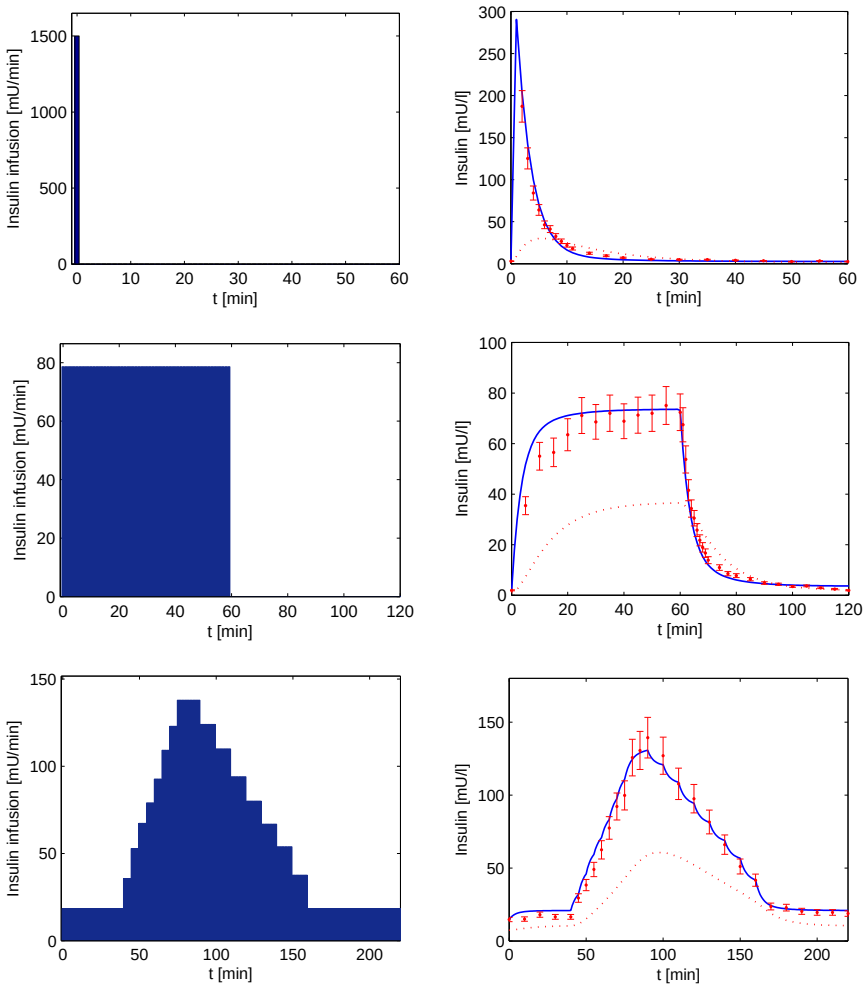


Figure 5.4 Experimental protocols from Polonsky et al. [1986a] (Protocol 1-3, from top to bottom). Insulin input (left) and resulting plasma insulin concentration with model fit (solid) and modelled interstitial insulin $Q(t)$ (dashed) (right).

Interstitial Fluid Insulin Kinetics

In the validation of interstitial fluid kinetics, the mean steady state concentration gradient γ (with $\pm\,10\,\%$ error) is 0.65 (0.58 - 0.71) and 0.55 (0.5 - 0.6) in the lean and obese groups of the OGTT, respectively. In the clamp data, γ is 0.55 (0.5 - 0.6). Maintaining this ratio is important, as a variation outside of the $20\,\%$ band

reported would achieve inaccurate steady state values. Parameter n_C thus needs to be bound to n_I through γ, as defined in Equation 3.11.

In the OGTT studies, the value of n_I calculated a-priori results in a profile of interstitial insulin $Q(t)$ that is well within measurement error. The parameter is not very sensitive, as can be seen by the calculated profiles shown in Figure 5.5. Multiplying and dividing n_I by a factor of 2 and 3 still achieves reasonably good data fits of $Q(t)$. A factor of 2 is mostly still within measurement error.

In the clamp study, the plasma insulin concentration profile could be modelled in addition to the ISF profile, resulting in a good fit as shown in Figure 5.5. Estimated hepatic clearance was similar to the values identified in the plasma insulin validation, $n_L = 0.15$. The effect of n_I on plasma insulin is shown by the two dotted lines around the plasma insulin profile shown in Figure 5.5, representing an increase and a decrease of n_I by a factor of 3.

ISF insulin concentrations during the first 90 minutes could not be matched by the model fit, as the concentration gradient is significantly lower at $\gamma =\sim 0.2$. This lower gradient is identified by the authors of the study as a methodological sampling problem at lower insulin concentrations, and is thus not considered a modelling error in this study [Gudbjornsdottir et al., 2003]. The last sample of this first clamp step, at 120 minutes, is in line with the assumed $\gamma = 0.55$, as well as the remaining samples during the second clamp step. Increasing and decreasing n_I by a factor of 2 and 3 results in good model fits. The value of n_I has the biggest impact during the first 10 minutes, in which the insulin infusion loading protocol causes a very fast increase in concentrations.

5.2.4 Discussion and Conclusions

Plasma Insulin Kinetics

The insulin kinetics model, with the parameter estimation approach presented in Chapter 3, shows very good performance in fitting the frequently sampled plasma insulin data of Polonsky et al. [1986a]. This is a relatively remarkable performance, as only one parameter, the hepatic clearance n_L, is estimated from the data. All the other parameters are calculated from a-priori known subject

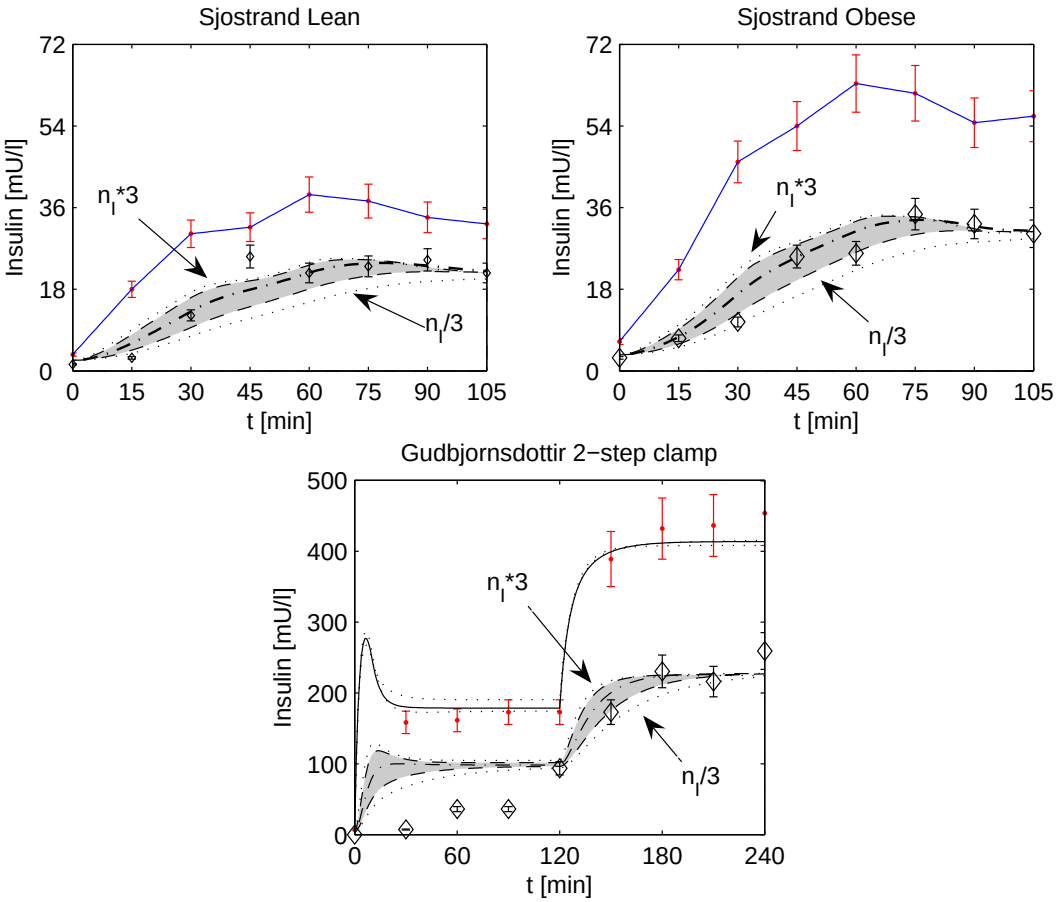


Figure 5.5 Sensitivity analysis on n_I performed on mean plots of lean (upper left) and obese (upper right) subjects from [Sjostrand et al., 2005a], and on the clamp data from [Gudbjornsdottir et al., 2003]. Average values for γ are used in each case, with $\gamma = 0.65$ used for the lean OGTT, $\gamma = 0.55$ for the obese OGTT and the clamp subgroups. Shown are plasma insulin samples and linear interpolation in OGTT or modelled insulin in clamp (solid), interstitial insulin concentration samples (diamonds) and modelled interstitial insulin, with $n_I = 1{\times}n_I$ (dash-dot), $2{\times}n_I$ and $1/2{\times}n_I$ (grey area) and $3{\times}n_I$ and $1/3{\times}n_I$ (outer dashed lines). The dotted plasma insulin profiles in the clamp plot show the modelled profiles at $3{\times}n_I$ and $1/3{\times}n_I$.

characteristics using population values or equations.

With more elaborate fitting approaches, and by allowing more parameters to vary, the fits could potentially be further improved, but only with significant additional effort. This is not desired in this study, as the methods are designed to be applicable on sparsely sampled experiments, and the model performance shown in this chapter is more than adequate for such an application. Note that an over-fitting of the data can potentially also reduce accuracy and increase variability, as is the case in the Minimal Model, in which S_G^{MM} is overestimated and S_I^{MM} underestimated, as discussed in more detail in Chapter 3.

Estimated n_L varied slightly during the different protocols, but not exceeding a natural physiological variability or assay error. The values were lower in the infusion protocols than in the bolus injection, which could have been caused by a different suppression of endogenous insulin secretion in the different types of insulin administration. In addition, hepatic extraction has been shown to be highly variable and saturable when exposed to high insulin concentrations, particularly over time [Ferrannini and Cobelli, 1987b; Thorsteinsson, 1990; Toffolo et al., 2006], a dynamic that was not accounted for in this validation study.

It should be noted that the plasma insulin data used in this validation can be much more variable than just the assay errors. The study by Polonsky et al. [1986a] was performed over 20 years ago, when insulin assays were not necessarily very accurate and had a high cross-reactivity to proinsulin [Chevenne et al., 1999; Robbins et al., 1996]. Even today, the insulin assay is not completely standardised and large inter-laboratory variations persist [Sapin, 2003].

To conclude, the insulin kinetics model and the a-priori parameter estimation approach described in Chapter 3 could be validated sufficiently well with the presented data. The variable protocols of an impulse response, a step increase and a gradual increasing infusion consistently show very good model performance. Frequently sampled plasma insulin samples allow validation of the fast and the slow components of insulin kinetics.

Interstitial Fluid Insulin Kinetics

Validation of interstitial insulin concentration modelling shows good performance and robustness. The key parameter to estimate in this case is the steady state concentration gradient $\gamma = Q_{SS}/I_{SS}$, which is in the reported range of 0.5-0.7. Estimating this parameter in individual tests would require separate sampling of interstitial fluid, which is not viable in a simple clinical test. Thus, fixing the parameter to a value within this range provides a reasonable and simple method to identify the model within physiological plausibility.

Testing robustness by modifying n_I by a factor of 2 and 3 results in the plots shown in Figure 5.5. As the data is very noisy, especially at lower concentrations [Gudbjornsdottir et al., 2003; Sjostrand et al., 2005a], a change by a factor of

2, shown by the grey area in the plots, does not compromise the quality of fit significantly. A further increase by a factor of 3 does not affect the plot as strongly as a reduction by a factor of 3. The parameter thus seems to be more robust on the high side, and as long as a large enough value is chosen, model performance is within expected bounds.

The interstitial insulin measurements done by Sjostrand et al. [2005a] and Gudbjornsdottir et al. [2003] are a novel and still experimental technique, requiring complicated calibration methodology [Gudbjornsdottir et al., 2003; Sjostrand et al., 1999]. Further validation of these methods are necessary until more accurate results are obtainable. Nonetheless, the parameters of interstitial fluid insulin kinetics result in very reasonable data fits, suggesting that they are potentially very close to the true values.

5.3 Summary

In the validation performed in this chapter, insulin and C-peptide models and identification methods presented in Chapter 3 could be satisfactorily validated. Pancreatic insulin secretion can be estimated with high accuracy and performance on bolus dose response data as found in an IVGTT. Plasma insulin kinetics can be estimated very accurately with the a-priori parameter identification proposed and by estimating only one parameter from data. Interstitial kinetics are well represented and robust with the population values estimated a-priori.

The insulin model and its parameter estimation method, mostly employing population parameters, thus provide not only a physiological insulin kinetics model, but also a method to identify its parameters with minimal data requirements. This approach performed very well as validated on the data in this chapter. Overall, the simplicity, robustness and physiological validity of the proposed approach make it ideal for use in an application designed for a clinical setting.

Chapter 6

Euglycaemic Clamp Validation

The euglycaemic-hyperinsulinaemic clamp [DeFronzo et al., 1979] is the gold standard to assess insulin sensitivity. Model validation is performed on clamp data to verify how the model-based metric for insulin sensitivity S_I compares to the clamp derived metric ISI. The correlation of both metrics gives an indication of the performance of the overall model, including the insulin, C-peptide and glucose components.

The clamp test is described in more detail in Chapter 2, but is briefly outlined here for improved clarity. The clamp test consists of a constant infusion of insulin and a variable infusion of glucose to maintain euglycaemia. The result is an eventual suppression of endogenous insulin and glucose with the goal to reach a metabolic steady state. The first hour of the test is highly dynamic in glucose concentration, as the system needs to be stabilised, which is achieved by proposed formulae [DeFronzo et al., 1979; Ferrannini and Mari, 1998] and/or experience from medical staff. The second hour is the steady state and ideally constant insulin and glucose concentrations are achieved, which are used for the calculation of ISI.

6.1 Experimental Data

The clamp data used in this validation have been kindly provided by Dr. Kirsten McAuley and Prof Jim Mann from the Edgar National Centre for Diabetes Research, Dunedin, New Zealand. The data have been previously published [McAuley et al., 2002], with full description of subjects and experimental proto-

col. Key aspects are briefly reproduced here for clarity.

The data consist of euglycaemic-hyperinsulinaemic clamp tests performed by McAuley et al. [2002] during a study assessing insulin resistance in normoglycaemic individuals before and after a 16 week lifestyle intervention. The study population was randomised into control, moderate and intense intervention subgroups. The subgroup results are not relevant in this validation and the cohort is thus simply split into the pre- and post-intervention groups. Cohort data are given in Table 6.1.

Table 6.1 Description of the intervention study population from McAuley et al. [2002]. ISI is the clamp based metric of insulin sensitivity.

	Mean (SD)	**Range**	**Mean (SD)**	**Range**
Age (All N=146)	46.8 (8.9)	30-68		
	pre-interv. (N=73)		**post-interv. (N=73)**	
Fast. glucose $[\frac{mmol}{l}]$	4.9 (0.6)	4.0-6.8	4.8 (0.6)	3.5-6.9
Fast. insulin $[\frac{mU}{l}]$	19.9 (12.1)	6.6-84.3	17.2 (11.2)	5.7-65
BMI $[\frac{kg}{m^2}]$	34.4 (4.9)	24.5-45.2	33.2 (5.0)	23.6-44.8
Weight $[kg]$	96.7 (15.3)	67.9-140.8	93.4 (15.5)	62.5-142.4
ISI $[\frac{mg/kg/min}{mU/l}]$	3.03 (0.9)	1.16-5.15	3.79 (1.3)	1.74-8.37
HOMA-IR $[\frac{mU}{mmol}]$	4.4 (3.2)	1.4-24.4	3.8 (3.0)	0.9-19.9

The clamp was performed after an overnight fast, and was run over 120 minutes. The protocol infused insulin (Actrapid) at 40 mU/m^2/min between 10 and 120 minutes and tried to maintain the blood glucose levels at a goal of 4.6 mmol/l. An insulin loading protocol was applied during the first 10 minutes, starting at 127 mU/m^2/min with an 11 % reduction per minute, as described by DeFronzo et al. [1979]. The glucose infusion was adjusted at 10 minute intervals. Glucose concentration was sampled every 10 minutes and insulin at 0, 60, 90, 120 minutes. Further details are described in [McAuley et al., 2001].

Glucose clearance (M-value, mg/kg/min) is the average glucose infusion during the last 60 minutes, with a space correction to compensate for deviations in glucose concentrations between 60 and 120 minutes [DeFronzo et al., 1979; McAuley et al., 2001]. The insulin sensitivity index ISI is M divided by the

average measured plasma insulin concentration during the last 60 minutes:

$$ISI = \frac{M}{\frac{I_{60}+I_{90}+I_{120}}{3}} \tag{6.1}$$

6.2 Methods

6.2.1 Comparison of Insulin Sensitivity Metrics

When comparing ISI and S_I, it is important to first understand the extent of similarity between these two measures of insulin sensitivity to avoid a comparison of "apples and oranges". The ISI calculation of the clamp is the steady state ratio of glucose infusion rate M per kg body weight (mg/kg/min), divided by the average plasma insulin concentration (mU/l) at a balances homeostatic state. It is assumed that during the clamp steady state all endogenous glucose and insulin secretion is fully suppressed, and that all glucose and insulin in the body is being infused externally [Bergman et al., 1985]. The test metric thus tells the clinician how much glucose the body can metabolise at a given plasma insulin concentration. More importantly, it assumes all glucose uptake to be mediated by insulin and that the uptake rate is proportional to plasma insulin concentration.

This assumption is not physiologically accurate, as it neglects insulin independent glucose uptake. This uptake can be dependent on glucose concentration or can be constant, as is the uptake by the brain and central nervous system, which at ∼100 mg/min is quite substantial [Baron et al., 1988; Zierler, 1999]. The mean glucose infusion rate in this study population is 890 mg/min, so brain uptake accounts for over 10 % of the infused glucose.

A further physiological misassumption in the clamp calculation is the lack of glucose clearance saturation [Chase et al., 2004; Natali et al., 2000; Prigeon et al., 1996]. At the high rate of glucose and insulin infusion, glucose clearance saturation is very likely, thus resulting in an underestimation of true insulin sensitivity. As saturation and insulin sensitivity trade off and cannot uniquely be identified on data from one test [Natali et al., 2000], the model saturation parameter is set to α_G=0 to match the clamp assumption. This dynamic should

thus not further cause additional bias between ISI and S_I.

The model-based S_I (l/mU/min) is in principle similar to ISI, as it relates the rate of glucose uptake to an insulin concentration. The main difference is that insulin in interstitium, $Q(t)$, instead of insulin in plasma, $I(t)$, is used. This approach is physiologically more accurate, but results in a systematic shift when comparing absolute metrics.

A further important difference is the fact that the glucose model accounts for additional glucose uptake that is not dependent on insulin, such as glucose-only dependent uptake, p_{GU}, and constant uptake, GU_G. As these two effects are physiologically valid and included in the model used in this research, they are also used to validate the model against the clamp, to improve comparability between the metrics attained in both tests. Removing these two effects, and relating all glucose uptake to insulin dependent effects, results in more similar assumptions to the clamp and thus likely in a tighter correlation.

When comparing the units of both metrics, it is evident that a correction is necessary to compare absolute values. In this example, ISI is corrected to match the units of S_I:

$$ISI \left[\frac{\text{mg} \cdot \text{l}}{\text{kg} \cdot \text{min} \cdot \text{mU}}\right] \times \frac{\text{weight}}{G_{clamp} V_G} \left[\frac{\text{kg}}{\text{mg/l} \cdot \text{l}}\right] = S_I \left[\frac{\text{l}}{\text{mU} \cdot \text{min}}\right] \tag{6.2}$$

where the weight/V_G term is used to normalise to body mass and volume. Similarly, the $1/G_{clamp}$ term normalises the clearance to the steady state glucose value achieved. This is a common approach to reduce the dose dependency of the clamped glucose level and to correct for tests that deviate slightly from this clamp goal [Bergman et al., 1985].

As can be seen in Equation 6.2, further variability can be introduced by the unit conversion. The volume V_G cannot be measured nor estimated from the clamp data and an estimation of its value has to be made. A common choice is $V_G = 0.19 \times$weight [DeFronzo et al., 1979]. Using a weight-dependent volume causes the effect of weight and volume to cancel out of Equation 6.2, and merely introduces a constant factor. A more conservative choice is to estimate

the volume dependent on the estimations for insulin distribution, V_P and V_Q, as this decorrelates weight and volume and simulates a more realistic situation.

6.2.2 Parameter Estimation

Fitting of data was performed with the integral-based fitting method described in Chapter 4. In addition to the parameters identified a-priori, V_G was set to $V_G = 1.2 \cdot (V_P + V_Q)$, as the clamp data are not dense enough to allow a unique identification. A 20 % larger volume than the total insulin distribution volume was chosen, as apparent glucose distribution volume has been found to be larger than the total distribution volume for insulin, due to fast hepatic storage and non-insulin dependent uptake by the brain [Despopoulos and Silbernagl, 2003; Waterhouse and Keilson, 1972].

Hepatic insulin clearance n_L is estimated as a constant over 120 minutes. Glucose uptake at basal insulin, p_{GU}, cannot be estimated from these data, as a high insulin concentration is present throughout the test and was fixed at a constant value of $p_{GU} = 0.004$ over 120 minutes, as described in Chapter 4. Insulin sensitivity, S_I, is fitted as a constant over each 60-minute period to assess any differences during transient S_{I-TR} and steady S_{I-SS} states.

During a clamp test EGP is reduced and eventually suppressed by the high infusions of glucose and insulin [Bergman et al., 1985]. As the profile of EGP cannot be measured from the available data, an assumption of its profile is made. The assumption is a linear suppression during the transient 0-60 minutes and full suppression thereafter, in line with observed metabolic responses during this test [DeFronzo et al., 1979; Ferrannini and Mari, 1998]. If this assumption is physiologically feasible, S_{I-TR} and S_{I-SS} should be very similar. In contrast, if no suppression of EGP is actually the case, S_{I-SS} will likely be overestimated by seeing more glucose removal than actually occurs.

The goodness of the model fit at steady state is assessed as the relative difference between ISI values calculated from experimental data and ISI values calculated from glucose and insulin levels simulated by the model, given as a percentile root mean square error (RMSE). Errors in fitted glucose and insulin profiles are given to assess fit accuracy at transient states.

6.2.3 Correlation Analysis

Modelled insulin sensitivity parameter S_I is correlated (Pearson correlation coefficient) to clamp derived ISI, normalised by the average plasma glucose concentration during the final hour steady state ($ISI_G = ISI/G$) [Bergman et al., 1985]. ISI is normalised to account for trials in which a steady state glucose concentration is not fully attained and the mean steady state value deviates from the target of 4.6 mmol/l. It also matches the units for ISI_G and S_I, making comparisons clearer, per Equation 6.2.

For the steady state comparison, S_I during the last 60 minutes (S_{I-SS}) was used, as ISI_G is calculated over the same interval. The transient value of S_I is fitted over 0-60 minutes (S_{I-TR}) to assess the potential of the model for a dynamic insulin sensitivity test. Pearson correlation coefficients were obtained for the whole data set and the pre- and post-intervention subgroups.

Statistical Methods

Probability distributions of the insulin sensitivity parameters could not be assumed to be normal, as assessed with the Shapiro-Wilk test. To assess the significance of the correlations, their 95 % confidence intervals (CI) in the form of percentile intervals were calculated using the nonparametric bootstrap, which does not require a parametric distribution model for the data [Efron and Tibshirani, 1993]. Where data are log-normally distributed, the geometric mean and multiplicative standard deviation (MSD) are used to describe the spread [Limpert et al., 2001].

6.3 Results

Mean S_I at steady state was $S_{I-SS} = 4.85 \times 10^{-4}$ (MSD 1.54) l/mU/min and at transient state $S_{I-TR} = 4.82 \times 10^{-4}$ (MSD 1.51) l/mU/min. The mean values and distributions of steady state and transient state S_I are statistically equal ($P = 0.71$). In contrast, assumption of no suppression of EGP resulted in a larger S_I during steady state, $S_{I-SS} = 7.34 \times 10^{-4}$ (MSD 1.48) l/mU/min, as

compared to the transient state, $S_{I-TR} = 6.01 \times 10^{-4}$ (MSD 1.43) l/mU/min, as hypothesised. Both S_I values were significantly different in the unsuppressed EGP assumption case, as assessed by the Wilcoxon rank sum test ($P < 0.001$).

The root mean square error (RMSE), shown in percent, between clamp ISI from experimental and modelled data for all 146 tests was 1.8 % (MSD 2.8). The expected error in the calculation of ISI, due to error propagation of sensor errors and device inaccuracies, was calculated from the data as 6.8 % (SD 0.45 %, range 6.24 - 9.32 %) over all 146 tests. The RMSE between experimental and modelled glucose and insulin values were 2.9 % (MSD 3.5) and 3.9 % (MSD 3.4), respectively. Hence, all ISI errors are within measurement error propagation.

Mean estimated hepatic insulin clearance rate was $n_L = 3.68 \times 10^{-2}$ (SD 2.04×10^{-2}) min^{-1}. Further estimated parameters are given in Table 6.2 for the pre- and post-intervention subgroups. A sample model fit is shown in Figure 6.1.

Table 6.2 Parameter estimates from clamp data fit, given for the pre- and post-intervention subgroups. Data given as mean and SD and geometric mean and multiplicative SD (MSD) where noted specifically.

	Mean	**SD**	**Mean**	**SD**
	pre-interv.	**(N=73)**	**post-interv.**	**(N=73)**
V_G [l]	12.22	1.06	11.99	1.08
V_P [l]	4.52	0.37	4.46	0.36
V_Q [l]	5.67	0.54	5.54	0.57
n_K [$10^{-2}\,\text{min}^{-1}$]	6.00	0.24	5.95	0.28
n_L [$10^{-2}\,\text{min}^{-1}$]	3.24	2.14	4.12	1.84
n_I [10^{-2} l/min]	27.56	2.70	26.90	2.86
n_C [$10^{-2}\,\text{min}^{-1}$]	4.86	0.06	4.86	0.06
EGP_b [mg/kg/min]	1.47	0.29	1.51	0.29
S_{I-SS} [10^{-4} l/mU/min]	4.25	MSD 1.54	5.54	MSD 1.49
p_{GU} [min^{-1}]	0.004			

Correlation between clamp derived ISI and steady state S_{I-SS} is $r = 0.953$ (95 % CI: 0.933 - 0.968), and transient state S_{I-TR} is $r = 0.920$ (95 % CI: 0.881 - 0.950). To reduce variability introduced by a deviation from the clamped glucose concentration, ISI is commonly normalised by the steady state glucose concentration [DeFronzo et al., 1979; Ferrannini and Mari, 1998], denoted ISI_G. ISI_G is further unit-corrected to reduce variability introduced by model assumptions for V_G and to match the units of S_I (l/mU/min). The resulting correlation between

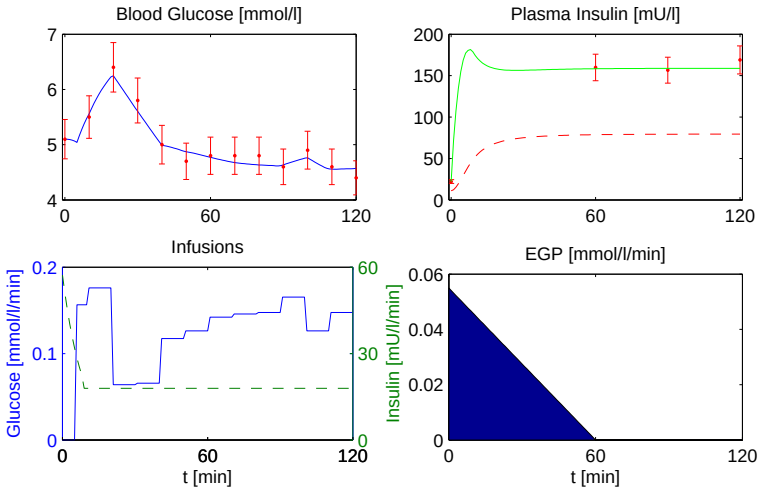


Figure 6.1 Example of a clamp data model fit and corresponding samples. Shown are glucose concentration (upper left), insulin concentration (upper right) with plasma $I(t)$ (solid) and interstitial $Q(t)$ (dashed) concentrations, infusions (lower left) of glucose (solid) and insulin (dashed), and profile of EGP.

ISI_G and steady state S_{I-SS} is $r = 0.995$ (95 % CI: 0.992 - 0.997). At transient state S_{I-TR} the correlation is $r = 0.924$ (95 % CI: 0.887 - 0.951), thus lower and with a broader CI. Both regression lines have a y-intercept indifferent from zero and a slope of 0.52 and 0.51 for steady and transient state, respectively. The slopes are very close to $\gamma = Q_{ss}/I_{ss} = 0.5$, the expected ratio between ISI_G and S_I. Correlations for the steady and transient states are shown in Figure 6.2 for all 146 clamp tests.

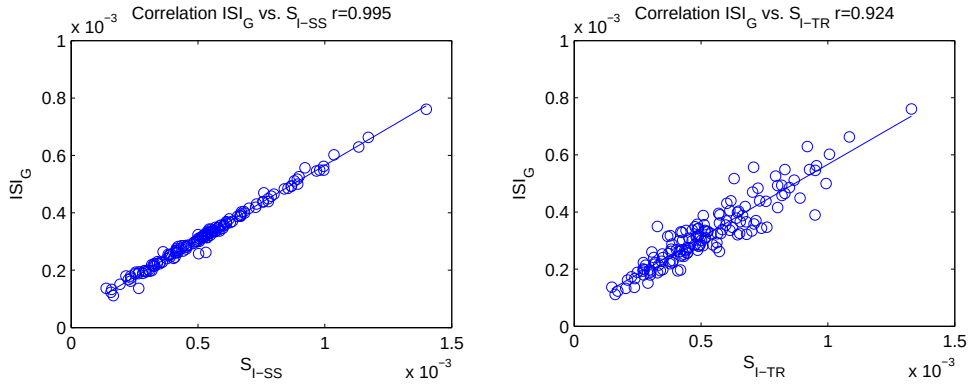


Figure 6.2 Correlation of ISI_G and S_{I-SS} (left) and ISI_G and S_{I-TR} (right).

As steady and transient state S_I are nearly identical, fitting the data with a constant value of S_I over 120 minutes should result in an equally good fit. The RMSE between experimental and modelled glucose and insulin values were

slightly larger at transient state (3.8 % (MSD 3.2)), but equally accurate at steady state (3.9 % (MSD 3.4)). Correlation between ISI_G and S_I in this case is $r = 0.981$ (95 % CI: 0.971 - 0.988). This result is more accurate than the transient only correlation, as the steady state data are taken into account in the fitting. In addition, S_I would be physiologically expected to be constant over a 2-hour test.

Pre- and post-intervention subgroups showed a similar trend when analysed independently. More specifically, there were higher correlations at steady state and at post-intervention. These results are given in Table 6.3, along with correlations of ISI_G. Finally, correlations to log-HOMA, the logarithmic transformation of HOMA-IR, are also shown, given its wide clinical use [Wallace et al., 2004b]. Note that the logarithmic transformation of HOMA-IR is required to enable a comparison of the linear trend between both metrics, as described in Section 2.2.

Table 6.3 Results of correlation analysis for the whole population and the pre- and post-intervention subgroups, given for steady and transient states.

Metric	**All (95 %CI)**	**Pre (95 %CI)**	**Post (95 %CI)**
S_{I-SS} vs. ISI_G	0.995 (0.992-0.997)	0.988 (0.978-0.995)	0.998 (0.997-0.999)
S_{I-TR} vs. ISI_G	0.924 (0.889-0.950)	0.912 (0.869-0.951)	0.922 (0.873-0.955)
ΔS_{I-SS} vs. ΔISI_G	0.990 (0.984-0.995)		
ΔS_{I-TR} vs. ΔISI_G	0.897 (0.833-0.938)		
log-HOMA vs. ISI_G	-0.48 (-0.59- -0.35)	-0.49 (-0.64- -0.32)	-0.44 (-0.60- -0.25)

A further analysis is done to see how the model captures the change in insulin sensitivity after the intervention, as compared to the clamp ISI. This result is also shown in Table 6.3. Correlation of ΔS_{I-SS}, between pre- and post-intervention, and ΔISI_G is $r = 0.990$ (95 % CI: 0.984 - 0.995), and between ΔS_{I-TR} and ΔISI_G is $r = 0.897$ (95 % CI: 0.833 - 0.938). The regression lines have y-intercepts indistinguishable from zero, and slopes of 0.54 and 0.50 in steady and transient states, respectively, where a slope of 0.5 is expected due to $\gamma = 0.5$. The correlation plots in steady and transient states are shown in Figure 6.3.

Relative changes in ISI_G and S_{I-SS} are shown in Figure 6.4, along with

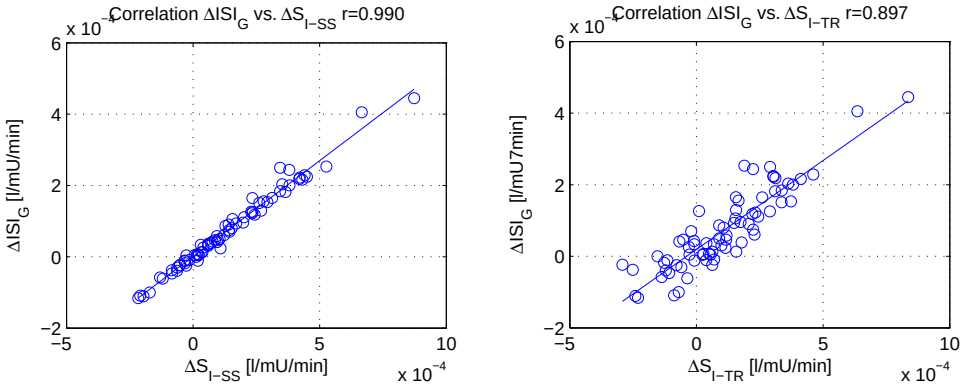


Figure 6.3 Correlation of ΔISI_G and ΔS_{I-SS} (left) and ΔISI_G and ΔS_{I-TR} (right).

relative changes in log-HOMA. These plots clearly show the accuracy of S_I in capturing the change in insulin sensitivity as compared to a common and typical fasting measure (log-HOMA) that clearly does not capture the trend. Note that log-HOMA is also one of the clinically highly regarded fasting measures [Ferrannini and Mari, 1998; Monzillo and Hamdy, 2003].

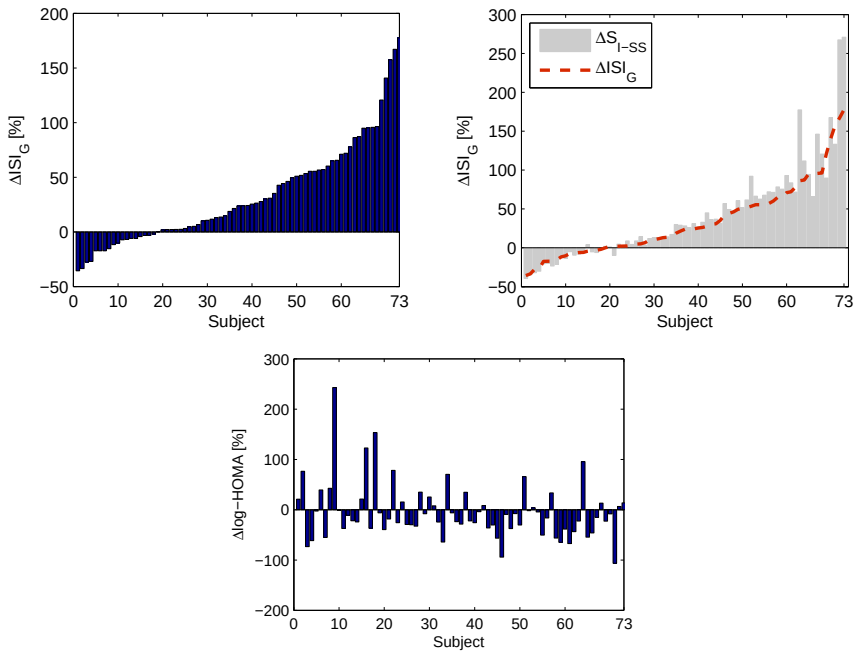


Figure 6.4 Relative percentile change in ISI_G (upper left), S_{I-SS} (upper right) and log-HOMA (bottom).

6.4 Discussion and Conclusions

The error in modelled vs. calculated ISI over all 146 trials is well within the expected accuracy from assay errors and reflects the ability of the model to match the glucose and insulin dynamics during steady state. The mean fitting error of the glucose profile of 2.9 % (MSD 3.5) shows that the model also captures the transient dynamics in glucose during the first 60 minutes. Errors in modelled glucose are attributed to highly dynamic metabolic changes at the beginning of the clamp, such as EGP, which are likely not fully captured by the model.

The assumption of a steady reduction of EGP during the transient state and full suppression during steady state proved to be a physiologically sensible approach. The difference in transient and steady state S_I is statistically insignificant, validating this approach. This result is further confirmed with the good correlation of S_I ($r = 0.98$) when fitted as a value constant over the whole 120 minute trial.

The fact that the model fit with constant parameters is within measurement noise, validates the model dynamics. In particular, it shows that all variability can be accounted for by its structure leaving only measurement error. Hence, these results validate the overall model, its structure, and the fitting methods, values and assumptions. A separate fitting of the transient state is still important to validate the model's transient performance. This value assesses the estimation of S_I when only transient data are considered. Such a transient form of data would be the case in a simple clinical test.

When EGP is kept constant throughout the clamp, estimated S_I is increased during steady state, which is a result of overestimating modelled glucose input. In particular, small deviations of γ from the expected or ideal value $\gamma = 0.5$ could be attributed to errors or deviations in the 60-minute, linear EGP suppression assumed. However, the overall good validation results obtained indicate that this assumption is also not far from reality in the absence of better data that are typically not available.

The insulin profile in steady state was captured by fitting only one parameter of the insulin kinetics equations, hepatic clearance n_L. The mean fitted value of $n_L = 3.68 \times 10^{-2}$ (SD 2.04×10^{-2}) min^{-1} results in a hepatic metabolic clearance

rate of 4.6 ml/min/kg (range 2.7-6.9), which is slightly lower than the reported range of 5.0-8.5 ml/min/kg found by others [Ferrannini et al., 1983; Sherwin et al., 1974]. This lower clearance could be caused by various factors, such as heavy saturation of the liver being exposed to such large supra-physiological concentrations [Thorsteinsson, 1990], reduced clearance in obesity [Valera Mora et al., 2003], or incomplete suppression of pancreatic insulin during the clamp test, resulting in an apparent lower clearance rate. None of these effects are accounted for in the model or data fit and result in an underestimated n_L. Incomplete suppression of pancreatic insulin secretion is particularly likely, given the shorter and lower dose method used in this specific clamp study [Ferrannini and Mari, 1998].

The goodness of fit for the first hour of the insulin profile could not be assessed, as measurements were taken at only 0 and 60 minutes. However, the shape of the modelled transient insulin profile matches profiles reported in similar studies [Bergman et al., 1985; DeFronzo et al., 1979]. The glucose profile was fitted by estimating only one parameter, S_I. The quality of fit is not compromised by keeping p_{GU} constant throughout the cohort. Estimation of p_{GU} is not possible on this data, as the insulin concentration is high throughout the test and does thus not allow insulin-independent effects to be identifiable. Furthermore, the effect of p_{GU} is small in this study, as it is not effective near the basal glucose concentrations, which are close to the clamped 4.6 mmol/l.

The mean value of S_{I-SS} is higher than the unit-corrected ISI_G ($S_{I-SS} = 1.59 \times ISI_G$). This factor is due to the difference in the assumptions of the two calculations. The clamp calculation of ISI assumes all insulin in plasma ($I(t)$) is active to enable glucose uptake by the cells. The model uses the modelled insulin concentration in interstitial fluid, $Q(t)$, which has been shown to be correlated to glucose uptake in dogs [Miles et al., 1995; Yang et al., 1989] and humans [Castillo et al., 1994]. It is also a more physiological assumption.

As the steady state insulin concentration gradient is $\gamma = 0.5$, ideally S_{I-SS} should be $2 \times ISI_G$ if both metrics were otherwise equal. The further reduction of this factor to 0.51 is caused by the constant glucose uptake GU_G and the suppression of EGP (discussed in detail in Section 6.2), which is included in the model but not the clamp calculation. This constant uptake reduces the amount of glucose that is to be cleared by insulin, thus reducing the value of S_I.

Correlations between ISI_G and S_{I-TR} at transient points in the trial are useful to see how well the model performs in estimating insulin sensitivity when the steady state assumptions of the ISI_G calculation are not met and a steady state is not yet reached. It provides a means to verify if the model-based S_I would be equally accurate if determined from a short 30-60 minute dynamic test. Correlations between ISI_G and S_{I-TR} were slightly lower than at steady state, but at $r = 0.92$ still very high and higher than similar dynamic metrics. As a comparison, Table 6.4 shows various correlation coefficients of the similar model-based IVGTT compared to the clamp. Different IVGTT protocols are shown with a wide range of correlation values, mostly in the range of $r = 0.5$ to $r = 0.7$.

Table 6.4 Correlation coefficients reported in various studies comparing the IVGTT with the euglycaemic clamp test. A standard IVGTT includes only a glucose bolus. A tolbutamide-modified IVGTT additionally includes added tolbutamide to trigger pancreatic insulin secretion. An insulin-modified IVGTT additionally includes an insulin bolus. Subgroups are normal glucose tolerant (NGT), impaired glucose tolerant (IGT), obese (OB) and type 2 diabetes (T2).

IVGTT	**Weak**	**Strong**	**Subjects**	**Refs**
Standard	$r = 0.44$		12 NGT	[Donner et al., 1985]
180 min	$r = 0.53$		9 NGT, 3 IGT, 8 T2	[Foley et al., 1985]
	$r = 0.54$		10 NGT	[Beard et al., 1986]
Tolbut.		$r = 0.84$	10 NGT	[Beard et al., 1986]
180 min		$r = 0.89$	5 NGT, 5 OB	[Bergman et al., 1987]
	$r = 0.71$		35 NGT	[Saad et al., 1997]
Insulin	$r = 0.57$		28 NGT, 13 OB, 15 T2	[Katz et al., 2000]
180 min	$r = 0.48$		20 IGT	[Saad et al., 1994]
	$r = 0.41$		12 T2	[Saad et al., 1994]
	$r = 0.70$		35 NGT	[Saad et al., 1997]
	$r = 0.73$		12 T2	[Coates et al., 1995]

Comparing the correlations between S_I and ISI_G in the pre- and post-intervention subgroups in Table 6.3, a slightly better correlation can be seen in both states after the intervention. This increase in correlation is very small and not statistically significant ($P < 0.001$) A reduction in the correlations can be seen when correlating the transient states, as would be expected due to this highly dynamic perturbation. The correlations decrease to $r = 0.91$ in the pre-intervention group, with a wider CI of $r = 0.87 - 0.95$. The still comparably narrow 95 % CIs in all subgroups highlight the positive correlations.

One reason for the lower correlations at transient state is a greater variability in S_{I-TR}, which compensates for endogenous insulin and glucose production

insufficiently accounted for in the model. This estimation could be improved by modelling or estimating endogenous insulin, through C-peptide measurements [Van Cauter et al., 1992] and/or estimating a more correct hepatic glucose output through tracer experiments [Vicini et al., 1999]. While the first option is readily applicable without significantly adding to test complexity, tracer experiments are too complex and expensive to perform without highly specialised and trained personnel [Carson and Cobelli, 2001].

The effect of the intervention, measured as a change in insulin sensitivity, was well captured by the model, with correlations of the change in sensitivity of $r = 0.99$ and $r = 0.90$ in steady and transient states, respectively (Figure 6.3). The regression line of the changes had a linear relationship with a y-intercept very close to zero and slopes of 0.54 and 0.50, respectively. These slopes should ideally be 0.5, and thus show very close similarity of both metrics. The deviation from the ideal line is due to experimental problems resulting in insufficiently clamping the steady state, thus resulting in incorrect clamp ISIs. In addition, model assumptions about the suppression profile of EGP may play a small role. How well the model captures the change in S_I is visualised better in Figure 6.4, in which the relative change in S_I is shown in a sorted order. The change in S_{I-SS}, though more noisy than ISI_G, captures the major trend in changing sensitivity (Figure 6.4, upper right).

One of the more widely used surrogates for estimating insulin sensitivity in clinical practice is the HOMA-IR measure [Wallace et al., 2004b]. This fasting measure does not correlate as well with experimental ISI_G in this population, with $r = -0.48$ over all 146 tests. Other studies have reported a wide range of correlations $r = -0.2$ to $r = -0.8$ in different subgroups [Bonora et al., 2000; Lewanczuk et al., 2004; Mather et al., 2001], matching the wide 95 % CIs in Table 6.3. Figure 6.4 (lower plot) shows the change in sensitivity as assessed by log-HOMA, which clearly does not capture the trend seen in the clamp ISI and the model S_I, rendering it clinically infeasible for such a study or use.

The results of this validation study, especially during the transient state of the trial, show the performance of the validated system model when correlated to the clamp derived ISI_G. Unlike the clamp or IVGTT, which rely on a steady state to assess insulin sensitivity, this model and fitting method has the potential to perform similarly well as the clamp in a much shorter transient test. These

results show great potential for the performance of the model in such a short, dynamic test.

A key factor in the increased variability during transient state is incompletely modelled dynamics, mainly endogenous insulin and glucose. They are a main cause for previously reported over- and underestimation of the Minimal Model's parameters S_G^{MM} and S_I^{MM} [Caumo et al., 1999; Mari, 1997]. Errors in these effects are lumped into S_{I-TR}, causing a greater variability in the transient state. However, for these clamp fits, with the assumed suppression profile of EGP, the equality of S_I in both states indicates these effects were captured sufficiently well.

Suppression of EGP is not as strong during a low-dose bolus injection of glucose and insulin and should not have such an impact on S_I estimated from such a test [Jefferson and Cherrington, 2001]. Overall, these clamp validation results show great model performance in this highly dynamic test and very good correlations in insulin sensitivity metrics as compared to the gold standard metric, even in a transient state.

6.5 Summary

In the validation performed in this chapter, the pharmaco-dynamic models and identification methods presented in Chapters 3-4 could be satisfactorily validated by fitting them to euglycaemic-hyperinsulinaemic clamp trials. Performance was very good, with correlations between model- and experimental metrics for insulin sensitivity of $r = 0.92$ and $r = 0.99$ in transient and steady states. Estimated insulin sensitivity during steady and transient states was nearly equal, further validation the modelling assumptions.

Correlation coefficients between this model's S_I and the clamp ISI are better than in other insulin sensitivity tests. This better comparative performance is a resulting consequence of the specific design aspects of this PD model. Overall, the model performance validated in this chapter shows great promise for the intended insulin sensitivity test.

Chapter 7

Proposed Insulin Sensitivity Test

The ability to sensitively and accurately identify individuals with insulin resistance (IR) is critical for the implementation and assessment of intervention programmes in high risk groups. To diagnose IR in population studies and to be applicable in clinical settings, a test has to be simple and cost effective. However, it must also be accurate enough to assess small changes in IR or the progression of treatment.

In this chapter, a clinical test protocol for a model-based assessment of insulin sensitivity is proposed. The protocol applies the models and methods described in Chapters 3-4 to estimate insulin sensitivity and β-cell function from clinically sampled data. A Monte Carlo error analysis is performed on a simulated virtual cohort to assess the expected accuracy in repeatability and in correlation to the euglycaemic clamp test, in the face of clinical and assay noise.

7.1 Overview and Goal

The target environment for the proposed protocol is a clinical setting with commonly available blood sampling and assay facilities. The protocol should thus be simple and not require specially trained personnel and equipment such as glucose tracers [Caumo and Cobelli, 1993] or hand arterialisation equipment (i.e. heated hand technique [Bergman et al., 1985; Godsland et al., 1993]). In essence, it must be of low clinical intensity and effort.

A dose response test capturing the metabolic response to a glucose and in-

sulin injection is very rich in information about the analysed system and less noise-affected than a static or constant infusion sample. The impulse response can be fitted with a model of the relevant physiology to derive metabolic information from its parameters. Hence, a simple dose-response format has significant potential in both a clinical and modelling scene.

The IVGTT with Minimal Model assessment is such a test, and is commonly used in research studies [Bergman et al., 1981]. The main disadvantages of the IVGTT are its duration (3 hours), number of samples (22+ samples), non-physiological dosing (20-30 g glucose, 2-5 U insulin), and triggering of regulatory responses, such as suppression of EGP. Furthermore it usually requires arterialisation of venous blood and very frequent sampling (1-2 minutes) during the first 10-20 minutes. All these factors limit its use to highly controlled research settings.

The Minimal Model identification of an IVGTT with commonly used fitting methods [Carson and Cobelli, 2001] generally overestimates insulin independent clearance S_G^{MM} and thus overestimates S_I^{MM} [Quon et al., 1994b]. The main reason identified for this behaviour is the simplified description of glucose kinetics by a mono-compartmental model [Caumo et al., 1999]. As explained in Section 4.2.1, this problem can be addressed by understanding the source of the fitting error, namely trying to fit the fast decay in the initial minutes of the test with an unsuitable model.

The proposed test is based on the same dose-response principle, but should be significantly shortened in duration (< 60 minutes) and sampling (< 10 samples). Dosing should be physiological (~ 10 g glucose, ~ 1 U insulin) to assess a more accurate effect and minimise endogenous regulatory responses that can negatively affect the results. No specialised knowledge or test equipment (beyond standard clinical practice) should be required. The application of improved modelling and fitting approaches should thus be able to improve test performance enough to meet the clinical accuracy requirements.

Additional information about endogenous insulin response to glucose, or β-cell function, can also be gathered in this type of data by sampling and modelling C-peptide kinetics. To assess this response, a gap of at least 10 minutes is required between glucose and insulin administration, as exogenous insulin injection

suppresses pancreatic insulin secretion [Jefferson and Cherrington, 2001]. The key secretory response of interest after such a bolus injection of glucose is the first-phase secretion of insulin [Ferrannini and Mari, 2004], which lasts approximately 10 minutes. Therefore, this very valuable diagnosis of β-cell function can be easily obtained in addition to insulin sensitivity.

7.2 Protocol Design

7.2.1 Clinical Aspects

The test protocol is designed with physiological, computational and practical aspects in mind, and limited in time to be less than 60 minutes long. A more extensive sampling and dosing protocol is proposed first, which is then simulated before it is tested in a clinical pilot study. From this more extensive protocol, shortened versions are derived in a further step in Chapter 9 and their performance compared to the full protocol. The protocol is shown schematically in Figure 7.1 and briefly defined in the following steps:

1. Inject a fixed dose of glucose (5 g, 10 g or 20 g) at 0 minutes.
2. Inject a fixed dose of insulin (0.5 U, 1 U or 2 U) at 10 minutes.
3. Sample blood at -10, 0, 5, 10, 15, 20, 25, 30, 35, 45 minutes and assay for glucose, insulin and C-peptide concentrations.
4. Fit metabolic models of glucose, insulin and C-peptide to dose response curves.
5. Determine insulin sensitivity from model parameter S_I.

Sampling frequency is initially chosen in 5 minute steps during the more dynamic stages and 10 minute steps during the initial fasting period and the near-fasting condition at the end. A more frequent sampling directly after the injections, as done in an IVGTT, is not clinically practicable. In addition, the mixing process in plasma can take up to 10 minutes to complete [Bergman et al.,

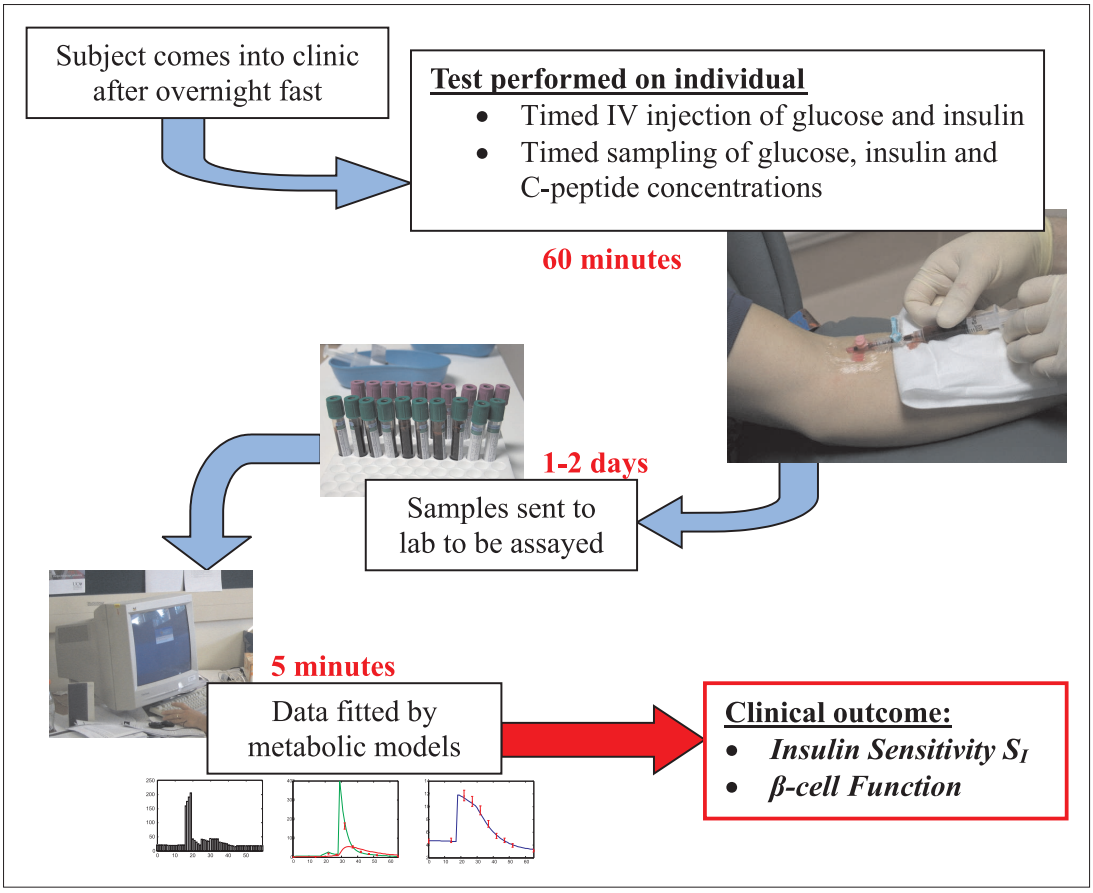


Figure 7.1 Schematic of the steps involved when applying the insulin sensitivity test.

1985; Caumo and Cobelli, 1993], and earlier measurements may thus be inaccurate [Ader and Bergman, 1987].

Blood is to be taken from a venous access for increased safety and simpler protocol. Arterialisation of venous blood, as performed in an IVGTT, should not be a requirement, as specialised equipment would be needed. Sampling venous instead of arterial blood has been shown not to affect Minimal Model parameters in an IVGTT [Godsland et al., 1993].

Glucose and insulin dosing should be as small as possible to measure the necessary effect without assay errors dominating the resulting profiles. However, they should also be smaller than a standard IVGTT to minimise intensity and counter-regulatory responses. These basic requirements ensure a less intense, yet physiological test, as per Figure 2.7.

Three fixed steps in dosing are chosen: 5 g/0.5 U, 10 g/1 U, 20 g/2 U glucose/insulin respectively. The dosing of 10 g of glucose and 1 U of insulin is used as the main option in the following study, as it is $> 50\,\%$ lower than the dose used in an IVGTT, but likely yielding a better signal to noise ratio than the very low dose (5 g/0.5 U). The protocol was also simulated with the low and high dose to assess any differences in results and expected accuracy.

After two fasting samples, taken 10 minutes apart, the glucose is administered as a bolus at $t = 0$ min. At $t = 10$ min, insulin is administered as a bolus. This approach enables the separate information and assessment of a fasting situation, the pancreatic insulin response after the glucose bolus, and the final decay of glucose in the presence of exogenous insulin. The steps involved result in the response profiles shown for glucose and insulin in Figure 7.2.

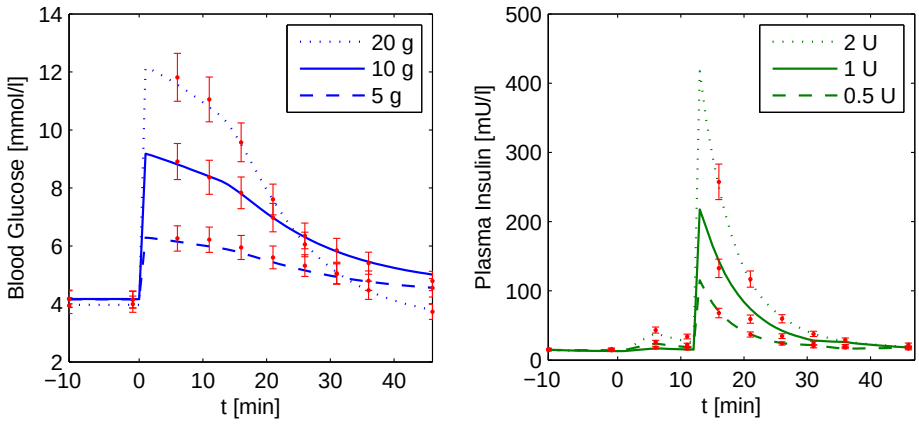


Figure 7.2 Example of simulated profiles of glucose (left) and insulin (right) responses to the low-, medium- and high-dose test protocols on the same virtual subject. Discrete measurements are shown with error bars along with model fits (continuous curves).

7.2.2 Model Fitting

The model fitting of sampled glucose, insulin and C-peptide data is done as described in Chapters 3-4. Some parameters need to be fixed, as the information in the data does not allow a unique identification and inaccurately identified parameters can affect accuracy of the others [Caumo et al., 1999; Chase et al., 2004; Finegood and Tzur, 1996].

Insulin independent glucose clearance p_{GU} cannot be estimated accurately

from the data, and is fixed to an approximate population value for healthy individuals, $p_{GU} = 0.004$, as described in Chapter 4. It is also not a dominant dynamic in the presence of low glucose doses and exogenous insulin [Natali et al., 2000; Prigeon et al., 1996]. Equilibrium glucose concentration, G_E, is set to the fasting glucose level of each subject, as shown in the cohort description in Table 6.1. Glucose clearance saturation is set to $\alpha_G = 0$, as the subjects are fasted, and with the low dose insulin bolus saturation is not likely. This value also better matches the assumptions in calculating ISI for the supra-physiological clamp test [DeFronzo et al., 1979].

Parameters identified from the data are n_L and x_L for the insulin model and S_I and V_G for glucose. The measured data are the plasma glucose, insulin and C-peptide concentrations at each time point. The integral-based fitting method employed is described in detail in Chapters 3-4.

7.3 Monte Carlo Error Analysis

To assess the expected accuracy and repeatability of the test, a Monte Carlo analysis is performed on test simulations, taking into account significant errors and potential unmodelled dynamics or effects. These errors include:

1. Errors in laboratory assays
2. Dilution of input solutions
3. Timing of samples
4. Errors due to unmodelled dynamics

Errors in unmodelled dynamics could include poorly or unmodelled endogenous glucose production (EGP) and first-pass hepatic insulin extraction. The simulations are based on model-based insulin sensitivity (S_I) values obtained from fitting a cohort of 146 euglycaemic-hyperinsulinaemic clamp tests by McAuley et al. [2002], covering a range of metabolic responses. Rather than assessing clinical or physiological validity of the estimated insulin sensitivity value, this specific

study aims at validating the robustness of the proposed test in a noisy clinical test environment.

The simulation procedure used is shown schematically in Figure 7.3, and explained in more detail in this chapter:

1. Insulin sensitivity from 146 euglycaemic-hyperinsulinaemic clamp tests by McAuley et al. [2002] is calculated from the test data (ISI).

2. A virtual cohort is created by fitting the metabolic model to the clamp tests, resulting in a model-based insulin sensitivity for each subject (S_I).

3. Monte Carlo simulations on the proposed test protocol are run on the virtual cohort by adding random noise from published error levels to measurements and inputs, and accounting for potentially unmodelled regulatory dynamics in a randomised function.

4. The metabolic model is fitted to the simulated test profiles (glucose, insulin and C-peptide concentrations), resulting in insulin sensitivity from the Monte Carlo analysis of the proposed test (S_{I-MC}).

5. Performance of the method is assessed by the coefficient of variation (CV) of S_{I-MC} and by correlating S_{I-MC} with ISI and S_I. Additional comparisons are made to HOMA-IR.

7.3.1 Generation of Virtual Clamp Cohort

To simulate the proposed test and make it comparable to the clamp, a simulation cohort was created using metabolic information estimated from a set of clamp trials performed by McAuley et al. [2002] to study the effects of lifestyle interventions on insulin resistance. The data consist of 146 trials performed on 73 individuals, once before and once after a 16 week intervention. The population is identical to the data presented in the clamp validation in Chapter 6 and described in more detail in that chapter. Population characteristics are given in Table 6.1 on Page 96.

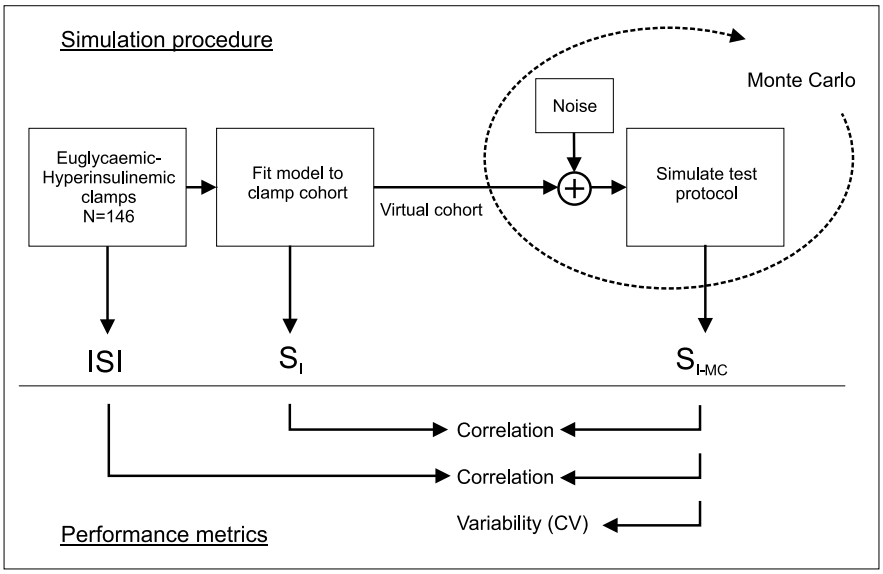


Figure 7.3 Simulation procedure and performance metrics used in this Monte Carlo simulation.

The clamp trials were fitted by the model described in Chapter 4 by estimating parameters S_I and n_L. Mean absolute errors of the fits were 5.9 % (SD 6.6 %) for glucose and 6.2 % (SD 6.4 %) for insulin [Lotz et al., 2006a], as also described in Chapter 6. Insulin sensitivity, S_I, was estimated as time-varying, piecewise constant during transient and steady state [Lotz et al., 2006b]. The steady state value was taken for the subsequent simulations.

Mean n_L estimated from the clamps was very low for this cohort compared to that seen on dose-response tests. This result may be caused by various factors, such as heavy saturation of the liver being exposed to such large supraphysiological concentrations [Thorsteinsson, 1990], reduced clearance in obesity [Valera Mora et al., 2003] or incomplete suppression of pancreatic insulin secretion during the clamp. None of these effects are accounted for in the model or fitting methods, and result in an underestimated n_L. Incomplete suppression of pancreatic insulin secretion is particularly likely, given the shorter and lower dose method used in this clamp study [Ferrannini and Mari, 1998].

To achieve a more realistic insulin profile, n_L was thus increased by 0.1 min^{-1} for all individuals based on empirical testing on various dose-response data. This increase results in a more realistic simulated insulin profile and does not affect the performance of the insulin sensitivity value obtained. Thus, the outcome of

the test and the performance of the simulations are not affected.

Due to the low resolution of the clamp data, further parameters had to be identified a-priori in creating this virtual cohort, as described in Chapter 4. In addition, V_G was set to $V_G = 1.2 \cdot (V_P + V_Q)$, as the clamp data are not dense enough to allow a unique identification. A 20 % larger volume than the total insulin distribution volume was chosen, as glucose distribution volume has been found to be larger than for insulin, due to fast hepatic storage and non-insulin dependent uptake by the brain [Despopoulos and Silbernagl, 2003; Waterhouse and Keilson, 1972]. This choice also does not affect the outcome of the study, as S_I and V_G are subsequently identified from the test profiles, as described in Chapter 4.

All of the model parameters derived from the clamp data and population are summarised in Table 7.1. These parameter values are used in the models of Equations 3.7-3.8 and Equation 4.8 to create the virtual cohort on which the test protocol is simulated.

Table 7.1 Simulation model parameters calculated and estimated as described in Chapters 3-4 to generate the virtual simulation cohort.

	Mean (SD)	**Range**	**Mean (SD)**	**Range**
	pre-interv. (N=73)		**post-interv. (N=73)**	
V_P [l]	4.52 (0.37)	3.98-5.93	4.46 (0.36)	3.90-5.96
V_Q [l]	5.67 (0.54)	4.52-7.47	5.54 (0.57)	4.44-7.26
V_G [l]	12.22 (1.06)	10.20-15.67	12.00 (1.08)	10.00-15.75
n_K [10^{-2} min^{-1}]	6.0 (0.24)	5.3-6.4	6.0 (0.28)	5.3-6.4
n_L [10^{-2} min^{-1}]	15 (2.7)	10-21	16 (2.2)	10-20
n_I [10^{-2} l/min]	28 (2.7)	22-36	27 (2.9)	21-36
n_C [10^{-2} min^{-1}]	4.9 (0.06)	4.7-5.0	4.9 (0.06)	4.7-5.0
S_I	4.25 (MSD 1.54)	1.37-8.63	5.54 (MSD 1.49)	2.22-13.95
[10^{-4} l/mU/min]				
p_{GU} [min^{-1}]	0.004 (fixed)			
α_I [l/mU]	0.0017 (fixed)			
α_G [l/mU]	0 (fixed)			

Pancreatic insulin secretion is not known for this cohort, as C-peptide data are not available. Insulin secretion can be suppressed or reduced by exogenous insulin. However, full suppression is only achievable by a prolonged infusion of large amounts of insulin [Ferrannini and Mari, 1998]. In the protocol for this study, an insulin bolus is injected 10 minutes after glucose, thus not affecting

the first phase endogenous insulin burst, but suppressing second phase insulin secretion. Simulated total insulin secretion rate is thus reduced back to its basal rate after the bolus injection of exogenous insulin [Argoud et al., 1987; Jefferson and Cherrington, 2001; Lotz et al., 2005b].

Pre-hepatic endogenous insulin secretion can be simulated by a basal secretion rate, superimposed by a first-phase burst. The burst peaks at a rate of 72 mU/min/m^2·BSA [Eaton et al., 1980; Mari, 1998], which is dependent on body surface area (BSA), and is followed by an exponential decay lasting 10 minutes. For the lower and higher dose protocol, this first-phase burst is halved and doubled, respectively [Carson and Cobelli, 2001]. Basal endogenous secretion u_b is calculated from the steady state fasting insulin balance using Equation 3.7 with basal insulin concentrations I_b and $Q_b = \gamma I_b$ ($\gamma = 0.5$), and a randomly generated first pass hepatic extraction x_L:

$$u_b = \frac{V_P I_b}{1 - x_L} \left(n_K + \frac{n_L}{1 + \alpha_I I_b} + \frac{1}{2} \frac{n_I}{V_P} \right) \tag{7.1}$$

Total prehepatic endogenous insulin secretion is thus modelled as:

$$u_{en}(t) = \begin{cases} u_b + (72 \cdot \text{BSA}) \cdot e^{-0.3t} & 0 \leq t < 10 \; min \\ u_b & t < 0 \quad and \quad t \geq 10 \; min \end{cases} \tag{7.2}$$

First pass hepatic extraction, x_L, is often approximated around 50 %, but is generally higher in the fasting state [Ferrannini and Cobelli, 1987b; Meier et al., 2005; Toffolo et al., 2006], often reaching values of over 90 % [Meier et al., 2005]. As a conservative choice, x_L is thus determined from a uniform distribution of values between 0.5 and 0.95 (almost complete extraction). Using this model, the total insulin secreted and the peak during the first phase match values reported in the literature [Eaton et al., 1980; Mari, 1998].

Basal endogenous glucose production EGP_b (Equation 4.7) can be split up into insulin dependent, EGP_{Ib} and insulin independent production, EGP_{GE}. As EGP_{GE} is not affected by an exogenous input, as described in Chapter 4, only EGP_{Ib} is taken into account. EGP_b is thus calculated from the fasting steady

state glucose balance in Equation 4.8, where $Q_b = (1/2)I_b$ and $G(0) = 0$:

$$EGP_b = S_I G_E \frac{\frac{1}{2}I_b}{1 + \alpha_G \frac{1}{2}I_b} \tag{7.3}$$

where I_b is the basal plasma insulin concentration, α_G is known in this case (or assumed), and S_I and G_E are also known for the virtual cohort simulation via the data summarised in Table 7.1.

7.3.2 Monte Carlo Simulation Method

The proposed protocol is simulated on the virtual cohort by generating noisy glucose, insulin and C-peptide data and fitting the models to determine their parameters.

Assay errors are assumed normally distributed with inter- and intra-batch coefficients of variation ($\mathrm{CV}_{inter}, \mathrm{CV}_{intra}$) reported by the assay manufacturers. Random intra-batch errors are generated for each sample of a test and added to an inter-batch error, equal for all samples of a given test. As CV_{intra} is assumed to be included in the reported CV_{inter}, the CV to be superimposed on CV_{intra} (CV_{add}) is calculated:

$$\mathrm{CV}_{add} = \sqrt{{\mathrm{CV}_{inter}}^2 - {\mathrm{CV}_{intra}}^2} \tag{7.4}$$

Errors in the timing of samples are caused by variations in blood sampling procedure and are assumed to be normally distributed between ±30 seconds around the sampling time. Due to anticipation of these small complications during sampling, the sampling procedure in similar studies is usually initiated early, thus sometimes resulting in early sampling.

Dilution errors can occur when drawing up glucose in a syringe or when diluting insulin, which is typically distributed in highly concentrated form (e.g. 100 U/ml). Insulin has also been reported to bind to the inner walls of syringes and tubes when being administered, causing a loss of insulin during the dilution

process [NovoNordisk, 2002]. All of these issues are well known problems and usually taken into account by the investigator and the choice of equipment. The errors in this case are also assumed to be normally distributed around the mean.

Suppression of EGP is caused by increases in plasma insulin or glucose [Jefferson and Cherrington, 2001]. The amount and efficiency of suppression is dependent on the administered dose [Vella et al., 2003]. With the low dose this test aims at, the suppression is likely not as large as during an IVGTT ($\sim 75\,\% - 100\,\%$ [Nagasaka et al., 1999; Vicini et al., 1999]), but also cannot be neglected. Since this level of suppression cannot be easily measured, a linear reduction of EGP is assumed from the time of insulin input, reaching a randomly generated maximal suppression EGP_{suppr} at the end of the test. It is defined:

$$EGP(t) = \begin{cases} EGP_b \left(1 - EGP_{suppr} \frac{t}{t_{end}}\right) & 0\ min \leq t < t_{end} \\ EGP_b & t \leq 0\ min \end{cases} \tag{7.5}$$

where the maximal suppression, EGP_{suppr}, at the 10 g/1 U dose was chosen randomly from a normal distribution from values between $25\,\% - 75\,\%$ with a mean value of $50\,\%$. For the lower (5 g/0.5 U) and higher (20 g/2 U) dose variants, EGP_{suppr} was shifted to $0\,\% - 50\,\%$ and $50\,\% - 100\,\%$, respectively. Studies have shown a direct dose-dependent relationship between glucose concentration and suppression of EGP [Vella et al., 2003], validating this basic approach.

The random disturbances thus assumed in this Monte Carlo analysis are summarised:

- Glucose assay errors: CV_{intra}=1 %; CV_{inter}=2 % [Wallace et al., 2004a]
- Insulin assay errors: CV_{intra}=2 %; CV_{inter}=2.8 % [Roche, 2004; Wallace et al., 2004a]
- C-peptide assay errors: CV_{intra}=3 %; CV_{inter}=3.4 % [Roche, 2005]
- Glucose input error: CV=1.67 %
- Insulin input error (dilution): CV=3.33 %

- Sample timing error: SD 10 seconds
- First pass hepatic insulin extraction: $x_L \in [0.50, 0.95]$
- Mean maximal suppression of EGP: EGP_{suppr}=50 %(10 g/1 U); 25 %(5 g/0.5 U); 75 %(20 g/2 U) (SD 8.3 %)

The required number of Monte Carlo simulations was identified to be 500 in a convergence test, as the variability in the standard deviation (SD) of the resulting S_I value identified did not change significantly with more runs.

7.3.3 Monte Carlo Results

Performance of the method was assessed by correlation (Pearson correlation) of the estimated insulin sensitivity S_I with the gold standard clamp test ISI derived clinically. Accuracy in the estimation of S_I is given as its coefficient of variation (CV=SD/mean). The distribution of S_I can be assumed to be normal, as assessed by the single sample Kolmogorov-Smirnov (KS) test.

Accuracy of ISI was assessed by Monte Carlo analysis with assay errors as described above and a glucose infusion error of 10 %. Accuracy of HOMA-IR is also affected by assay errors, as well as pulsatile basal insulin secretion. Therefore, the HOMA-IR criteria is also estimated for comparison using Monte Carlo analysis with a CV of 10 %, as reported by Wallace et al. [2004b], accounting for both assay and natural variability.

The model parameter for insulin sensitivity fit from clamp trials is $S_I = 4.85$ $(MSD\ 1.54) \cdot 10^{-4}$ l/mU/min. This value is higher than clinically measured clamp ISI normalised by steady state glucose and corrected for units $(ISI_G = ISI/G{\cdot}weight/V_G)$ $ISI_G = 3.23$ $(SD\ 1.16) \cdot 10^{-4}$ l/mU/min. This difference is due to the different compartmental insulin concentrations used in the respective calculations. The clamp uses plasma insulin (I) and the modelled S_I uses interstitial insulin (Q). Clamp fitted S_I and measured ISI correlate $r = 0.95$. However, S_I and ISI_G correlate much better $r = 0.995$. The higher correlation with ISI_G is a result of the unit correction, which reduces variability introduced by other parameters and imperfect clamping to a basal glucose level [Bergman et al., 1985].

Mean insulin sensitivity resulting from Monte Carlo analysis is S_{I-MC} = 4.86 (SD 1.55) $\cdot 10^{-4}$ l/mU/min and thus identical to S_I. Correlations with clinically measured ISI and ISI_G are slightly lower, at $r = 0.91$ (90 % CI: 0.90-0.92) and $r = 0.98$ (90 % CI: 0.97-0.98), respectively. Figure 7.4 shows the correlation plot of S_{I-MC} and ISI_G with the 90 % CI's of each metric over all 500 Monte Carlo runs, shown by the cross.

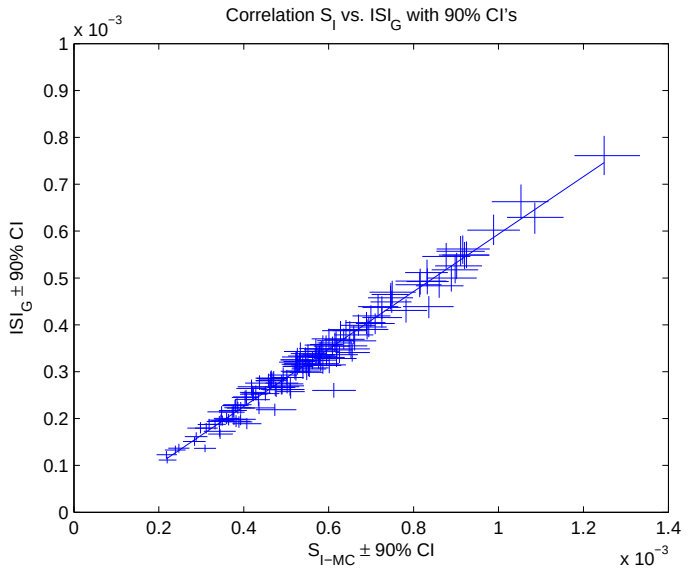


Figure 7.4 Correlation of S_{I-MC} and clinically measured ISI_G with 90 % CI's of each metric and mean regression line. The CI for S_I is assessed by Monte Carlo simulation, the CI for ISI_G by error propagation calculation.

Intra-individual CV in S_{I-MC} using the proposed low intensity test method is CV_{SI}=4.5 % (90 % CI: 3.8 % - 5.7 %). This value is larger than the CV for ISI, CV_{ISI}=3.3 % (90 % CI: 3.0 % - 4.0 %), calculated by error propagation of assay errors, but significantly lower than the CV for HOMA-IR, CV_{HOMA}=10.0 % (90 % CI: 9.1 % - 10.8 %). The three intra-individual CVs are shown in Figure 7.5 for all N=146 subjects.

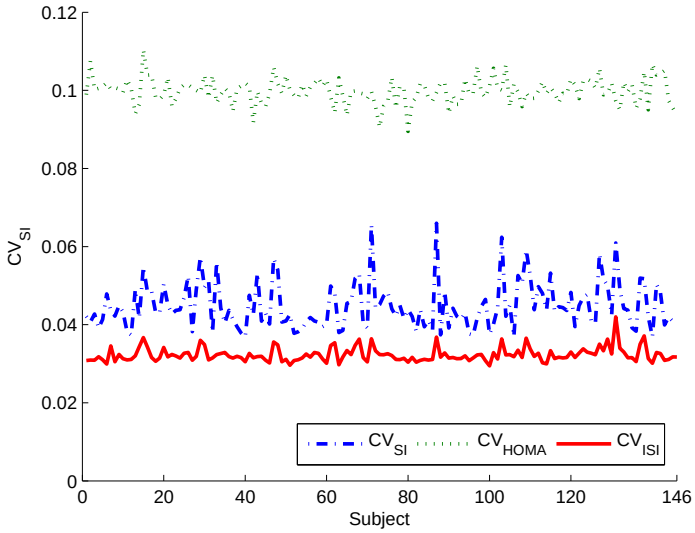


Figure 7.5 Intra-individual coefficients of variation for S_{I-MC} (CV_{SI}) at 10 g/1 U dosing, ISI_G (CV_{ISI}) and HOMA-IR (CV_{HOMA}).

The increase in insulin sensitivity after lifestyle intervention [McAuley et al., 2002] was captured by the model, with S_I increasing from $S_{I-MC(BEFORE)}$=4.34 (MSD 1.47) 10^{-4} l/mU/min to $S_{I-MC(AFTER)}$=5.57 (MSD 1.48) 10^{-4} l/mU/min. This value matches the increase in ISI shown in Table 6.1. Correlation between the change in S_{I-MC} and ISI_G, ΔS_{I-MC} and ΔISI_G is $r = 0.96$ (90 % CI: 0.96 - 0.97) with a mean regression line of $ISI_G = 0 + 0.48 S_{I-MC}$. Note that the ~ 50 % slope is due to the fixed $\gamma = 1/2$ ratio of insulin concentration in interstitium (Q) and plasma (I) during steady state. Specifically, ISI is calculated using I and S_I is identified using Q.

The intra-individual CV of S_{I-MC} decreased slightly from $\mathrm{CV}_{SI(BEFORE)}$=4.6 % (90 % CI: 3.8 % - 5.9 %) to $\mathrm{CV}_{SI(AFTER)}$=4.3 % (90 % CI: 3.7 % - 5.2 %). A strong correlation of $r = -0.83$ could be seen between a decrease in insulin sensitivity ISI and increased intra-individual CV in S_{I-MC}. Figure 7.6 shows the linear relationships between ISI_G and CV_{SI} before and after intervention. A clear reduction in accuracy of estimated S_I can thus be seen in subjects with very low insulin sensitivities.

Re-simulating the low intensity test protocol with different doses of glucose and insulin showed a clear dependence of accuracy of the method on the dose employed, as can be seen in Figure 7.7. Administering 5 g glucose and 0.5 U

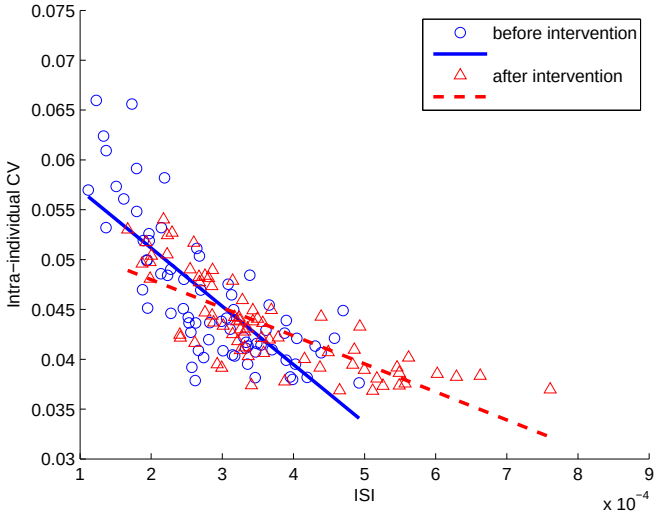


Figure 7.6 Relationship between ISI_G and intra-individual coefficients of variation CV_{SI} before and after intervention.

insulin resulted in CV_{SI}=6.9 % (90 % CI: 4.9 % - 9.9 %). The high dose variant with 20 g glucose and 2 U insulin resulted in a more accurate measure with CV_{SI}=3.6 % (90 % CI: 3.0 % - 4.5 %), which is very close to the accuracy of ISI. Correlation of CV_{SI} with ISI_G was stronger in the low dose protocol ($r = 0.90$) but showed a weaker linear relationship in the high dose variant ($r = 0.46$), indicating the reduced effect of assay variation and other errors at higher doses.

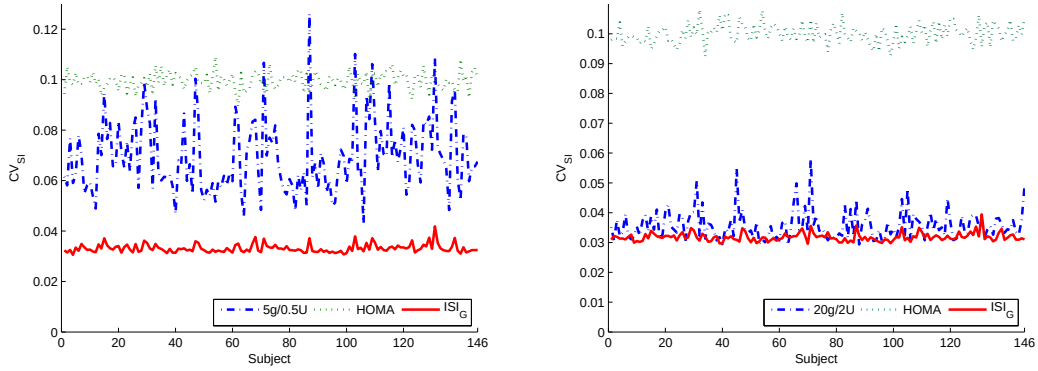


Figure 7.7 CV_{SI} compared to CV_{ISI} and CV_{HOMA} simulating the protocol with 5 g glucose and 0.5 U insulin (left), and 20 g glucose and 2 U insulin (right).

Simulated hepatic insulin clearance n_L and simulated first pass hepatic insulin extraction x_L were underestimated slightly in the Monte Carlo analysis, by -4.4 % (90 % CI: -16.5 % - 8.1 %) and -2.2 % (90 % CI: -12.4 % - 7.3 %), respectively.

Simulated glucose distribution volume V_G was overestimated by 1.7 % (90 % CI: 0.7 % - 3.5 %). However, these results are relatively small and not likely to have significant clinical impact.

7.4 Discussion

The test protocol presented is developed with the main goal to provide a clinically useful, highly accurate method to diagnose insulin resistance (IR) that is highly correlated to the gold standard euglycaemic-hyperinsulinaemic clamp. To be clinically useful, a test must be accurate, short and simple. To correlate highly to the clamp, a test must measure the same effects as the clamp and be equally accurate. The most widely used and accepted tests developed so far (e.g. IVGTT, OGTT and HOMA-IR), are all judged by their ability to correlate to the clamp. This goal has been achieved only with some significant variability, yielding a wide range of IVGTT-clamp correlations, for example, between $r = 0.44 - 0.89$ [e.g. Bergman et al., 1987; Donner et al., 1985; Saad et al., 1994].

One major obstacle is that every test effectively measures a different effect [Radziuk, 2000], as discussed in Chapter 2. The clamp relies on a steady state glucose concentration during supra-physiological insulin and glucose infusions, in which endogenous insulin and glucose are assumed to be completely suppressed. Its metric for insulin sensitivity is the rate at which glucose is disposed in the body with a given plasma insulin concentration. In contrast, the IVGTT fits the Minimal Model [Bergman et al., 1979] to the glucose response curve after a high-dose injection of glucose and insulin by estimating three model parameters (p_1, p_2, p_3), with insulin sensitivity being the ratio $S_I^{MM} = p_3/p_2$. Fitting three parameters has the disadvantage that a longer test is required to allow for enough resolution and data, and thus inter-subject variability is distributed amongst these three parameters. In general, the IVGTT is considered the best clamp-correlated method, with correlation values up to $r = 0.89$ being reported [Bergman et al., 1987]. However, lower results, as low as $r = 0.44$, have also been reported [Donner et al., 1985].

Other popular methods, widely used due to their simplicity, are surrogate measures such as the OGTT (measuring the rate of glucose decay after an oral

glucose load) and HOMA-IR (based on one fasting glucose and insulin sample). These methods are far less correlated to the clamp [Monzillo and Hamdy, 2003], as they too measure different effects. In particular, HOMA-IR can be very variable due to a pulsatile secretion of insulin [Song et al., 2000] and assay inaccuracies, leading to a CV $> 10\,\%$ [Wallace et al., 2004b].

The proposed low intensity protocol presented was designed to specifically measure the same effects as the clamp in a much shorter and less intense transient test. Variability is constrained to insulin dependent effects in the periphery, controlled by the insulin sensitivity parameter S_I. Modelled S_I is lower than clamp ISI, but it does not introduce additional variability. The difference is consistent across all individuals, due to the fixed ratio of steady state plasma (I) and interstitial (Q) insulin in the model. The model and fitting method employed have been well validated [Hann et al., 2005b; Lotz et al., 2006a] and correlated to clamp data in transient and steady state, resulting in very high correlations ($r = 0.92$ in transient state, $r = 0.99$ in steady state) [Lotz et al., 2006b], as also shown in Chapter 6.

The proposed method was able to estimate S_I with high accuracy, given the assay errors and unmodelled suppression of EGP. CV_{SI} was slightly larger than CV_{ISI}. This larger CV can be expected given the highly dynamic state of the proposed test. Accuracy decreased drastically by 53 % in the lower dose test (5 g glucose, 0.5 U insulin), although accuracy was still better than HOMA-IR. The higher dose test (20 g glucose, 2 U insulin) improved accuracy by 20 %. As suppression of EGP was adjusted to the dose accordingly, being higher in the high dose test, the still improved accuracy suggests a strong dependence on the signal to noise ratio of the test, with EGP playing a minor role.

In spite of the improved accuracy at a higher dose, this high dose is not as practical for a simple clinical test, for a variety of reasons. As IV glucose is commonly available in 50 % solution, a 20 g bolus requires a 40 ml injection of a very viscous solution, which can cause discomfort for the test subject. The 2 U insulin dose also increases risk of hypoglycaemia, particularly in lean subjects. Finally, an intravenous glucose bolus of 20 g is on the upper physiological range, possibly triggering other glucose regulatory effects not accounted for in this model and simulation, which could in a real clinical test worsen the results.

The 10 g glucose and 1 U insulin dose is only slightly less accurate, but a lot easier and safer to administer in clinical practice. Mean and range of CV_{SI} are greatly reduced in the step from low to medium dose, whereas the improvement from medium to high dose is less pronounced. This decay is shown in Figure 7.8, which illustrates that the medium dose of 10 g glucose and 1 U insulin appears to be the best compromise in clinical practicability, safety and accuracy.

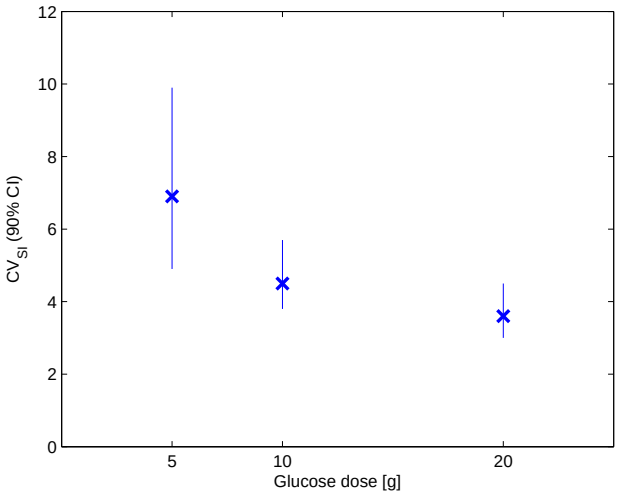


Figure 7.8 CV_{SI} with the 90 % confidence intervals for the low (5 g glucose, 0.5 U insulin), medium (10 g glucose, 1 U insulin) and high (20 g glucose, 2 U insulin) dose test variants.

A strong negative correlation was seen between a decrease in insulin sensitivity ISI_G and a resulting increased CV_{SI}. This correlation was even stronger with the low dose test, but was markedly reduced in the high dose test. The origin of this effect is likely physiological, as insulin-dependent effects are less dominant in subjects with low insulin sensitivity, leading to a reduced signal to noise ratio. As can be seen with the high dose test, this correlation can be reduced by increasing the signal. In contrast, the correlation is stronger with a weaker signal as shown in the low dose test.

Overall, the method is able to estimate the underlying insulin sensitivity with high accuracy from the proposed test protocol. Whether the estimated S_I is a true marker of insulin sensitivity will require further clinical validation with the clamp and other methods in a new data set. However, judging from the accurate simulation of clinically observed dynamics, it is very likely that the effect described by the model parameter S_I is physiological and that insulin sensitivity can be estimated with similar accuracy in clinical data. This result

is supported by the high correlation between S_I and ISI using clamp test data [Lotz et al., 2006b]. More specifically, because the proposed low intensity test was intentionally designed to measure the same physiological effect as the clamp using highly correlated models and methods, the test should be highly correlated to the clamp.

Even if the most prominent unmodelled dynamic (suppression of EGP) is included in this Monte Carlo analysis, real results could still be affected by other effects not simulated here. Inaccuracies in the simulated test protocol were identified in initial trials [Lotz et al., 2005b], such as in sample timing and imperfect cannula flushing, or incomplete mixing of glucose and insulin in plasma during the first 10 minutes. These effects are more likely in a clinical, non-research setting with a simple protocol, where special considerations common in research settings cannot be met. These factors have to be taken into account when designing a robust clinical test.

Finally, additional variability could be introduced by less accurate assay methods, especially for insulin and C-peptide. The assays used in this study are run by the authors' collaborating laboratory and are amongst the most accurate methods to date. Less accurate insulin assays with more cross reactivity to proinsulin are still widely used and could increase the test's variability or introduce a systematic error [Chevenne et al., 1999; Robbins et al., 1996].

7.5 Summary

The proposed test to assess insulin sensitivity is short and simple enough to be applicable in a clinical setting. Factors and problems apparent in previous similar attempts, such as the IVGTT, have been addressed and solved to improve overall system performance. The method proved to be very accurate in Monte Carlo simulation, and only slightly less accurate than the gold standard clamp test. As a result of its design to measure the same effects as the clamp, it is highly correlated to the gold standard clamp ISI metric.

The physiological dosing, simple and robust protocol and high accuracy make the test very attractive for early diagnosis and monitoring of interventions. Accuracy and correlation to gold standard tests must still be assessed in a clinical

validation on a new cohort. However, this study has indicated that the proposed test should possess the accuracy and robustness required, as compared to a large cohort of clamp results.

Chapter 8

Clinical Pilot Test

The test proposed in Chapter 7 shows very good Monte Carlo simulation results for diagnosing the underlying insulin sensitivity with great accuracy in a noisy clinical test environment. To assess its performance in a clinical setting and to find additional practical aspects to be optimised, a clinical pilot study on a limited number of subjects is required. This chapter describes the study design and presents its results.

The study was performed in collaboration with Dr. Kirsten McAuley and Prof. Jim Mann from the *Edgar National Centre for Diabetes Research* in Dunedin. Ethics approval for the study was granted by the *Upper South A Regional Ethics Committee*. Tests were performed both in Dunedin and Christchurch.

8.1 Objectives and Design

The pilot study can yield information on effects of different dosing and on intra-individual variability. It further provides an opportunity to assess practical aspects such as sampling procedure, ergonomics and comfort for the test subject. Sources of error that have not been addressed in the simulation study can be identified and the fitting algorithm optimised to improve overall method robustness.

The pilot study is designed to investigate these aspects and produce insights into the practical procedures, which can then be used to improve the protocol.

8.1.1 Aims

The primary aims of the pilot study were to investigate the following aspects:

1. Assess difference in the clinically estimated insulin sensitivity S_I at different dosing of glucose and insulin.
2. Determine the minimum dose of insulin and glucose to produce adequate resolution in the sampled data.
3. Assess intra-individual variability in S_I at the same dose.
4. Obtain an initial estimate of the inter-individual range in S_I and its relevant diagnostic ranges.
5. Assess the overall clinical potential of the test prior to a full scale validation.

The study was performed on a range of normal and overweight men and women over the age of 18 years, that were recruited from the general population. Only those with a current major medical illness were excluded.

8.1.2 Study Design

The study is divided into two main parts, primarily to investigate Aims 1-3. Aims 4-5 can be evaluated on data gathered from all tests.

Part 1

Study Part 1 addressed Aims 1 and 2 (and 4-5), mainly the effect of different dosing of glucose and insulin, and the minimum dosing required to minimise the effect of noise in data. Recruited subjects had two tests on two occasions, no more than two to three weeks apart. The test was performed as outlined in Chapter 7.2. On each occasion, a different dose of glucose and insulin was administered. The levels of glucose and insulin were defined in three steps for added simplicity. The steps were as used in the simulation study in Chapter 7, namely 0.5 U/5 g (low),

1 U/10 g (medium) and 2 U/20 g (high) of glucose and insulin, respectively. These steps were chosen as they are still lower than a standard IVGTT dosing [Bergman et al., 1981], and thus safer and more physiological.

As each subject was tested only twice, dosing steps for each subject were chosen as either low + medium or medium + high. A systematic bias in estimated S_I is an indication of a different result obtained at different dosing. From the noise in the resulting data and its affect on model fitting, a minimum dose for the final test design can be chosen, that is more robust to noise. This aspect was assessed in the Monte Carlo analysis in Chapter 7 and is initially validated on the clinical data in this chapter.

Part 2

Study Part 2 assessed Aim 3 (and 4-5), mainly the accuracy in repeatability of the same dose test on the same subject. The test was repeated on the same subject two to three times, each test two to three weeks apart. Part 2 overlapped with Part 1, as some of these subjects were simply tested a third time at one of the doses already used in Part 1. This part yielded information on the variability to be expected from the test. From model fitting performance, it could be estimated if this variability is caused by computational or natural factors.

Additional Analysis

To investigate Aim 4, the test data gathered from Parts 1 and 2 were further analysed computationally. The range of S_I estimated in this test was compared to the values obtained from the clamp population used in Chapter 6.

Further metabolic information that can be gathered from this test includes the pancreatic secretory response to the glucose injection (β-cell function). These data can be calculated from the estimated C-peptide secretion rate. Metrics of interest are basal fasting insulin secretion rate, u_b in mU/min, peak secretion rate above u_b after the bolus, S_{max} in mU/min, and total insulin secreted above basal during the 10 minutes after glucose input, AUC_{10} in mU, calculated as area under the secretion profile above the basal rate u_B. All of these metrics have significant

diagnostic merit and can be combined with the S_I results obtained to provide a more complete picture of the metabolic defect [Ferrannini and Mari, 2004].

8.1.3 Recruitment and Subjects

Recruitment of subjects was done in parallel in Dunedin and Christchurch by the respective clinical research teams, mainly by advertisement flyers around the university and the hospital. Interested subjects were informed about the study and their characteristics and contact details recorded. Subjects were picked from this list to obtain individuals within a wide range of age and BMIs. The recruitment flyer used in Christchurch is shown in Figure 8.1.

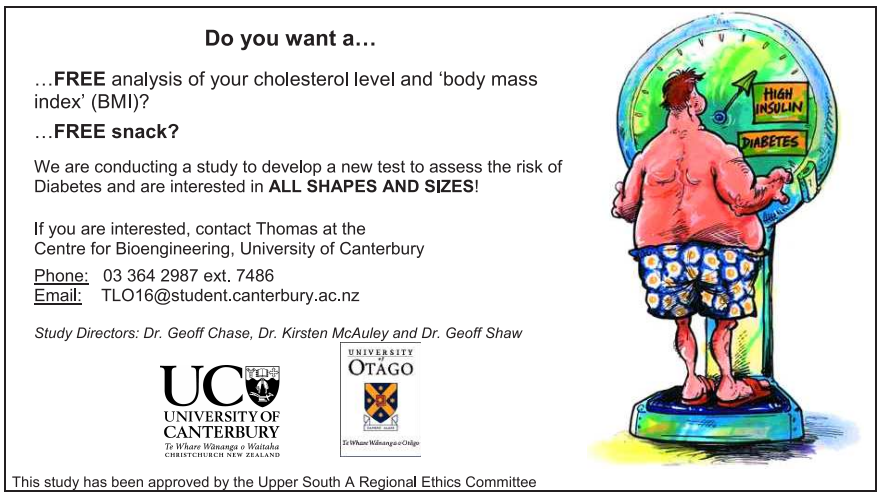


Figure 8.1 Advertisement used to recruit volunteers for the study.

The test was performed on a total of 17 subjects, 7 in Christchurch and 10 in Dunedin. One subject in Christchurch came only once and could thus not be further included in the analysis. The characteristics of the subjects included in both parts of the study are presented in Table 8.1.

8.1.4 Test Procedure

All tests were performed between 9-11 am after an overnight fast from 10 pm. The subject's weight, height and blood pressure were taken. Before the first

Table 8.1 Pilot study population in each study part. Age in years, weight in kg, BMI in kg/m^2, fasting glucose in mmol/l, fasting insulin in mU/l. 'IFG' denotes that the subject was not previously diagnosed with type 2 diabetes but has elevated fasting glucose concentrations qualifying for an ADA diagnosis of impaired fasting glucose (> 5.6 mmol/l) [ADA, 2006]. $^+$ denotes geometric mean and multiplicative SD, as data has log-normal distribution.

Part 1							
Subject	Gender	Age [years]	weight [kg]	BMI [kg/m^2]	Fast. G [mmol/l]	Fast. I [mU/l]	Type 2 or IFG
1	f	57	89	33.9	5.75	30.8	IFG
2	f	59	67	25.5	5.85	1.4	IFG
4	f	21	78	25.2	5	5.2	
5	m	41	76	21.7	4	0.5	
6	f	45	76	25.4	4.1	1.7	
7	m	55	73	24.1	4.5	4.4	
8	f	51	67	27.2	4.3	1.4	
9	f	35	66	24	4.8	6.6	
10	f	30	50	19.5	4.2	3.2	
11	f	55	85	30.1	6.8	9.2	Type 2
14	f	41	111	41.3	4.5	3.9	
15	m	29	84	25.9	5	2.5	
16	m	49	105	35.1	6.3	16.6	IFG
17	f	25	60	25.3	4.5	3	
mean	10 f/4 m	42.36	77.64	27.44	4.91^+	3.75^+	
SD		12.66	16.52	5.81	1.18^+	2.90^+	
Part 2							
3	f	59	87	39.2	4.7	12.5	
5	m	41	76	21.7	4	0.5	
6	f	45	76	25.4	4.1	1.7	
10	f	30	50	19.5	4.2	3.2	
11	f	55	85	30.1	6.8	9.2	Type 2
13	f	48	91	33.4	5.2	9.5	
14	f	41	111	41.3	4.5	3.9	
15	m	29	84	25.9	5	2.5	
16	m	49	105	35.1	6.3	16.6	IFG
mean	6 f/3 m	44.11	85.00	30.18	4.90^+	4.28^+	
SD		10.17	17.69	7.64	1.21^+	3.07^+	

visit, informed consent was obtained and any medical history recorded. Tests were performed in a seated, slightly reclined position.

Glucose was administered as a 50 % solution. Insulin was diluted in two steps to minimise dilution errors: First, 0.5 ml (50 U) of insulin (Actrapid, NovoNordisk) is drawn in a 1 ml syringe and diluted with 49.5 ml saline to obtain a 1 U/ml solution. From this result, the required amount for the test was drawn (0.5 ml, 1 ml or 2 ml) in a 3 ml syringe.

A cannula was inserted into the antecubital fossa. Two baseline blood samples (8 ml) were taken at $t = -10$ and $t = 0$ minutes. Glucose was administered at $t = 0$ min and insulin at $t = 10$ min (just after the samples at those times). In addition to the baseline samples, blood was sampled during the test at 5, 10, 15, 20, 25, 30, 35 and 45 minutes. Sampling and administration was performed on the same cannula, which was flushed first with the subject's own blood, then with saline after each bolus input. This approach minimises errors due to insulin and glucose binding to inner walls of tubes [NovoNordisk, 2002]. After the test, the subject was given a snack.

Blood samples were centrifuged and plasma serum separated for insulin and C-peptide analysis, and sent to the laboratory immediately or frozen at $-80\,^{\circ}\mathrm{C}$. Glucose was analysed by an enzymatic glucose hexokinase assay (Abbott). Insulin and C-peptide were analysed with an ECLIA immunoassay (Roche Diagnostics Elecsys).

The analysed data were fitted by the models and methods described in Chapters 3-4 and insulin sensitivity determined from the glucose model parameter S_I. Further parameters estimated from the fitting are n_L, x_L and V_G. Estimation of V_G is bound to a realistic range, between 15 % and 25 % body weight to avoid misidentification by noisy data. The estimation of endogenous insulin secretion rate was performed without the corrected peak proposed in Section 5.1.3.

8.2 Results

The experimentally sampled profiles of glucose, insulin and C-peptide were distinct enough in all dose options to achieve a good model fit. The model fits of

glucose and insulin concentrations and estimated endogenous insulin secretion rate on all subjects are shown in Appendix B. Where samples were within the expected ranges and not affected by incomplete mixing or contamination, a standard fit with all data points and no weighting was possible. Likely erroneous samples were evident in some tests and could result in incorrect estimation of insulin sensitivity, unless identified and removed. Some typical examples of these errors are shown in Figure 8.2.

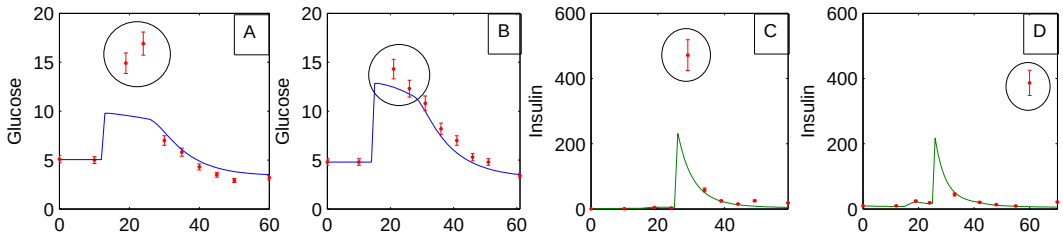


Figure 8.2 Errors in samples evident in some tests. Source of the errors could be incomplete systemic mixing, assay error or contamination, especially in the unrealistically high values seen in the two insulin plots.

Identified evident individual measurement errors in all 43 tests included:

1. Glucose

   - 4 tests had the first one or two samples after glucose input still affected by incomplete mixing, as shown in Figure 8.2 (B).
   - 4 tests had first samples after glucose input that were affected by very slow mixing or possible contamination, as shown in Figure 8.2 (A), resulting in unrealistically high values.
   - 2 tests had further samples later in the test that were larger than physiologically possible. These were most likely contaminated.

2. Insulin

   - 6 tests had the first sample after insulin input affected by incomplete mixing processes.
   - 5 tests had the first sample after insulin input outside of a realistic physiological range, most likely due to contamination or very slow systemic mixing, as shown in Figure 8.2 (C).

- 3 tests had a sample later in the test larger than physiologically possible. These were most likely contaminated. An example of such a test is shown in Figure 8.2 (D)
- Two tests, on subjects 6 and 9, had too noisy insulin profiles, caused by unexplained contamination of samples, and were disregarded for the further analysis, as the data did not allow a sensible model fit.

3. C-peptide

   - No problems were evident in C-peptide samples.

As the first respective sample after glucose or insulin administration is usually higher than expected by the model structure, a significant error can be introduced by the fitting algorithm, as it tries to compensate for this difference. The difference is caused by incomplete mixing between plasma and interstitial fluid and not accounted for by the model [Caumo and Cobelli, 1993; Regittnig et al., 1999]. As the goal for the algorithm is to be generic without prior manual filtering of the data, an automated filtering of the samples is undertaken to minimise the error introduced by noisy data. The automated filtering steps include:

- Replace the two fasting samples with their mean value.
- Remove first glucose sample after glucose administration at $t = 5$ min and first insulin sample after insulin administration at $t = 15$ min.
- Further samples are expected to decay with time. If a sample is 10 % higher than the previous one, it is removed. This removes all samples that are clearly contaminated.

This simple filtering eliminates all of the issues described in the ∼30 samples noted (of 1290 total). The results presented for Part 1 and Part 2 were obtained with this generic algorithm, without prior manual data analysis. As mentioned before, two tests, one on Subject 6 and one on Subject 9, resulted in unreliable insulin data, due to unexplainable data errors and were excluded from the subsequent analysis.

8.2.1 Study Part 1

The study population for Part 1 consisted of 13 subjects, with the characteristics shown in Table 8.1. 7 subjects had a low and medium dose test, 6 subjects the medium and high dose test. The difference in estimated S_I is shown in Table 8.2, along with estimated parameters V_G, n_L and x_L. In subjects with two tests at the same dose, the mean value was taken.

Estimated S_I is lower in 9/13 subjects at the higher dose test, but the differences are not statistically significant (low/medium P=0.50, medium/high P=0.52), as assessed by the two sample t-test. The volume V_G is estimated with high repeatability in all but 2 subjects (subjects 2 and 4). No relationship between the difference in V_G and in S_I is apparent. Hepatic clearance parameters n_L and x_L were consistent in subjects, with significantly reduced clearance rates in low S_I subjects, in accordance with their hyperinsulinaemia [Ferrannini and Cobelli, 1987a; Li et al., 2006; Valera Mora et al., 2003].

Pancreatic secretory metrics were consistent within individuals, within the expected accuracy due to assay errors calculated in Chapter 5 (CV$= 4\,\% - 10\,\%$), and are given in Table 8.3. Basal secretion was consistently higher in subjects with lower S_I, as would be physiologically expected. Secreted insulin above basal during the first 10 minutes after glucose input, AUC_{10}, is increased at the higher dose in all but one subject, with a wide range in changes of $-7.1\,\% - 213.8\,\%$. The same is the case for the difference in maximal secretion rate, S_{max}, which is in the range of $-20.7\,\% - 180.9\,\%$, and positive for all but two subjects.

8.2.2 Study Part 2

The study population for Part 2 consisted of 8 subjects, with 4 repeated for the low dose test and 4 repeated for the medium dose test. Two subjects at the medium dose had the test performed on three occasions, all others only twice. Estimated S_I and the error around the mean are given in Table 8.4, along with the estimated parameters V_G, n_L and x_L.

Errors in S_I are in the range of $1.7\,\% - 24.7\,\%$ with a geometric mean value of 6.0 % (MSD 4.9). The volume V_G was estimated consistently in all subjects

Table 8.2 Results in estimated S_I in study Part 1, effect of dosing. Also shown are further estimated model parameters, n_L, x_L and V_G, and the relative difference in S_I at the higher dose.

Subject	dose	S_I [l/mU/min]	V_G [l]	n_L [min^{-1}]	x_L [-]	Diff S_I [%]
low/medium dose						
4	5 g	13.39	15.85	0.16	0.78	
	10 g	16.49	11.70	0.11	0.75	23.14 %
7	5 g	19.33	16.44	0.22	0.74	
	10 g	18.06	17.82	0.21	0.74	-6.60 %
8	5 g	18.64	14.29	0.15	0.86	
	10 g	13.61	16.12	0.07	0.80	-26.98 %
10	5 g	43.73	12.33	0.20	0.80	
	10 g	17.40	11.56	0.13	0.77	
	5 g	29.19	10.13	0.18	0.78	-52.27 %
11	5 g	6.88	12.75	0.14	0.72	
	10 g	6.73	12.75	0.16	0.70	
	5 g	5.75	13.14	0.11	0.74	6.53 %
15	5 g	8.28	16.57	0.16	0.78	
	10 g	7.39	16.45	0.12	0.78	
	5 g	8.99	16.81	0.17	0.79	-14.40 %
16	5 g	3.27	15.75	0.09	0.77	
	10 g	3.17	15.75	0.05	0.75	
	5 g	3.16	15.75	0.05	0.81	-1.40 %
mean		13.53	14.55	0.14	0.77	-10.28 %
SD		10.34	2.24	0.05	0.04	24.33 %
medium/high dose						
1	10 g	3.13	13.35	0.05	0.74	
	20 g	2.69	13.35	0.06	0.67	-14.15 %
2	10 g	19.47	16.19	0.10	0.82	
	20 g	13.43	12.95	0.15	0.75	-31.02 %
5	10 g	26.45	16.04	0.21	0.81	
	20 g	25.07	18.19	0.34	0.74	
	10 g	19.97	16.96	0.13	0.79	8.02 %
6	10 g	14.84	16.10	0.14	0.82	
	20 g	12.83	18.30	0.20	0.73	-13.56 %
14	10 g	11.70	16.65	0.19	0.69	
	20 g	14.12	16.99	0.18	0.73	
	10 g	11.65	17.59	0.14	0.71	20.89 %
17	10 g	36.85	14.21	0.20	0.52	
	20 g	12.60	12.25	0.12	0.70	-65.82 %
mean		16.06	15.65	0.16	0.73	-15.94 %
SD		9.06	2.04	0.07	0.08	30.51 %

Table 8.3 Results in estimated pancreatic secretion metrics in study Part 1, effect of dosing. Also shown are the relative changes in AUC_{10} and S_{max} at the higher dose.

Subject	dose	u_B [mU/l]	AUC_{10} [mU]	Diff AUC_{10} [%]	S_{max} [mU/l]	Diff S_{max} [%]
low/medium dose						
4	5 g	19.61	221.16		35.42	
	10 g	20.90	253.99	14.84 %	42.08	18.81 %
7	5 g	24.94	419.04		107.69	
	10 g	24.74	785.88	87.54 %	152.82	41.91 %
8	5 g	11.39	379.94		87.61	
	10 g	12.83	832.57	119.13 %	190.98	118.00 %
10	5 g	13.68	479.54		107.34	
	10 g	13.49	628.42	25.75 %	149.70	
	5 g	15.62	519.90		122.65	30.18 %
11	5 g	36.15	201.59		27.23	
	10 g	33.86	226.68	16.24 %	29.22	
	5 g	42.18	188.43		31.72	-0.87 %
15	5 g	20.00	399.71		114.45	
	10 g	20.80	648.11	69.42 %	144.98	
	5 g	22.06	365.39		104.75	32.28 %
16	5 g	62.70	244.98		43.03	
	10 g	66.22	577.64	213.77 %	81.89	
	5 g	56.98	123.21		25.63	138.58 %
mean		28.79	416.45	78.10 %	88.84	54.13 %
SD		17.42	212.44	71.67 %	51.98	52.73 %
medium/high dose						
1	10 g	70.90	393.26		62.60	
	20 g	68.90	1027.10	161.18 %	175.85	180.92 %
2	10 g	8.94	174.78		32.51	
	20 g	12.02	225.09	28.79 %	39.02	20.00 %
5	10 g	14.28	410.45		76.09	
	20 g	10.85	564.21	48.55 %	130.27	
	10 g	11.06	349.18		69.20	79.32 %
6	10 g	17.04	344.69		63.39	
	20 g	17.16	554.53	60.88 %	81.06	27.87 %
14	10 g	21.05	696.51		183.96	
	20 g	19.13	666.65	-7.09 %	144.39	
	10 g	25.75	738.51		180.18	-20.69 %
17	10 g	9.62	383.14		111.86	
	20 g	11.43	607.46	58.55 %	120.78	7.97 %
mean		22.72	509.68	58.48 %	105.08	49.23 %
SD		20.56	228.66	56.27 %	52.05	72.32 %

Table 8.4 Results of Part 2, accuracy in repeatability. Given are estimated parameters S_I, V_G, n_L and x_L, and the error around the mean S_I.

Subject	S_I	V_G [l]	n_L [min^{-1}]	x_L [-]	Diff S_I [%]
low dose					
10	43.73	12.33	0.20	0.80	
	29.19	10.13	0.18	0.78	19.94 %
11	6.88	12.75	0.14	0.72	
	5.75	13.14	0.11	0.74	8.90 %
15	8.28	16.57	0.16	0.78	
	8.99	16.81	0.17	0.79	4.13 %
16	3.27	15.75	0.09	0.77	
	3.16	15.75	0.05	0.81	1.71 %
medium dose					
3	10.18	18.16	0.12	0.67	
	8.59	13.82	0.10	0.64	
	7.37	13.05	0.06	0.69	16.80 %
5	26.45	16.04	0.21	0.81	
	19.97	16.96	0.13	0.79	13.95 %
13	16.31	14.55	0.13	0.73	
	13.51	13.65	0.14	0.77	
	21.20	13.65	0.13	0.85	24.65 %
14	11.70	16.65	0.19	0.69	
	11.65	17.59	0.14	0.71	0.22 %

but subject 3, in which the first test resulted in a much larger volume estimate. Hepatic clearance parameters were also consistent across tests, with errors within expected physiological variability.

Repeatability in pancreatic secretion metrics are given in Table 8.5. Error in basal secretion rate u_b was in the range of $2.6\,\% - 11.7\,\%$, well within the assay errors as estimated in Chapter 5. Total first phase insulin AUC_{10} was estimated with high accuracy in repeatability, with a mean value of $9.5\,\%$ and a range of $2.9\,\% - 33.1\,\%$. Only in one subject the deviation of $33.1\,\%$ was larger than the expected accuracy estimated by Monte Carlo analysis in Section 5.1.4. Repeatability in maximal secretion rate, S_{max}, was good, with a mean error of $11\,\%$ and only two errors above $15\,\%$.

Table 8.5 Estimated pancreatic secretion metrics from C-peptide data with errors around their mean values.

low dose						
Subject	u_b [mU/l]	Diff u_b [%]	AUC_{10} [mU]	Diff AUC_{10} [%]	S_{max} [mU/l]	Diff S_{max} [%]
10	13.68		479.54		107.34	
	15.62	6.62 %	519.90	4.04 %	122.65	6.66 %
11	36.15		201.59		27.23	
	42.18	7.71 %	188.43	3.37 %	31.72	7.62 %
15	20.00		399.71		114.45	
	22.06	4.90 %	365.39	4.48 %	104.75	4.42 %
16	62.70		244.98		43.03	
	56.98	4.78 %	123.21	33.07 %	25.63	25.34 %
medium dose						
Subject						
3	34.08		1208.07		241.84	
	38.79	11.65 %	1424.32	8.22 %	270.62	14.43 %
	43.20		1316.04		315.99	
5	14.28		410.45		76.09	
	11.06	12.71 %	349.18	8.07 %	69.20	4.74 %
13	35.61		454.27		72.76	
	36.23	2.59 %	544.59	11.86 %	121.58	23.22 %
	34.10		593.89		101.68	
14	21.05		696.51		183.96	
	25.75	10.03 %	738.51	2.93 %	180.18	1.04 %

The distribution of estimated S_I in the pilot has the same model assumptions

as the clamp data fitting in Chapter 6 and its absolute S_I values should thus be comparable. The distributions of S_I from the clamp and the lower dose in each subject in this pilot study are shown in Figure 8.3 with log-normal distribution fits. Geometric mean of the pilot $S_I = 11.7 \times 10^{-4}$ (MSD 2.3) and of the clamp $S_{I-clamp} = 4.9 \times 10^{-4}$ (MSD 1.5). Note that this figure is not meant to compare the populations, just relate the ranges in S_I metrics obtained in these two population cohorts. The absolute values of S_I have the same units and result from the same model assumptions and should thus theoretically be comparable. The two cohorts are different per design, the clamp cohort being specifically an insulin resistant population, thus the tighter distribution at the lower mean value.

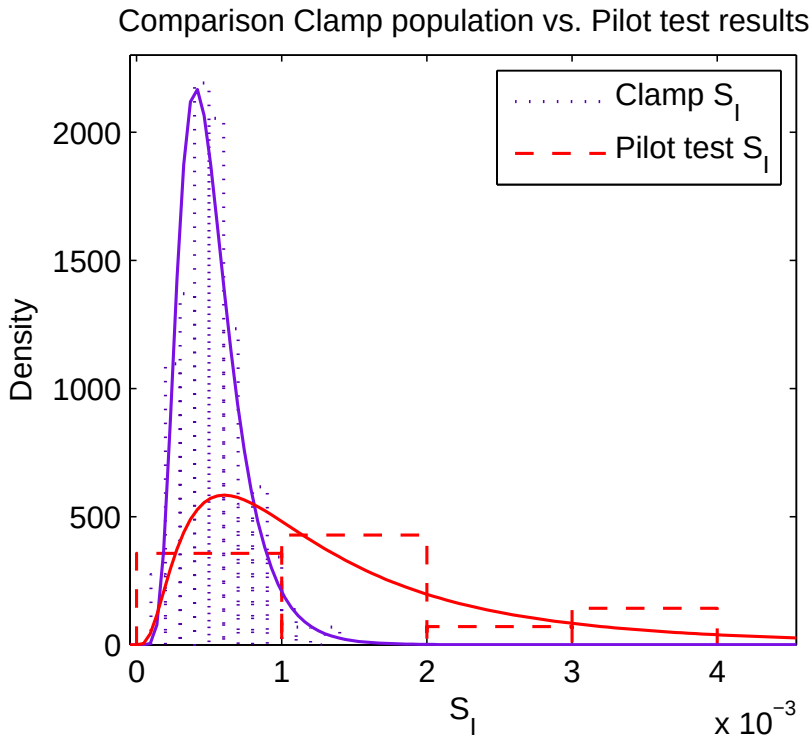


Figure 8.3 Distribution of S_I at the lower dose in each subject and corresponding log-normal distribution (dashed), compared to the clamp population (n=146) from Chapter 6 (dotted).

8.3 Clinical Diagnostic Relevance

The diagnostic value of the proposed test is far greater than just one metric of insulin sensitivity, as is usually the case with fasting or simple clinical tests. By assessing the whole dynamic metabolic response and including the physiological

models and methods, a broad picture of the state of the metabolic defect can be derived. To visualise these aspects, the resulting glucose and insulin concentration profiles and estimated endogenous insulin secretion rates are exemplified in Figure 8.4 for three subjects, with NGT, IFG and type 2 diabetes. All three tests were performed at the medium dose (10 g glucose, 1 U insulin).

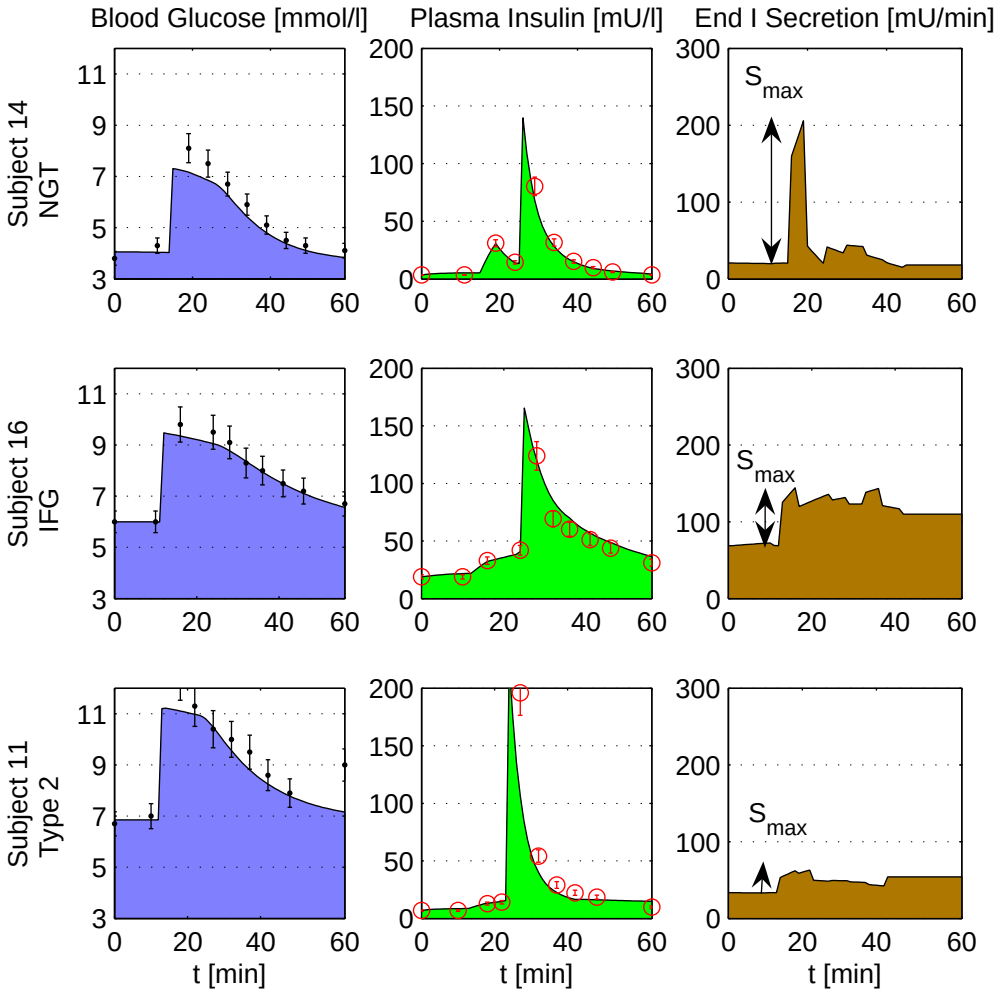


Figure 8.4 Three exemplary tests (medium dose, 10 g/1 U), from top to bottom, a normal glucose tolerant (NGT), impaired fasting glucose (IFG) and type 2 diabetes subject. Shown are, from left to right, the blood glucose concentration, the plasma insulin concentration and the estimated endogenous insulin secretion rate. Samples are given with error bars and areas show the model fits.

The progression of the disease can be described well on these examples. The NGT example, Subject 14, has an insulin sensitivity of $S_I = 11.7{\times}10^{-4}$ l/mU/min, a fasting glucose level of 4.5 mmol/l and fasting insulin level of 3 mU/l. Basal insulin secretion rate is $u_b = 21.1$ mU/min. The first phase β-cell response (en-

dogenous insulin secretion) to a bolus injection of glucose is very distinct and large, peaking at $S_{max} = 184$ mU/min above the basal rate u_b and releasing a total amount of insulin above the basal rate of $AUC_{10} = 697$ mU. The first phase insulin secretion lasts about 5-10 minutes, after which the secretion rate immediately drops back to nearly its basal rate. This is a typical healthy metabolic behaviour in all aspects.

The second example shows an IFG individual, Subject 16. The insulin sensitivity is very low at $S_I = 3.2 \times 10^{-4}$ l/mU/min, fasting glucose is elevated at 6.3 mmol/l, and as a compensatory result, fasting insulin is also elevated at 16.6 mU/l. Basal insulin secretion rate is consequently three times as high as in the NGT subject, at $u_b = 66.2$ mU/min. In response to the glucose bolus, the pancreas increases its output, but a distinct first phase secretion peak is not pronounced anymore. Insulin secretion peaks at $S_{max} = 82$ mU/min above its basal secretion rate u_b and continues to produce at this rate until the end of the test. The β-cells are producing at their limit but can only release additional $AUC_{10} = 578$ mU over the basal rate during the first phase. The pancreas is not able to fully compensate the low insulin sensitivity and blood glucose levels drop slowly. This subject has probably been insulin resistant and IFG for some years without being screened, and has now, as a result, significant damage in β-cell function.

The third example shows Subject 11, who is diagnosed with type 2 diabetes. The insulin sensitivity is higher than in the IFG example at $S_I = 6.7 \times 10^{-4}$ l/mU/min, which could be due to lasting effects of Metformin, a sensitivity enhancing drug normally taken by this subject. The fasting glucose level is at 6.8 mmol/l just below the type 2 diabetes diagnostic threshold of 7 mmol/l [ADA, 2006], and fasting insulin is elevated from normal at 9.2 mU/l. Basal insulin secretion rate is $u_b = 33.9$ mU/min and increased over the NGT subject, but not nearly as high as the IFG subject, a sign of β-cell exhaustion. Insulin secretion rate is slightly increased in response to the glucose bolus, but only $AUC_{10} = 227$ mU are produced above the basal rate u_b, with a secretion peak of only $S_{max} = 29$ mU/min above the basal rate. The strongly diminished β-cell function cannot compensate for the insulin resistance, resulting in fasting hyperglycaemia.

These three examples show all the major stages in the progression from nor-

mal glucose tolerance (NGT) to impaired fasting glucose (IFG) and type 2 diabetes. The information gathered from the test is able to describe and diagnose all key metabolic defects. Note that although insulin sensitivity in the type 2 diabetic subject is twice as high as in the IFG subject, glucose uptake is still slower due to the significantly deteriorated β-cell function in this subject. This shows that the information from only one metric might not be enough to fully describe the metabolic defect [Ferrannini and Mari, 2004]. Similarly, an individual could have a strong β-cell function that is able to compensate for a low insulin sensitivity, resulting in normal fasting glucose levels and giving normal fasting screening test results.

8.4 Additional Tests

Effect of Exercise

An additional test was performed on Subject 5 at the medium dose to assess the difference in S_I at a different level of exercise. The subject exercised regularly and extensively during the time of the original three tests, but stopped exercising due to injury and was tested again after three weeks of no exercise. Estimated S_I after lack of exercise was $S_I = 14.8 \times 10^{-4}$ l/mU/min. This value represents a 36 % reduction from the mean $S_I = 23.2 \times 10^{-4}$ l/mU/min at the same dose in two prior tests.

Effect of Systemic Mixing

The high errors on the first samples after glucose and insulin administration suggest either contamination or incomplete mixing in plasma. It is known that systemic mixing takes ∼10 minutes to complete [Bergman et al., 1985; Caumo and Cobelli, 1993; Regittnig et al., 1999]. To further analyse these effects and their impact on the fitting algorithm, the test was repeated a fourth time at the medium dose on Subject 5, while sampling simultaneously from both arms. In addition to the sampling protocol described above, samples were also taken at +7 and +17 minutes to increase the resolution in the first 10 minutes after glucose and insulin administration. The resulting samples and model fits are shown in Figure 8.5.

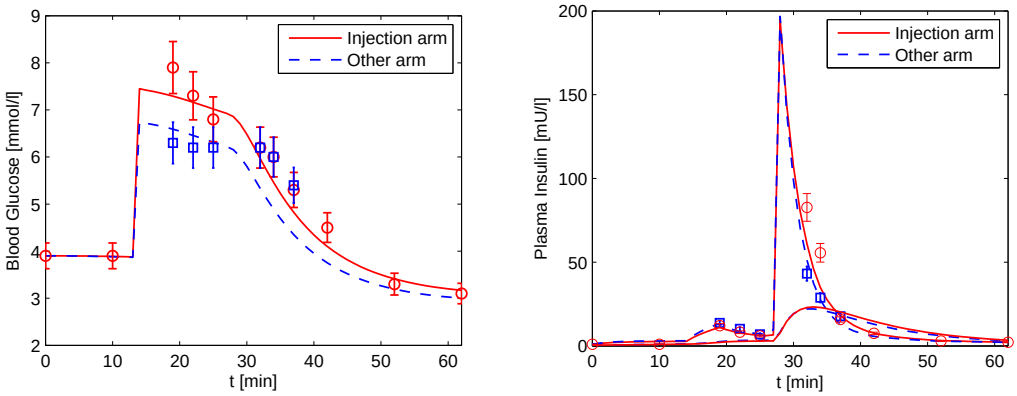


Figure 8.5 Test samples obtained on Subject 5 by sampling from both arms during the test. Double sampling was performed at +5, +7, +10, +15, +17 and +20 minutes. Shown are samples from the arm of administration (circle) and model fit (solid), and from the other arm (square) and model fit (dashed).

Samples taken from the arm of glucose and insulin administration (arm A) are clearly higher than from the other arm (arm B) after the respective input, but equalise after ∼10 minutes. The volume V_G was overestimated in arm B at $V_{G-B} = 19$ l, compared to $V_{G-A} = 15.3$ l, which matches the volume from the previous tests on this subject. No differences are evident in the C-peptide samples, which is expected as it is secreted by the pancreas and appears equally diluted at both arms. Estimated S_I are almost equal at $S_{I-A} = 18.9 \times 10^{-4}$ l/mU/min and $S_{I-B} = 19.6 \times 10^{-4}$ l/mU/min. This equal result is because the algorithm accounts for noisy data within the first 10 minutes after administration and is not sensitive to errors in estimated volume V_G. This test thus further validates the robustness of the overall method.

8.5 Discussion and Conclusions

The main goal of the pilot study was to assess the feasibility of the proposed test in a clinical setting and to get a feel for practical aspects in performing it. The study design consisted of two main parts, one to assess the effect of dosing and the second to assess its repeatability. The test was performed a total of 43 times, both in Christchurch and in Dunedin.

Overall the pilot study was successful and no major problems with tests or

subjects occurred. The protocol and fitting algorithm proved to be reliable and robust, and were able to produce results with considerable accuracy. The subjects were comfortable with the procedure. Some subjects receiving the 20 g dose of glucose complained about discomfort during the injection, which is understandable as it is a 40 ml injection of a rather viscous solution. Note that this is an important advantage over tests such as the IVGTT, in which a glucose dose of 20-30 g is standard procedure.

One aspect that was evident in many tests when analysing the lab results was the high noise in the first, and sometimes second, sample of glucose and insulin after their respective administration. It was hypothesised that the reasons could be mainly due to incomplete systemic mixing [Bergman et al., 1985; Caumo and Cobelli, 1993; Regittnig et al., 1999] and/or contamination of the cannula due to improper flushing, as it is well known that insulin binds to equipment [NovoNordisk, 2002]. A reduction in this contamination was seen in studies in which the cannula was flushed with the patient's own blood before the saline flush. A similar approach, adding 1 % serum albumin to the solution, has been shown to reduce binding [NovoNordisk, 2002; Polonsky et al., 1986a]. This method could possibly not be applicable in a widespread clinical test, as the practice of re-injecting a patient's own recently drawn blood introduces further risk of blood clotting and thus reduced safety. Instead, the algorithm thus needs to be robust enough to account for these errors.

The effect of systemic mixing is seen very clearly in the tests performed on Subject 5, in which blood was sampled from both arms. Mixing takes about 10 minutes to complete, which is in accordance with other studies [Bergman et al., 1985; Caumo and Cobelli, 1993; Regittnig et al., 1999]. This factor points out again, that samples taken between 0-10 minutes are not necessarily reliable.

This effect is also evident in the IVGTT with Minimal Model assessment and was discussed as a cause of poor estimation in the Minimal Model in Chapter 4. To overcome this problem, Caumo and Cobelli [1993] propose a two compartment description of glucose kinetics, but as that approach imposes further identifiability problems, it is not an option. Instead, as already mentioned to overcome contamination, the algorithm needs to be able to perform without the 5 minute sample. This limitation was imposed on the fitting method in the simple filtering used in the pilot study data.

In Part 1 of the study the effect of glucose and insulin dosing was analysed. Some subjects received a 5 g / 0.5 U and a 10 g / 1 U test and some a 10 g / 1 U and a 20 g / 2 U test. The dosing was chosen in these steps for practicality and ease of used, and as they are all lower and more physiological than the commonly used 20-30 g glucose and 2-5 U insulin used in the IVGTT. A dosing according to body weight or BSA, which is commonly done [Bergman et al., 1985], was not used as it would require individual adjustments and dilutions for each subject, which is not practicable in a simple clinical test for widespread use where consistency is important.

All doses were large enough to cause a significant rise in concentrations and thus provided enough information for a model fit. Estimated S_I was lower in 9/13 subjects in the higher dose test as compared to the lower dose test, but the difference was not statistically significant. This effect has been reported previously, where a significantly lower value in S_I was found by Prigeon et al. [1996] in an IVGTT performed at different doses. Injecting 2 U of insulin (in an 80 kg individual) resulted in a 32 % reduced S_I value compared to injecting 4 U of insulin. The number of tests in this pilot study is likely too small to get a significant result, and more controlled testing is necessary to isolate this effect from other introduced variability.

Possible sources of error, such as insulin dilution errors, or insulin binding to the equipment walls, can also cause a deviation in the results. These factors were not closely monitored in this study, and will have to be addressed in any follow-up study. Even if a systematic shift of the results is not significant, the effect of dosing is apparent in the increased variability between doses, as compared to the variability of repeated doses. This latter point is important to know when comparing results obtained with the test, as they might vary at different dosing. This effect of dosing has also been apparent in clamp tests performed at different dosing, and has been attributed mainly to insulin saturation effects in glucose uptake [Ferrannini and Mari, 1998; Prigeon et al., 1996].

The differences in S_I were not attributed to a variability in V_G or the hepatic insulin clearance parameters n_L and x_L, which were very consistent in each subject, mostly unaffected by the dose used. An inter-subject difference was seen, mainly a decrease in insulin clearance in obese and diagnosed type 2 diabetes subjects, which is in line with the compensatory hypersecretion of insulin in re-

sponse to an increased resistance [Ferrannini and Cobelli, 1987a; Li et al., 2006; Valera Mora et al., 2003].

Pancreatic secretory metrics were not affected by mixing or contamination issues, as they are estimated from C-peptide, which is secreted by the pancreas and is thus well mixed by the time it is sampled. Basal secretion of insulin was estimated consistently within each subject, within the expected assay noise calculated in Chapter 5. Total first phase insulin, AUC_{10}, was significantly larger in all but one subject, and maximal secretion rate, S_{max} was larger in all but two subjects. These results are also in accordance with the published literature, confirming a dose dependance of insulin release [Jefferson and Cherrington, 2001].

Part 2 of the study aimed at assessing the repeatability by performing the same dose test on each subject two or three times. The dosing was either low or medium. The errors around the mean in each subject were in the range of $0\,\% - 25\,\%$, with a geometric mean of $6.0\,\%$ (MSD 4.9). The model fits were good in most cases, and rarely affected by large noise in the data. The expected accuracy assessed by the Monte Carlo simulation in Chapter 7 resulted in a mean $\mathrm{CV}_{SI-MC} = 4.5\,\%$. In other words, considering 2 SD, a mean $9\,\%$ deviation from the mean can be attributable to assay and protocol errors in $\sim$95 % of the subjects. The natural variability in S_I, which was not included in the Monte Carlo simulation, can be a source of additional variability in this pilot study [e.g., Van Cauter et al., 1997]. The pilot results are thus in good accordance with the Monte Carlo simulation results, possibly being slightly more variable due to additional sources of variability.

The differences in estimated S_I are not necessarily attributable to the method itself, but can also be caused by natural variability, such as time of day [Van Cauter et al., 1997], state of health [Hollenbeck and Reaven, 1987; Van den Berghe et al., 2006], menstrual cycle [Trout et al., 2007] or exercise [Nishida et al., 2004; O'Gorman D et al., 2006]. The effect of these factors would have to be assessed in a separate study to further quantify them. However, that study is outside the scope of this thesis.

One opportunity was taken to re-test Subject 5, who exercises regularly and did so before each of his tests. Due to an injury, the subject had to cease all exercise for over two weeks. The test showed a reduction in S_I of $36\,\%$ as com-

pared to the mean value at the same dose on two prior occasions. Even though only one test was performed to assess this dynamic, the result is again in line with published results of a very significant increase in insulin sensitivity during exercise [Nishida et al., 2004; O'Gorman D et al., 2006]. It also further shows the resolution of the test by capturing this change. Note that only one test cannot be used as a reliable result and more validation of this effect is required.

Pancreatic secretory metrics were estimated with good consistency, within the expected accuracy due to assay errors. This result shows that the greater variability in Part 1 is mainly attributable to the different dosing used, rather than the model, methods or protocol. These metrics also provide further diagnostic data, such as the increased basal endogenous insulin u_b and blunted first phase response AUC_{10} seen in low S_I and diagnosed type 2 diabetes subjects, as discussed in detail in Section 8.3. Combined with S_I, a more complete diagnostic picture of the metabolic defect can be drawn.

Estimated insulin secretion rate peak, S_{max} is likely underestimated, due to the lack of samples during 0-5 minutes, as discussed in Section 5.1.3. By introducing a corrected peak at 1 minute, this metric could be estimated more accurately. This approach was not applied in this pilot study, as the basic methodology was to be tested. Even so, an inter-subject comparison is viable, as the introduced error due to reduced sampling is systematic and should not add random variability.

The estimated insulin sensitivities can be compared to the distribution estimated from the clamp population in Chapter 6. The clamp study was performed on insulin resistant individuals and the geometric mean is clearly lower at $S_{I-clamp} = 4.9 \times 10^{-4}$ (MSD 1.5) l/mU/min than in this study $S_I = 11.7 \times 10^{-4}$ (MSD 2.3) l/mU/min, when only the lower dose tests on each subject are considered. Even if this comparison has to be considered with caution, as the clamp values are highly dependent on assumptions of V_G and EGP, the ranges seem to be reasonably comparable.

More specifically, the type 2 diabetes subjects in the study have S_I values in the magnitude of 2-4$\times 10^{-4}$ l/mU/min, or 5-6$\times 10^{-4}$ l/mU/min for Subject 1 who was on Metformin, an insulin sensitivity enhancer. These latter values are in the lower range of the clamp population, which is still normoglycaemic and thus expected to be slightly less resistant. The range for normally sensitive

people seems to be from $\sim 7 - 20 \times 10^{-4}$ l/mU/min, and some highly sensitive outliers show values up to over $40{\times}10^{-4}$ l/mU/min. A complete validation of this relationship will have to be done in a separate validation study against the clamp, as the clamp results might also be affected by the higher dose or different method of administration.

The final test on Subject 5, in which the effect of mixing was investigated, was already discussed in part. One might consider changing the protocol to sample from a different arm than the one in which administration of glucose and insulin is done. In fact, this approach is what is done during an IVGTT [Bergman et al., 1985] and in other similar research studies. For a simple clinical test, this option would mean additional pain and stress for the person being tested and is also not a good option in terms of clinical effort. As can be seen in the equal results in S_I from both sampling arms, the approach to disregard the first 10 minutes is reliable and robust in dealing with this added noise. The contamination of samples could also be reduced by adding serum albumin to the insulin solution [NovoNordisk, 2002], or priming the equipment before use. In a final product, the insulin would be pre-diluted and this issue will most likely not be a significant problem.

Finally, the number of subjects and tests could have been larger to obtain statistically more significant results, especially in assessing the effect of dosing. Nonetheless, the study proved that the proposed test performs well and is very accurate, mostly within the simulated accuracy calculated in Chapter 7. The practical aspects learned from this pilot test allowed the fitting algorithm to be adapted to account for unpreventable inaccuracies, such as contamination and systemic mixing. The main sources of error were identified and accounted for by the algorithm as well as possible, and their further effect was characterised.

Further testing is required to address possible protocol improvements to reduce possible sources of error, or to improve practical aspects, such as ergonomics and sampling methods. A full validation study will also have to be performed to finally validate the method against the gold standard clamp test. This full study will yield experimental validation of the comparability of both metrics and the overall correlation between both tests.

8.6 Summary

The pilot study was designed as a proof of concept for the proposed insulin sensitivity test. The aims were to assess the feasibility of the protocol and fitting approach, the effect of different dosing of glucose and insulin and the accuracy in repeatability at the same dose. A total of 43 tests on 17 subjects were performed, both in Christchurch and in Dunedin.

The results show a greater variability in S_I when different dosing is employed, with an indication of a lower estimated S_I at a higher dose test, a result that is not statistically significant. Repeatability is very good, with accuracies within the expected ranges simulated in Chapter 7. Accuracy and repeatability in pancreatic secretory metrics u_b, AUC_{10} and S_{max} were very good, all within the expected accuracy defined by assay errors.

Overall, the test is accurate and the fitting method robust enough to account for identified sources of error. The protocol is simple and short enough to be useful in a clinical setting. Final validation against the euglycaemic clamp will be required to completely validate the equality of both metrics.

Chapter 9

Test Optimisation

The test developed shows good accuracy and robustness, both in simulation and in clinical testing. Optimisations of the test protocol can now be carried out to improve practical or ergonomic aspects, and reduce the test duration and number of samples. The overall goal is to improve its clinical applicability with no loss of performance. In this chapter, possible steps to reduce test complexity and cost are analysed. It concludes presenting some variations of the protocol that can be readily implemented without a significant loss in accuracy or robustness.

9.1 Clinical/Diagnostic Improvement Goals

To optimise the insulin sensitivity test proposed in Chapter 7, the intended improvements, goals and acceptable compromises in accuracy need to be defined. The key goals are:

Simplification of the protocol: For the test to be clinically practicable, it has to be as simple and as short as possible without compromising its performance. The proposed test is already simpler and shorter than the comparable IVGTT, but any further simplification can greatly enhance its clinical use. A systematic analysis is performed to assess the effects of targeted sample reduction steps on outcome metrics. A reduction in samples has the benefit of reducing assay costs, simplifying the protocol and reducing overall time requirements for the subject and clinical personnel.

Retain accuracy and clinical diagnostic relevance: With all possible simplifications and cost reductions, an important aspect should be kept in mind. This aspect is to not oversimplify the test and jeopardise its performance, affecting its clinical diagnostic relevance and accuracy. Slight variations in performance are acceptable, but should not alter the relevant diagnosis obtained from the test.

Accuracy can be assessed as a relative deviation from the metrics obtained from the full data set, and as a change in intra-individual repeatability. The latter should be comparable to the assessed repeatability in the Monte Carlo simulations of Chapter 7 and the pilot study results of Chapter 8.

Propose test protocol variations: The various simplified protocols do not necessarily have to be regarded as a replacement for the original full sample set, but rather as an expansion of the test's applicability. Simplified test protocols can be attractive for a clinical application that has cost or time limitations, whereas if higher repeatability is required, such as in a semi-research setting, a more complete sampling can be performed. A further advantage is that the outcome metrics of these various alternative protocols are equal and thus comparable. Clinical results can thus easily be compared to research results and viceversa, an aspect that is not possible with currently available methods [Ferrannini and Mari, 1998].

9.2 Sample/Cost Reduction

A reduction of the samples required has a triple benefit for the test. First, it reduces complexity and stress for the clinical personnel performing it. Secondly, assay costs can be reduced, making it more appealing for large population studies and frequent use. Finally, it would reduce the stress on the patient being tested.

The cost reduction is based on current assay prices charged by the Canterbury Health Laboratories, Christchurch, NZ (www.cdhb.govt.nz/chlabs). Inter-lab differences in pricing are likely, particularly if comparing to larger U.S. or European centres. However, relative differences between glucose, insulin and C-peptide assays are expected to be comparable. The price structure used is in NZ$ and comprises:

- Glucose: $ 2.50
- Insulin: $ 25.00 (10× glucose)
- C-peptide: $ 35.00 (14× glucose)

From these prices it is evident that any significant reduction in cost is only possible if insulin and C-peptide samples can be reduced. A reduction of glucose sampling would not significantly reduce costs, but could reduce complexity and possibly the overall time required. Hence, insulin and C-peptide sample reductions impact on cost, while concomitant glucose sample reductions would then reduce clinical intensity and improve ergonomics.

The current assay price for one test with 10 samples, as described in the previous chapters, is NZ$ 625. Time required to perform the test, as simulated and piloted (without overheads pre- and post-procedure), is 55 minutes. These reference values will be used to evaluate the protocol optimisations suggested in this chapter.

9.2.1 C-peptide Sample Reduction

Methods and Results

To minimise the number of C-peptide samples required to accurately describe insulin secretion characteristics, it is crucial to identify key points of discontinuity in the C-peptide concentration profile during this type of test. These points of discontinuity are caused by sudden changes in the appearance of C-peptide, either exogenous or endogenous. Identifying these critical points is important as they include the key information about β-cell function. Missing these points results in a loss of this important diagnostic outcome of the test. Common identified changes in secretion during the clinical pilot results are illustrated in Figure 9.1 and defined:

1. **Injection of glucose (D_1)**: A sudden increase in plasma glucose triggers a secretion burst of stored insulin (first phase) lasting 5-10 minutes that is

often reduced or blunted in type 2 diabetes [Davies et al., 1994; Del Prato et al., 2002]. In the C-peptide concentration profile, this dynamic is seen as a very steep rise immediately after administration of glucose. As glucose is administered between $t = 0$ and $t = 1$ minutes, a lag of one minute is chosen here to account for glucose injection and initial pancreatic response time.

2. **Peak first phase secretion rate (D_2)**: Peak C-peptide secretion rate determines peak C-peptide concentration during the first 10 minutes post glucose input. In the concentration profile, this point is the maximum value CP_{max}, located at t_{CPmax}, assumed between 0-10 minutes.

3. **Injection of insulin (D_3)**: A sudden increase in plasma insulin inhibits pancreatic insulin secretion [Jefferson and Cherrington, 2001]. This response can be significantly delayed or not evident in type 2 diabetes [Jefferson and Cherrington, 2001]. In the concentration profile, this point can be seen as a steepening of the negative downward slope soon after insulin input. In these pilot tests, this point is not very pronounced due to the low insulin dosing employed and might not be very critical for this study.

4. **End of test (D_4)**: The last sample is not back to the basal concentration level in most cases due to the relatively short test duration after glucose input. This sample thus provides an indication of the continuing secretion after the insulin administration.

These points are typically very pronounced and consistent in healthy individuals. However, they can be very gradual or blunted in individuals with diabetes, who have an impaired first phase secretion and often have delays in pancreatic response to glucose and insulin concentration changes. Figure 9.1 shows examples for a healthy and a type 2 diabetes subject from the pilot tests, with the identified points of discontinuity.

Note that points D_2 and D_3 can be very variable in different individuals and may introduce errors when generic points at specific times are chosen in place of referencing the actual observed behaviour. In particular, healthy and type 2 diabetes responses are very different in both shape and time to peak concentration, as seen in Figure 8.4. Thus, identifying patients in transition between these states, which is the goal of this test, requires the flexibility to

utilise or estimate the actual dynamics and their variability. Diagnostically, this requires any sample reductions to still account for these potential differences without the excluded measurements.

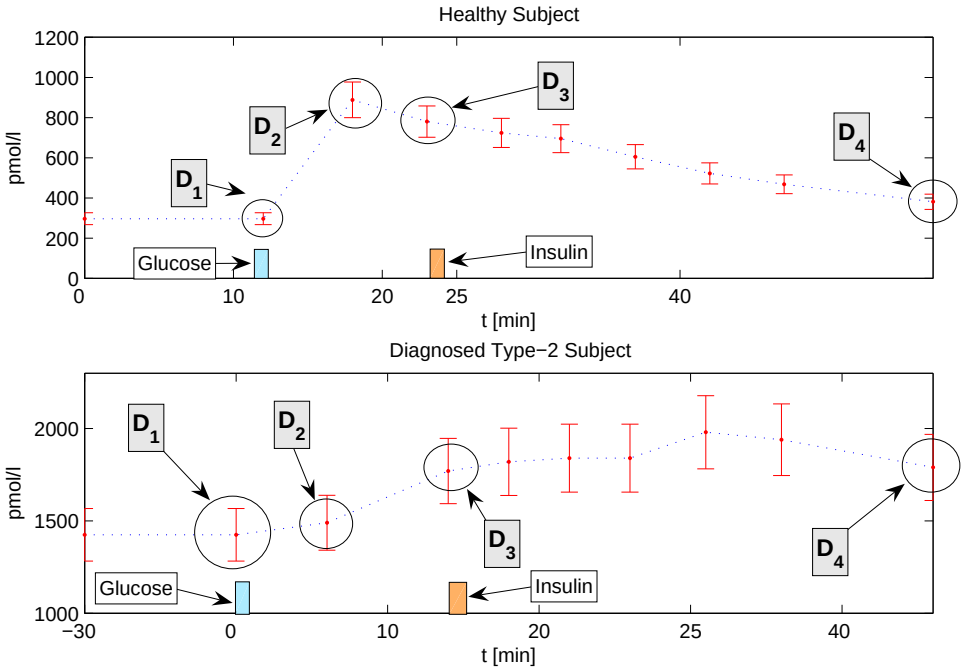


Figure 9.1 Example of points of discontinuity identified in the C-peptide profile during the pilot test in healthy (above) and diagnosed type 2 diabetes (below) subjects.

The reduction of samples is done in five steps, to individually assess the effect each step has on the final outcome. The steps are described and shown in Figure 9.2, and specifically defined:

CP1. Only one instead of two fasting samples. All other samples remain unchanged. This step reduces the total number of samples by one. However, it also simplifies the protocol. Assay and natural variability can result in different results due to this change, where two samples provided a better baseline estimate.

CP2. The samples are reduced to 5 by limiting the samples to the points of discontinuity. Both fasting samples are used.

CP3. A further reduction from Step CP2 to 4 samples by dropping D_3, which is not very distinct in most healthy subjects and may thus not provide too important information for early diagnosis.

CP4. A further reduction from Step CP3 by using only the first fasting sample. This step uses only 3 samples.

CP5. This step uses only the first fasting sample, the points of discontinuity $D_1 - D_4$ and the sample 10 minutes after insulin administration. This last sample is important for the insulin profile and can thus also be analysed for C-peptide. It can further improve accuracy in diagnosing and identifying type 2 diabetes subjects.

The performance of each of these steps is assessed by analysing the difference in performance and diagnostic metrics of the test, as compared to the full test result. These metrics are S_I, u_b, AUC_{10} and S_{max}. The analysis is done using the pilot data and simply excluding the selected points in the analysis. All other methods are the same. The resulting differences are shown in Table 9.1.

Table 9.1 Difference in test results for C-peptide sample reduction Steps CP1-5. The first column shows the number of samples (reduction from full set of 10), followed by the identified S_I, u_b, AUC_{10} and S_{max} values. The difference is shown as a percentile relative change and standard deviation (SD).

	# CP samp.	**Diff in S_I (SD)**	**Diff in u_b (SD)**	**Diff in AUC_{10} (SD)**	**Diff in S_{max} (SD)**
CP1	9 (-1)	-0.02 % (0.25)	0.56 % (3.61)	-2.07 % (6.00)	-1.97 % (6.20)
CP2	5 (-5)	-0.21 % (1.94)	0.00 % (0.00)	-0.01 % (0.05)	0.00 % (0.00)
CP3	4 (-6)	-0.44 % (3.19)	0.00 % (0.00)	2.43 % (19.60)	-2.99 % (13.15)
CP4	3 (-7)	-0.50 % (3.14)	0.56 % (3.61)	0.35 % (22.71)	-5.24 % (15.33)
CP5	5 (-5)	-0.03 % (1.85)	0.56 % (3.61)	-2.07 % (6.00)	-1.97 % (6.20)

The difference in intra-individual repeatability of the test introduced by the sample reduction steps CP1-5 was not different to the original full set analysis (6.0 % (MSD 4.9)) in steps CP1 (6.0 % (MSD 5.0)), CP2 (6.7 % (MSD 4.2)) and CP5 (5.8 % (MSD 5.1)). In the more extreme steps CP3 and CP4, the mean repeatability was larger at 9.1 % (MSD 2.8) and 9.1 % (MSD 2.8), respectively, but the MSD much tighter. The C-peptide sample reductions thus do not significantly reduce accuracy in repeatability of the test as assessed on these pilot study data.

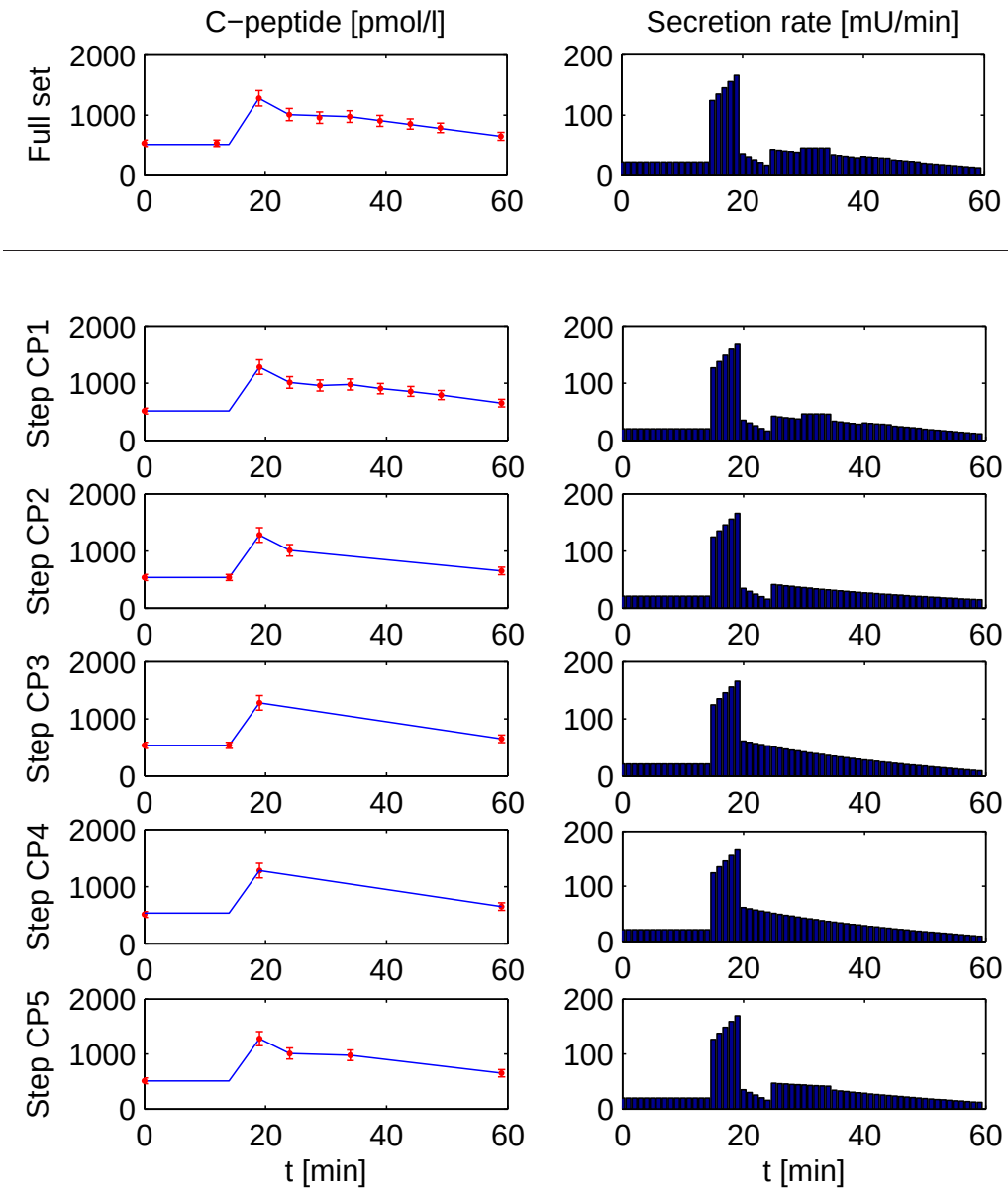


Figure 9.2 Original C-peptide full sample set and C-peptide sample reduction Steps CP1-5. Shown are the linearly interpolated C-peptide concentration samples (left) and the estimated insulin secretion rate (right). The profile is representative of a healthy subject.

Discussion and Conclusions

None of the five steps shown had a large effect on the estimated S_I value. The mean differences were insignificantly different from zero and the standard deviations relatively tight. The same is the case for the introduced changes in accuracy in repeatability in the different steps. Repeatability was not different to the original full data set analysis in steps CP1, CP2 and CP5, and slightly less accurate in steps CP3 and CP4. Clinically, these differences would have no diagnostic impact.

The reasons for this lack of effect are that the estimated secretion rate is only one input to the whole system. In addition, any errors in its estimation are smoothed out by the subsequent fitting of insulin and glucose. These minimal differences suggest that all five steps are viable alternatives if S_I is the main result of interest.

The estimated endogenous insulin secretion metrics u_b, AUC_{10} and S_{max}, are more affected by these steps, as they are directly calculated from the C-peptide data. Basal insulin secretion, u_b, is estimated from fasting information, and is thus only affected by using one fasting sample, instead of the mean of two. The effect on u_b is thus very small and of a similar magnitude to the effect on S_I. It is also well within C-peptide assay errors, as shown by Monte Carlo analysis in Section 5.1.4, and thus not significant.

The impact on AUC_{10} is not very large in steps CP1, CP2 and CP5, but significantly larger for steps CP3 and CP4. In particular, steps CP1, CP2 and CP5 include all samples in the first 10 minutes after glucose input (D_2 and D_3). These 3 steps are therefore only affected by a change in u_b, which is integrated over this time period. In contrast, steps CP3 and CP4 do not include D_3, a key sample in some subjects. The differences are especially large in type 2 diabetes subjects, for whom D_2 is not a peak in concentration. For example, these errors are especially large in Subject 16, but if Subject 16 is taken out of the calculation, the SD of Step CP3 reduces to $\sim$15 % and of Step CP4 to $\sim$18 %. Hence, for type 2 diabetes subjects, Steps CP3 and CP4 can introduce significant errors.

The same factors are important for S_{max}, as it is estimated from the same time period immediately after glucose infusion. The standard deviations for steps CP3

and CP4 are wide for S_{max}, which is again mainly caused by Subject 16. Without this subject, they are reduced to 0.76 % in Step CP3 and 5.4 % in Step CP4, with the mean values slightly improved.

The best overall approach is Step CP2, which is accurate in all metrics, but uses only half the original samples. For practical sampling reasons, Step CP5 could be better, as it uses only one fasting sample and the 10 minute post insulin sample, which is important for the insulin profile identification. This sample could be dropped to further reduce the sample number to four, with no effect on the pancreatic metrics and a slight, but not clinically relevant, difference in S_I.

Overall, all steps work well for individuals with healthy pancreatic responses, in which D_2 is a clear peak in the concentration profile. In type 2 diabetes individuals, the differences in pancreatic metrics can be much larger, especially if D_3 is left out. The differences in S_I are very small, and indicate that all of these approaches are feasible if only insulin sensitivity is of importance. Clinically, changes in endogenous insulin secretion rates may be important additional diagnostic markers of early insulin resistance. Hence, the choice of Step CP2 or CP5 represent the best options. Overall, the C-peptide assay costs can thus easily be reduced by $50\,\% - 60\,\%$ (NZ$ 175-210) without compromising test accuracy.

9.2.2 Insulin Sample Reduction

Methods and Results

As with C-peptide, to minimise the number of insulin samples, the timing of key samples in the dynamic profile have to be identified. There are two inputs to the insulin system, exogenous insulin from the bolus and endogenous insulin secreted by the pancreas. Thus, the considerations in reducing the number of samples must be done by including the C-peptide sampling utilised. The important insulin profile samples include:

- **Fasting sample**. Instead of using two fasting samples, one could be dropped.

- **Two samples, 5 and 10 minutes post glucose input**. These samples are important if pancreatic secretory metrics are of interest. Dropping them could also affect the quality of the estimated S_I.
- **10 min post insulin sample**. This sample is the first reliable sample that is not affected by mixing after the insulin input. As the fast component of insulin decay is not identifiable after 10 minutes, this sample is crucial.
- **Last sample**. The last sample shows how fast insulin concentrations return to basal levels, and thus reflects insulin sensitivity and uptake, as well as its effective removal rates via the kidney and liver.

If only these samples were used, the total number of samples could already be reduced to 5, a cost improvement of 50 % from the original 10 samples. However, the compromise in accuracy will have to be assessed, as a reduction in diagnostic power of the test is not feasible. This tradeoff is also affected by the assumptions or sample reductions made for C-peptide. In particular, eliminating both insulin and C-peptide samples at specific time points will reduce blood draw requirements.

A further possibility to reduce sampling numbers is to make use of steady state assumptions and attempt to identify insulin kinetics with a fasting sample of insulin and C-peptide. Since two kinetic parameters, n_L and x_L, are identified, a further physiological dependency between them needs to be worked out to achieve this goal. The parameter x_L describes the fractional extraction of insulin secreted by the pancreas, during the first pass through the liver before it reaches the systemic circulation. It is thus the same effect as described by the hepatic clearance rate n_L.

These two parameters can be combined into one parameter by incorporating hepatic blood flow, F_h, and total blood volume (plasma + haematocrit), V_B. The relationship between them can be defined:

$$x_L = n_L \frac{V_B}{F_h} \tag{9.1}$$

where the ratio V_B/F_h (l/(l/min)) provides the fraction of blood flow through the liver. The fractional extraction, x_L, is thus related to n_L by this constant.

Hepatic blood flow and blood volume can be estimated from the physiological literature [Guyton and Hall, 2000]. Hence, blood volume can be defined:

$$V_B = V_P/(1 - \text{haematocrit}) \tag{9.2}$$

with mean haematocrit levels of 0.4 and 0.45 for female and male respectively [Boron and Boulpaep, 2003]. Hepatic blood flow is defined [Guyton and Hall, 2000]:

$$F_h = 0.8 \text{ L/min/m}^2 \tag{9.3}$$

Pancreatic insulin thus appears in the plasma compartment at the rate

$$(1 - x_L)u_{en} = (1 - n_L V_B/F_h)u_{en} \tag{9.4}$$

where u_{en} is the secretion rate estimated through C-peptide kinetics prior to the first pass hepatic extraction. Rewriting Equation 3.7 in steady state form (subscript ss) utilising these terms thus yields:

$$0 = -n_K I_{ss} - n_L \frac{I_{ss}}{1 + \alpha_I I_{ss}} - \frac{n_I}{V_P}(I_{ss} - Q_{ss}) + \frac{u_{ex}}{V_P} + (1 - x_L)\frac{u_{en-ss}}{V_P} \tag{9.5}$$

Incorporating $\gamma = I_{ss}/Q_{ss}$ and the relationship between x_L and n_L from Equation 9.1 with $u_{ex} = 0$, results in the relationship defined:

$$0 = -\left(n_K + (1 - \gamma)\frac{n_I}{V_P}\right) I_{ss} - n_L \frac{I_{ss}}{1 + \alpha_I I_{ss}} + (1 - n_L \frac{V_B}{F_h})\frac{u_{en-ss}}{V_P} \tag{9.6}$$

For a fasted or measured steady state, the only unknown in Equation 9.6 is now n_L. Thus, n_L can be determined analytically by rearranging that equation:

$$n_L = \left[-\left(n_K + (1-\gamma)\frac{n_I}{V_P} \right) I_{ss} + \frac{u_{en-ss}}{V_P} \right] \left(\frac{1 + \alpha_I \left(I_{ss} + \frac{V_B u_{en-ss}}{V_P F_h} \right)}{I_{ss} + \frac{V_B u_{en-ss}}{V_P F_h}} \right) \quad (9.7)$$

The hepatic clearance saturation α_I is difficult to identify from this low dose data, and likely has little or no effect at such low doses based on prior work [Ferrannini and Cobelli, 1987a; Thorsteinsson, 1990; Thorsteinsson et al., 1987]. Hence, it was set to zero in the previous analysis. A good data fit could still be achieved as the parameters n_L and x_L were identified accordingly. In this case, $\alpha_I = 0$ results in an underestimation of insulin concentrations in 10/43 pilot tests, and is especially evident for those subjects with high fasting plasma insulin levels, as found in IGT or type 2 diabetes. Increasing the saturation level to $\alpha_I = 0.0017$, a mean value found in the literature [Thorsteinsson, 1990] and used in glycaemic control studies [Chase et al., 2005a; Wong et al., 2006b], the model fits are improved in the remaining subjects, with 40/43 insulin profiles from the pilot tests within measurement error. The remaining three tests had unreasonably large sample errors, greater than a multiple of normal assay error, which were likely due to contaminated samples rather than realistic assay results.

The fasting identification of insulin kinetics results in similar, but increased, values for n_L and x_L compared to the full data set identification. Two representative fits are shown in Figure 9.3 for a NGT subject and a type 2 diabetes subject. It is clear that the differences between fasting and full data identification are clinically irrelevant.

An analysis to consider further sample reductions is performed by assessing the reduced sample profiles in comparison to the fasting identification, due to its minimal differences compared to the full data identification. Different options of C-peptide profiles are also used to find an optimum, given the inter-relationship of C-peptide and insulin. The five steps are defined:

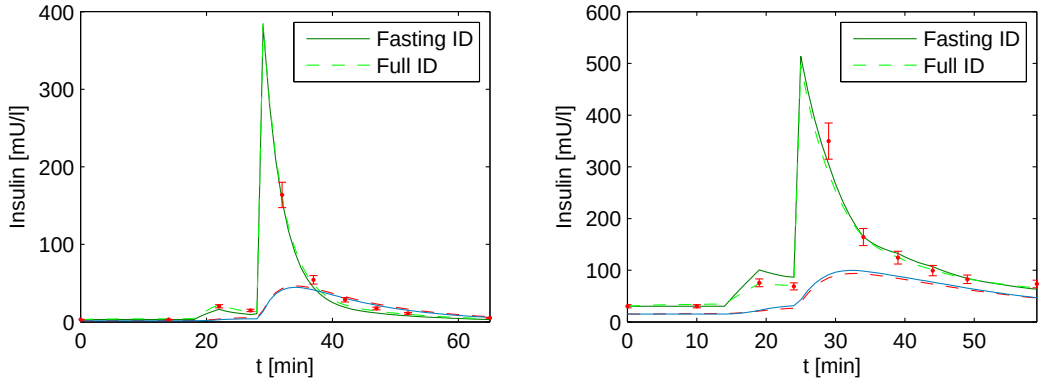


Figure 9.3 Two insulin profiles and modelled insulin identified with only one fasting insulin and C-peptide sample (solid) and with the full data set (dashed). The example on the left is a healthy subject, the one on the right a diagnosed type 2 diabetes subject.

INS1. The five critical insulin samples are used. These samples consist of only one fasting sample, the two post glucose input samples (5, 10 minutes), the 10 minute post insulin sample (20 minutes), and the last sample. The full 10-sample C-peptide profile is used, to isolate any differences to changes in insulin sampling alone.

INS2. The five insulin samples from Step INS1 are used. In addition, C-peptide samples are only obtained at the same five points.

INS3. Insulin kinetics are identified using the fasting insulin and C-peptide sample as described earlier in this section. No additional fitting of the insulin concentration curve is done. The full 10-sample C-peptide profile is used, to isolate any differences to changes in insulin sampling alone.

INS4. Insulin is only identified with its fasting sample, as in Step INS3. Additionally, only the fasting C-peptide sample is used. A constant basal endogenous insulin secretion rate is calculated from this sample and it is assumed to be constant over the duration of the test. This approach obviously does not capture pancreatic metrics, but it presents a low-cost, simple option if only the value of S_I is of interest.

INS5. This step uses the five insulin samples from Step INS1, but does not use any C-peptide information. The insulin equation cannot be identified without C-peptide information. Instead, plasma insulin is approximated from the sampled data and interstitial insulin $Q(t)$ is calculated using the approximated plasma insulin as the input to Equation 3.8. This option does not

include pancreatic metrics, but it presents a further option if a C-peptide assay is not available or too costly.

The resulting insulin and C-peptide profiles from these five sample reduction steps are shown in Figure 9.4. The insulin profiles are qualitatively very similar after the insulin bolus. A difference in the first phase insulin peak is seen in Step INS4, in which the C-peptide concentration peak is not sampled and thus endogenous insulin secretion is assumed to stay constant throughout the test.

The performance of the sample reduction steps is assessed by comparing estimated S_I, n_L and x_L. Pancreatic metrics u_b, AUC_{10} and S_{max} are not applicable, as they are calculated from C-peptide samples and are equivalent to the relevant steps CP1-CP5 in Section 9.2.1. As the differences to the original sample set can be quite large, especially during the fasting identification, a further comparison is made in each step between the mean percentile repeatability in each subject based on the clinical pilot test data in Chapter 8. The results are given in Table 9.2. The repeatability metrics shown in Table 9.2 are absolute values and are log-normally distributed. The multiplicative standard deviation (MSD) is thus used to describe their spread. Standard deviation (SD) is used for all other metrics, as they are normally distributed.

Table 9.2 Difference in test results for insulin sample reduction steps INS1-INS5. Given are the number of samples (reduction from full set of 10) for both insulin and C-peptide, the differences in S_I, n_L, x_L, and the intra-individual repeatability. The difference is shown as mean percent relative change and standard deviation. Repeatability is shown as mean absolute percentile deviation and multiplicative standard deviation (MSD), as the data was lognormal ($P < 0.05$).

	# I samp.	**# CP samp.**	**Diff in S_I (SD)**	**Diff in n_L (SD)**	**Diff in x_L (SD)**	**Repeat. (MSD)**
INS1	5 (-5)	10 (-0)	-1.8 % (14.9)	12.3 % (51.0)	-7.0 % (10.2)	9.4 % (2.5)
INS2	5 (-5)	5 (-5)	-2.1 % (15.2)	11.9 % (49.9)	-7.1 % (9.9)	8.9 % (2.6)
INS3	1 (-9)	10 (-0)	-6.8 % (17.4)	42.3 % (66.4)	12.7 % (12.7)	7.3 % (1.9)
INS4	1 (-9)	1 (-9)	21.0 % (30.0)	42.3 % (66.4)	12.7 % (12.7)	6.1 % (2.3)
INS5	5 (-5)	0 (-10)	-6.5 % (14.1)	-	-	6.3 % (6.7)
					Original full sample set	6.0 % (4.9)

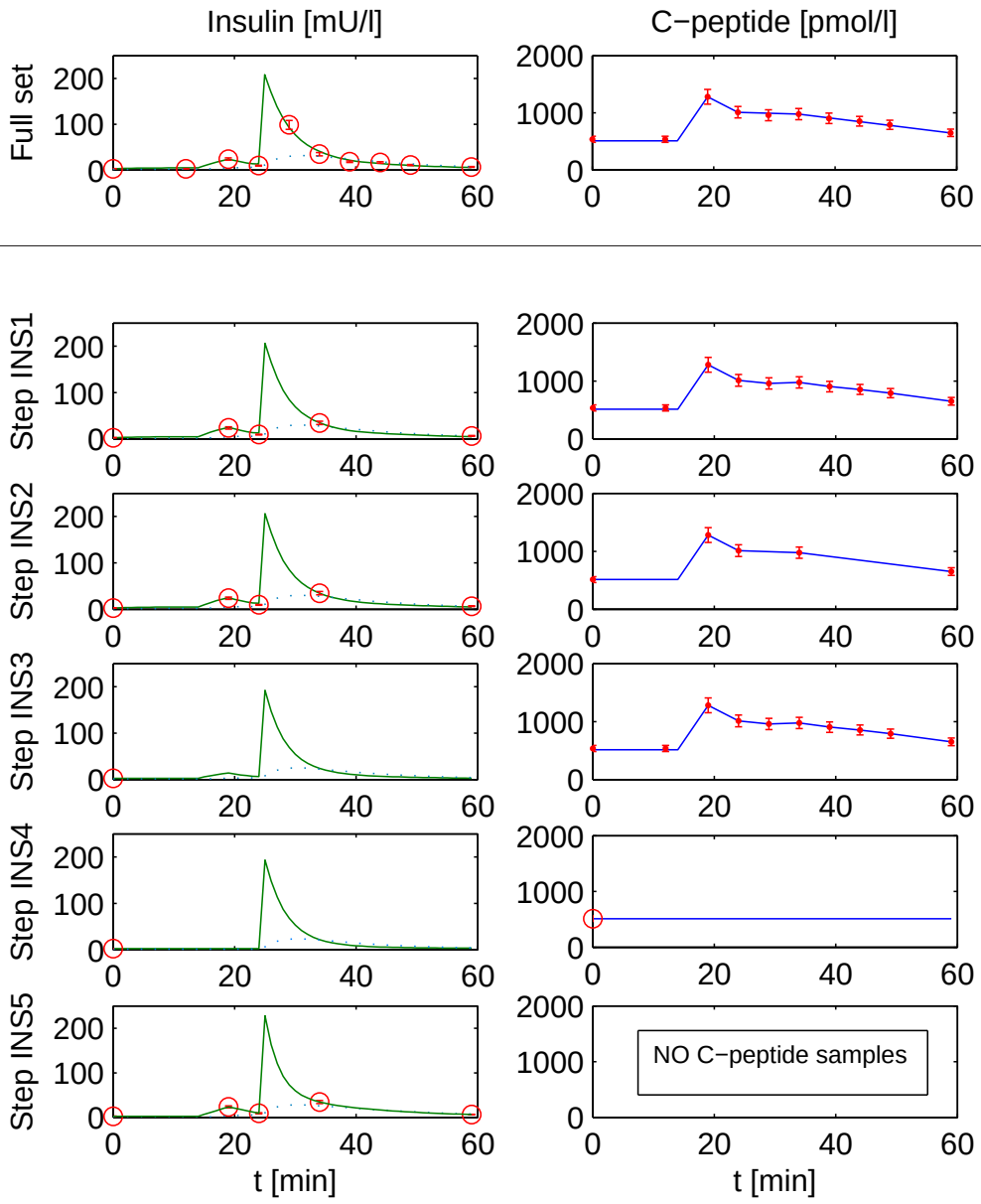


Figure 9.4 Original full sample set and insulin sample reduction steps INS1-INS5. The plasma insulin model fits (solid) and interstitial insulin (dotted) are shown on the left, and the linearly interpolated C-peptide concentration samples are shown on the right. The example on the left is a healthy subject, the one on the right a diagnosed type 2 diabetes subject.

Discussion and Conclusions

Insulin has much faster kinetics than C-peptide due to its multiple routes of clearance. It is thus a much more dynamic peptide with larger time constants. The insulin system has two inputs, one from an exogenous bolus injection and the other from endogenous secretion by the pancreas. A reduction in samples can thus not be performed without also considering C-peptide, which is used to estimate the endogenous insulin secretion. Five different options of sample reductions were analysed and their affect on S_I assessed, both in difference to the full sample set and in intra-individual repeatability over the cohort. All steps were able to perform well, some with more accuracy than others.

The reduction to the five key samples identified (steps INS1 and INS2) resulted in nearly identical results when either the full C-peptide sample set was used (INS1) or just the same insulin sampling times (INS2). The mean deviation in S_I is close to zero, but the standard deviation of 15 % is rather broad and may have a clinical diagnostic impact. This result should be expected, as the insulin decay curve is estimated from only two samples, which is much less robust to sampling or assay error than a larger number of samples. Mean n_L is slightly overestimated, while mean x_L is underestimated, both with similar SDs in both steps INS1 and INS2.

Intra-individual repeatability is larger than in the original sample set, but still very narrow at 9 %. The multiplicative standard deviation in this case is much tighter (2.5 vs. 4.9) than in the original set. This result indicates that the reduced sampling set is not necessarily less accurate, even with the larger variability, when S_I is compared directly between methods. With a reduction in insulin and C-peptide assay costs by 50 % (NZ$ 300 reduction), the approaches of steps INS1 and INS2 are very attractive.

The identification of insulin kinetics with just a fasting insulin and C-peptide sample (steps INS3 and INS4), assuming a steady state during fasting, performs surprisingly well given the generic assumptions used for hepatic blood flow F_h and blood volume V_B. When using $\alpha_I = 0$ some insulin profiles were slightly underestimated. The largest deviations from sampled data appear to occur after the insulin bolus, when concentrations are high. When the insulin levels get closer to their fasting values, accuracy increases. This result suggests an overesti-

mation of clearance rates at high insulin concentrations with this approach, due to the neglected saturation effect. Increasing $\alpha_I = 0.0017$ [Ellemann et al., 1987; Thorsteinsson, 1990] improved the underestimated fits, while maintaining a very good fit in the other subjects. The accurate patient-specific estimation of this parameter is not possible on a normal low dose data set of this type. However, merely including the effect using a population value is able to greatly improve model performance.

The fasting ID approach of steps INS3 and INS4 is very attractive, not only from a cost perspective (90 % reduction in insulin assay cost), but also in terms of the robustness of practical clinical aspects. Because no further insulin samples are required, the method is completely unaffected by the often very error affected plasma insulin samples immediately after the bolus insulin administration. These samples were often contaminated or affected by incomplete mixing, as discussed in Section 8.2.

The comparison of Step INS3 to the full sampling set shows larger errors in S_I and n_L and broader SDs, which does not automatically invalidate the approach. These deviations can also be caused by more variable original data due to sample errors, which does not affect the fasting ID. In fact, the repeatability is improved in this case, at nearly the same mean value, but with a much tighter MSD. The subjects in which the between test variation is large in the original assessment, mostly had large errors in portions of their sample sets. With the fasting ID, the results were thus more consistent. The drawback of the fasting ID is that the endogenous insulin input, estimated through C-peptide is still required. However, a reduction of C-peptide to five samples should not affect the performance, as can be seen in the result of Step INS2.

The two more extreme sample reduction steps, INS4 and INS5, were analysed to test two special scenarios. These approaches can be necessary if, due to cost or simplicity, only a fasting sample of C-peptide and insulin can be taken. The rest of the samples are then only assessed for glucose (Step INS4). The second case would occur if a C-peptide assay is not available and only insulin can be sampled (Step INS5).

In Step INS4, in which endogenous insulin secretion is assumed to stay constant throughout the test, the difference to the original assessment is largest due

to completely missing any first phase β-cell response. Interestingly, the repeatability is still very tight, even better than Step INS3 and much tighter than the original set. This result may occur because the incorrect assumption on endogenous insulin secretion is equally incorrect in all respective tests and may thus effectively cancel. Because no dynamic assessment of C-peptide is performed, no pancreatic insulin secretion metrics can be assessed. From a simplicity and cost perspective, this option can be very interesting if only the value of S_I is required.

Step INS5 has interesting results as well. In this case, no C-peptide sample is taken. This simplification does not allow an insulin model identification, as this significant endogenous insulin input to the system is not known. Nonetheless, the insulin profile can be estimated with the same approach used in the integral fitting method, as described in Chapter 3, and $Q(t)$ calculated using the approximated plasma insulin $I(t)$ as input to Equation 3.8. The approach is very accurate, both in relative S_I and in repeatability, although slightly broader than the original set in this limited pilot study.

To pick the optimal option, the requirements and facilities available have to be determined. If cost is not relevant and good accuracy is required, more samples can be included. As shown, a reduction to half the samples (5) can be readily undertaken without compromising diagnostic performance (Step INS2). Using only a fasting insulin sample (Step INS3, INS4) results in an increased repeatability for S_I, as compared to the original data set, but also in a reduction of accuracy for other potentially useful clinical metrics. Overall, this analysis adds to the robustness and versatility of the method by outlining all the potential tradeoffs in terms of the test and thus clinical or diagnostic outcomes. From these results, any form of the test could be created with an understanding of the tradeoffs involved. In terms of economical aspects, the insulin and C-peptide assay costs can now be reduced by at least 50 %, thus creating an initial 40+ % reduction in assay costs from the full test.

9.2.3 Glucose Sample Reduction

Methods and Results

The main incentive for the reduction of glucose samples is not cost as it was for insulin and C-peptide sample reductions. Glucose sample reductions are analysed to improve clinical practicality and shorten the overall test. The key samples for the glucose profile are a fasting sample, the 5 or 10 minute post glucose input, and two samples at least 10 minutes after that. The 5 minute sample is highly affected by mixing and/or contamination, as was seen during the pilot test, and is thus not considered to be always reliable. The 20 minute sample is chosen, as sampling at this time is required to identify the insulin kinetics. A further sample is needed, which can be either at 30 minutes or later, such as the last sample at 45 minutes.

This approach yields four key samples, at 0, 10, 20 and 30 (or 45) minutes. To approximate the glucose concentration decay, a linear interpolation is not reliable anymore, as not enough closely timed samples are available, which would introduce larger errors to the integral-based fitting method. Instead, a single exponential decay is approximated between the last three samples. In all the following glucose sample reduction analysis steps, the four glucose samples are used. In this analysis, different combinations of reduced insulin and C-peptide sampling protocols are tested while using only these limited key glucose samples. Six steps are assessed, as described here and shown in Figure 9.5:

G1. Glucose is sampled at $t = 0, 10, 20$ and 45 minutes. For insulin and C-peptide the full data sets are used, to isolate the effect of glucose sample reductions.

G2. Glucose is sampled at $t = 0, 10, 20$ and 45 minutes, but only five C-peptide and insulin samples are used, as described in Step INS2. This approach is clinically more practical and less intense, as sampling is only performed at five time steps.

G3. Total test time is reduced, by replacing the 45 minute sample with the 30 minute sample. This is also done for insulin and C-peptide. Test time is now only 30 minutes, a reduction of 25 minutes from the original protocol,

with glucose sampled at $t = 0, 10, 20$ and 30 minutes, and insulin and C-peptide at $t = 0, 5, 10, 20$ and 30 minutes.

G4. Glucose and C-peptide samples are sampled at $t = 0, 10, 20$ and 30 minutes, but only a fasting insulin sample is used.

G5. Glucose is sampled at $t = 0, 10, 20$ and 30 minutes, but only one fasting insulin and C-peptide sample is used. Insulin kinetics are identified from the fasting samples and basal C-peptide secretion is assumed to stay constant throughout the test as in Step INS3. This step introduces the option of only one complete fasting sample and possibly only capillary glucose testing thereafter. Thus, this test could remove the need for a cannula, as well.

G6. Glucose is sampled at $t = 0, 10, 20$ and 30 minutes. No C-peptide sample is taken and insulin is interpolated between these five samples, as described in Step INS5. This approach could be attractive for cases in which a C-peptide assay is not available.

Test performance is again assessed by comparing the mean relative percentile difference in estimated S_I. As a further comparison, correlations between the estimated S_I values are calculated. Finally, the mean repeatability for S_I in each subject (Clinical pilot test Part 2, Chapter 8) is presented. The results are given in Table 9.3. Correlation plots are shown in Figure 9.6. Estimated V_G was equal in all steps, and similar to the original full sample set, with a mean difference of -0.85 % (SD 3.8 %).

Discussion and Conclusions

The sample reduction analysis steps for glucose show that only four samples are necessary to achieve an almost equally good estimation of S_I, if insulin and C-peptide are fully sampled (Step G1). More samples could also be used, but enough information is found in these four when compared to the original results. All six steps were thus carried out with the same minimal glucose sampling protocol, while the combinations with different reduced insulin and C-peptide sampling protocols were tested.

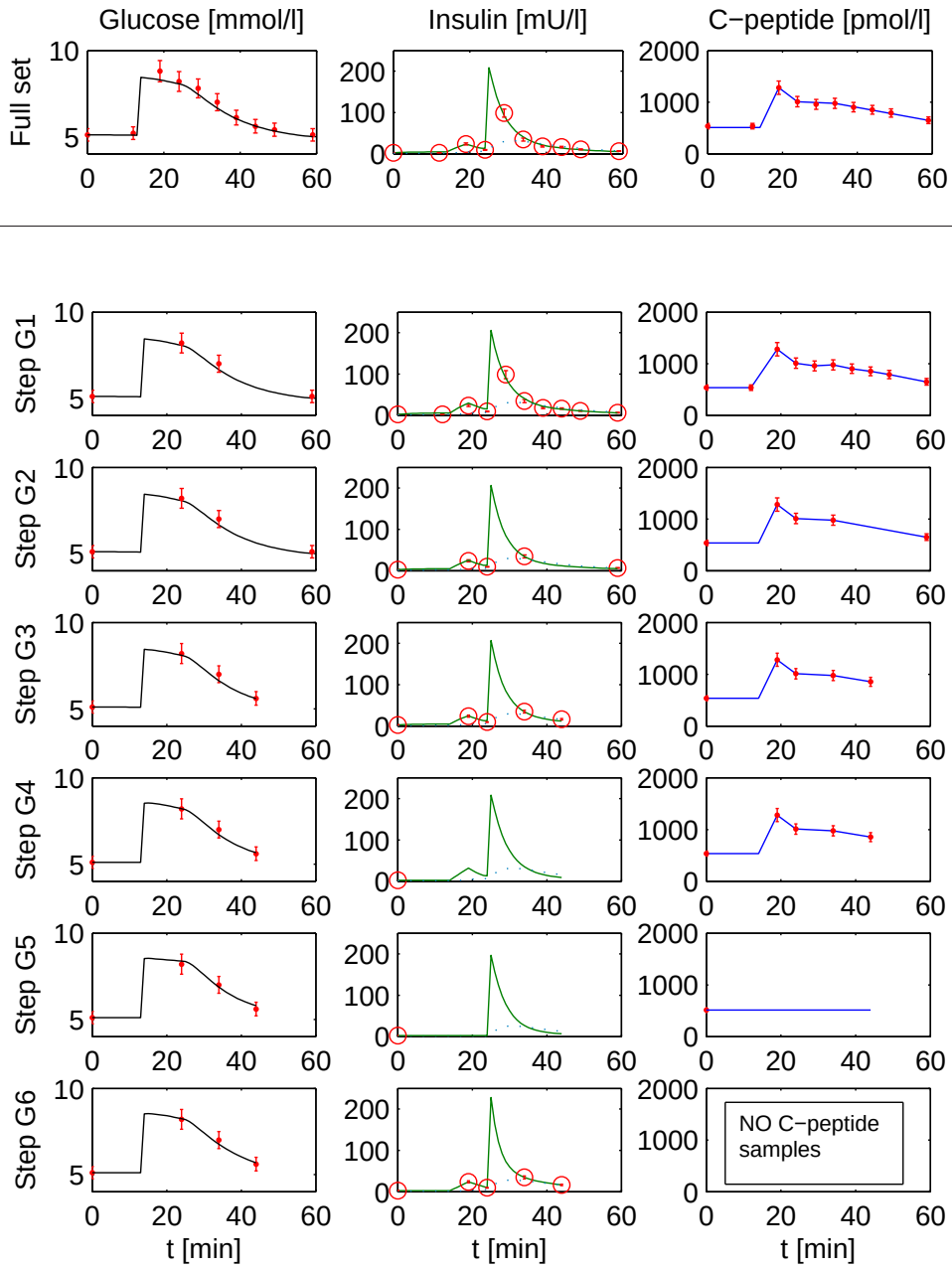


Figure 9.5 Original full sample set and glucose sample reduction steps G1-6. Shown are the glucose (left) and insulin model fits in plasma (solid) and interstitial fluid (dotted) (middle), and linearly interpolated C-peptide concentration samples (right).

Table 9.3 Difference in test results for glucose sample reduction steps G1-6. Given are the number of samples (reduction from full set of 10) for glucose, insulin and C-peptide, difference in S_I, correlation between S_I and original full set S_I and the intra-individual repeatability. The difference is shown as percentile relative change and standard deviation. Repeatability is shown as mean absolute percentile deviation and multiplicative standard deviation (MSD).

	# G samp.	# I samp.	# CP samp.	Diff in S_I (SD)	Correl. r	Repeat. (MSD)
G1	4 (-6)	10 (-0)	10 (-0)	-10.6 % (19.0)	0.96	7.0 % (2.9)
G2	4 (-6)	5 (-5)	5 (-5)	-6.3 % (18.3)	0.96	8.8 % (3.1)
G3	4 (-6)	5 (-5)	5 (-5)	-6.9 % (22.1)	0.89	10.5 % (3.7)
G4	4 (-6)	1 (-9)	5 (-5)	-8.7 % (28.1)	0.80	6.8 % (3.4)
G5	4 (-6)	1 (-9)	1 (-9)	15.3 % (35.9)	0.81	4.9 % (4.3)
G6	4 (-6)	5 (-5)	0 (-10)	-7.8 % (23.0)	0.90	7.3 % (6.5)
				Original full sample set		6.0 % (4.9)

The deviations from the original set get larger as less insulin and C-peptide samples are used. This result is expected, as errors accumulate from model to model, and the glucose model is affected by both insulin and C-peptide. This effect can be seen in the correlations calculated in the different sets. Whereas the correlation between the Step CP5 S_I and the full set S_I is $r = 1.0$, it is reduced to $r = 0.96$ in insulin Step I2, and is still $r = 0.96$ in glucose Step G2. All three steps use the same samples and accumulate their errors. The error in this case is mainly introduced by reduced insulin sampling, with reduced glucose and C-peptide sampling not having a large impact. These three specific correlations are shown in Figure 9.7 for clarity.

The intra-individual repeatability is still very good in all steps. In particular, most of the MSDs are even tighter than the original set. However, a tighter MSD may only imply a more repeatable, but also potentially more erroneous result. The mean repeatability values are larger than in the insulin steps, which is again due to the additional model included in this case.

Due to the low number of tests, only two or three in each subject, it is difficult to compare the intra-individual repeatability of these sample reduction steps to the repeatability assessed by the Monte Carlo simulations in Chapter 7, as any of these few tests could be an outlier. Nonetheless, a simple comparison is possible. The mean CV in S_I assessed in the 500 Monte Carlo runs was $\mathrm{CV}_{SI-MC} = 4.5\,\%$, meaning that ∼95 % of S_I values (2 SD) are within 9 % of the mean and ∼100 %

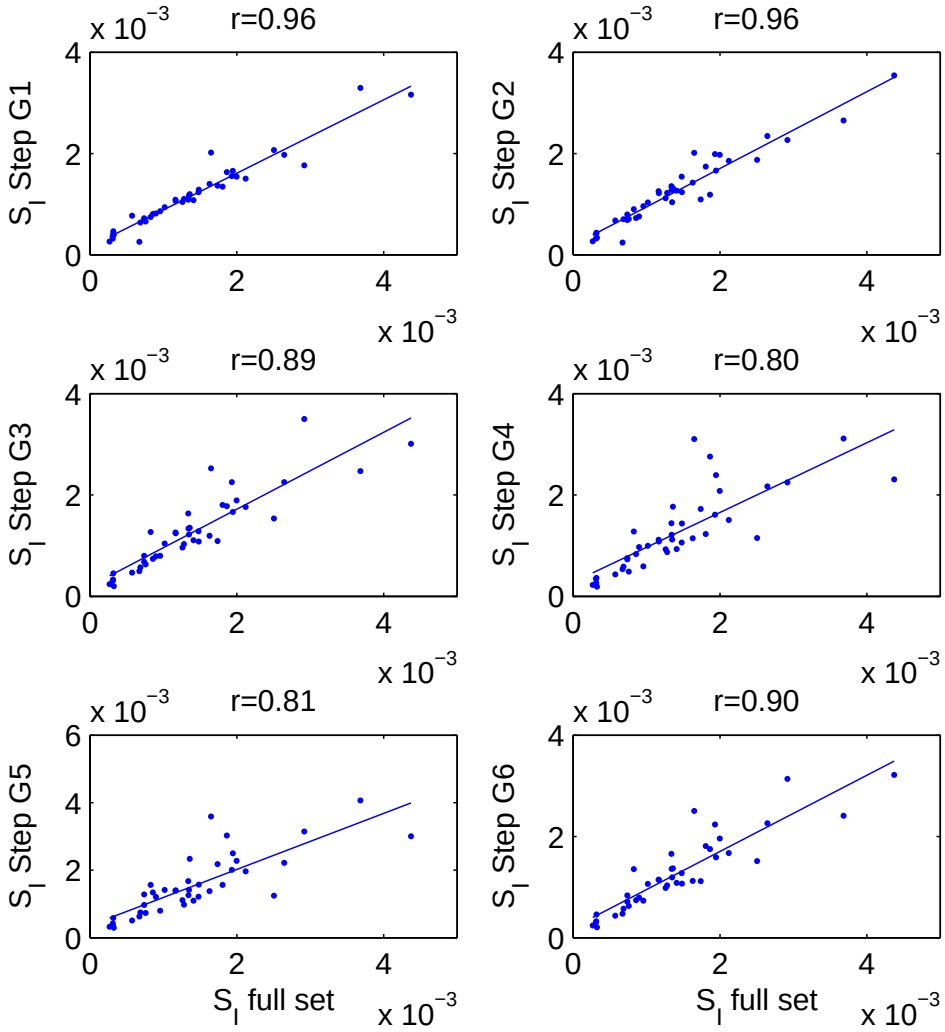


Figure 9.6 Correlations between the original full sample set and glucose sample reduction steps G1-6.

(3 SD) are within 13.5 %. All the mean sample reduction steps G1-5 are within 3 SD of CV_{SI-MC}, with only G3 at 10.5 % larger than 2 SD. Note that although the MSD's are relatively large, no subject had a larger deviation than 28.9 % in all analysed sample reduction steps. This comparison shows that a large part of the variability could potentially be attributable to assay and clinical protocol errors.

The reduction in total test time to only 30 minutes in steps G3-G5 is very attractive. In particular, this choice reduces personnel cost and subject time by

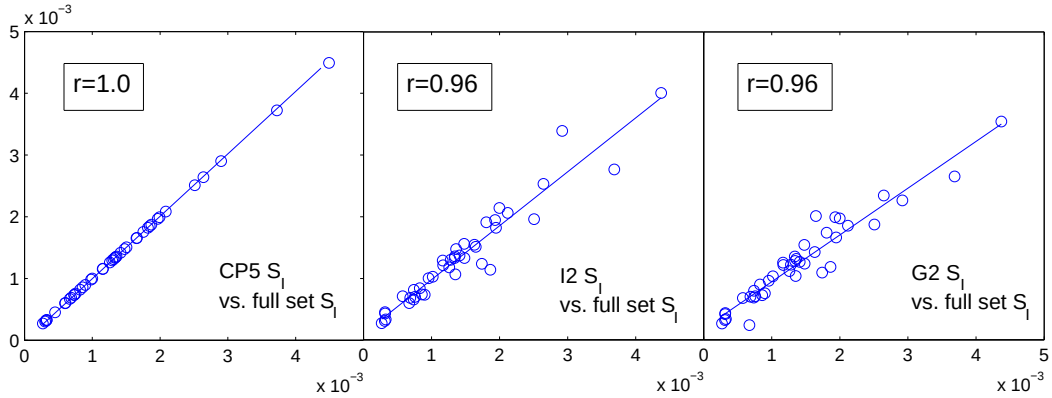


Figure 9.7 Reduction in correlation coefficients between sample reduction steps S_I and the full set S_I. The left plot shows only C-peptide sample reduction Step CP5, the middle plot including additional insulin sample reductions, Step INS2, and the right plot with additional glucose sample reductions, Step G2.

50 % as compared to the full protocol. The effect of this reduction is clear when comparing Step G3 to G2, as the only difference between them is the timing of the last sample (45 vs. 30 minutes. Repeatability is slightly worse in G3, but this result is within the expected variability introduced by assay errors, as a different sample is used.

One drawback that is evident is a loss in robustness. Reducing the samples to only the most critical four, leaves no redundancy if one of these is contaminated. Such contamination error occurs in three tests in which the 30 minute sample is clearly too high to be within expected noise, as discussed in Section 8.2. These three tests are still included in the analysis, producing the increased deviation in Step G3. Hence, a contaminated sample could effectively ruin the entire test result.

In contrast, a source of error in Step G2 is an increase in glucose concentrations if the test lasts too long and EGP begins to increase. In these cases, the decay in glucose cannot be estimated by a single exponential decay, as the real curve is affected by a changing EGP. This occurs only in highly insulin sensitive individuals, but negatively affects the accuracy of Step G2. This artefact speaks in favour of Step G3. However, it is clinically not critical as these individuals do not match the target group for this test.

The steps using a fasting identification of insulin (steps G4-G5) are not affected by contaminated insulin samples, as discussed in the insulin steps of Sec-

tion 9.2.2, and are thus much more robust in some ways. While glucose samples are still required, they appear to provide more stable S_I results than the pilot test. The deviations in S_I in Step G4 are not larger than in the preceding steps and the accuracy in repeatability is even increased. Even Step G5, which only makes use of a fasting C-peptide sample is reasonably accurate in S_I. Step G6 performs similar to G3, given the almost equal insulin information, and thus provides an option to perform the test with good accuracy without the need of a C-peptide assay.

The correlations in the first two steps are very high at $r = 0.96$. As discussed previously, this reduction from $r = 1.0$ seems to be attributable to reductions in insulin sampling. When an earlier sample is taken as the last sample, this correlation is reduced to $r = 0.89$, which reflects the measurement and mixing noise seen in the data used. The same correlation is seen in Step G3, which is expected because they share the same insulin sampling protocol. The two fasting identifications, steps G4 and G5 are less accurate at $r = 0.80$ and $r = 0.81$.

The reasons for a reduced correlation for any sample reduction steps versus the full pilot test case can be explained well when looking at Figure 9.6. As can be seen, steps G4 and G5 include some high deviation points caused by outliers in the high S_I range of $20 - 40 \times 10^{-4}$. As noted, this high range is not clinically or diagnostically relevant. The accuracy in the normal to low range targeted by this test is much tighter.

The compromises in accuracy are expected, given the heavy reduction of samples. With the lowest correlation at $r = 0.80$, the steps are still relatively accurate. More practically, these reduced protocols now provide a variety of options for different variations in the test protocol, depending on the available resources and expectations. Hence, these analyses provide not only cost and intensity reductions, but propose different protocol options for the same test metrics. A suite comprising a range of tests with varying degrees of intensity is thus presented, that enables a direct comparison of outcome metrics between them, an aspect that is not possible with other currently available tests [Ferrannini and Mari, 1998; Radziuk, 2000].

Overall, the further simplification of the test by reducing glucose sampling along with insulin and C-peptide sampling resulted in acceptable accuracy. The

losses in accuracy can be counterweighed by an increase in practicability and a reduction in assay cost and time, where reduced test time also reduces the cost of personnel. Even if higher accuracy is required, a 30 minute test with five samples of glucose, insulin and C-peptide results in almost equal performance, at half the cost and time, as long as no samples are contaminated. Finally, even with the larger spread in some extreme sample reduction steps, clinical diagnostic performance was not jeopardised. A diagnosis of an individual at risk of developing type 2 diabetes would not have differed if any of these variations in protocol were used.

9.3 Practical Aspects

Besides a reduction in samples, further aspects of the test can be optimised to improve robustness and ease of use with the goal of ensuring more ready acceptance by clinical personnel and subjects. Some aspects that came up in discussions during and after the pilot test are described here, with an eye towards possible test improvements:

Contamination and Systemic Mixing: As discussed in Chapter 8, samples taken within 10 minutes after glucose or insulin administration are strongly affected by mixing and sometimes contamination due to imperfect flushing. Sampling from the other arm can reduce these sources of error and give a more accurate picture of the true concentrations. This approach would not be very practical, as the test subject would have both arms punctured, making the test much more invasive. In a more research based setting, this approach can be feasible. Alternatively, a heating of the cannulated arm can be performed to improve blood flow and mixing [Ferrannini and Mari, 1998]. To reduce contamination, flushing can be improved by methods such as including serum albumin [NovoNordisk, 2002] or similar approaches. Nonetheless, this issue is not a major problem as the algorithm is already tuned to work without these samples if they are affected.

Dosing: The administration of glucose should be limited to ∼10 g or less. When 20 g of glucose was administered, many subjects complained of slight pain or uneasiness. This affect is due to the fact that glucose is diluted in 50 %

saline and 40 ml of this very viscous solution is required. Hence, its bolus injection is not always comfortable. The performance of the test method is not affected by a dose lower than 20 g, as seen in the results of Chapter 8.

Sample Timing: Sample timing accuracy is important to achieve a good model fit. If samples are timed too tightly, it becomes very difficult for clinical personnel to adhere to accurate sample timing. Due to unexpected and unpredictable delays in drawing the samples that were encountered in initial clinical practice, delays of 1-2 minutes can easily be introduced. The recommendation is thus not to time samples less than 5 minutes apart. A 10 minute gap is more practical. To further improve robustness, the timing should be linked to the respective administrations of glucose and insulin. For example, it is easier to obtain a better timed sample if the timer is restarted after the insulin is injected, as opposed to using the glucose bolus at $t = 0$ as a baseline.

Combined Injection of Glucose and Insulin: Glucose and insulin could be administered at the same time, ideally from the same syringe. Insulin is not degraded if diluted with glucose [NovoNordisk, 2002] and the two could potentially be mixed into a single bolus dose. This approach would increase robustness and ease of use. A drawback is that due to the immediate rise in exogenous insulin, no information about the initial first phase β-cell response would be available. In contrast, if this information is not required, the test time could be further reduced by 10 minutes to a minimum of only 20 minutes. How this method compares to the original protocol will have to be validated in pilot testing, but it is an aspect worth pursuing as part of a suite of such tests.

Capillary Glucose Sampling: If one of the fasting sample identified protocols is used, such as Step G5, only one full sample needs to be drawn at fasting state to assay for glucose, insulin and C-peptide. All other glucose samples could potentially be drawn from capillary blood and analysed on the spot with a home glucose monitor [Chase et al., 2006; Johnson and Baker, 1999]. The advantage would be that the fasting sample and the injections can be done with a venous puncture, with no need of a cannula to be inserted for the duration of the test. With only 3 additional glucose samples needed, this approach is potentially very cost efficient, simple and attractive to the subject. Differences in performance caused by capillary blood will have to

be assessed in a separate validation, however the sample errors for such devices are not too large [Johnson and Baker, 1999] to affect the basic models and methods utilised. Again, this approach has significant merits worth piloting clinically.

9.4 Summary

Possible optimisations of the test protocol were systematically analysed for all three assays. The focus was mainly on cost reduction, improved robustness, a simpler protocol and reduced overall test time. Special consideration was also given to limited or special situations, such as where no C-peptide assay is available or financial limitations only allow one sample of insulin and C-peptide.

The sample reduction analyses were carried out in a stepwise manner, first analysing C-peptide, then insulin and finally glucose. This approach is important, as the models build upon each other in that order in the fundamental methods presented. These assays thus affect overall variability in that same order.

Total sample numbers, and thus assay costs, could be halved to only five, without necessarily compromising test performance and thus diagnostic outcome. A further reduction of the much more costly insulin and C-peptide samples was presented, which identifies the insulin kinetic parameters with steady state analysis and only a fasting sample of both peptides. Financially limited scenarios can thus be catered for with a simple and low cost, although slightly less accurate, test.

By eliminating one of the fasting samples and the last two glucose samples, overall test time could be reduced by 25 minutes to only 30 minutes. This approach requires no C-peptide sampling but thus yields only an insulin sensitivity assessment S_I and no additional pancreatic performance measures or diagnosis. The compromise in accuracy is minimal and the correlation between S_I values of the full protocol and this reduced version is $r = 0.96$.

Further observations and suggestions for improvements experienced first hand during the pilot study are also discussed. These suggestions improve the ease of performing the test and overall test robustness. Overall, the results presented

in this chapter greatly improve the clinical practicability and cost of the test, without a great compromise in accuracy. The final result is effectively a suite of tests with known tradeoffs in accuracy, robustness, repeatability, cost and performance measures available.

Chapter 10

Conclusions

Diabetes has reached epidemic proportions worldwide. In particular, both the total number of affected individuals and the level of associated complications is growing for this chronic disease. Thus, the increasing number of major complications, such as polyneuropathy, blindness, kidney failure and limb amputations, are beginning to consume a major and increasing portion of worldwide healthcare costs.

One of the key pathological factors leading to type 2 diabetes is insulin resistance (IR), an impaired ability of the body to make use of available insulin. IR is evident up to 10 years before type 2 diabetes is diagnosed. Diagnosed early enough, there is the opportunity to initiate appropriate treatment and lifestyle interventions to prevent and significantly mitigate the effects of this disease. However, an accurate, yet simple, test to provide such early diagnosis of IR is not yet available for practical use in a clinical setting.

This thesis aimed at developing such a test, that is both accurate and repeatable, yet simple, cost effective and short enough to be accepted in a clinical setting. The test design incorporates physiological modelling and engineering techniques to match clinical requirements and provide outcome metrics that can support the clinician in a more complete diagnosis of metabolic defects associated with IR and pancreatic β-cell function.

Insulin Sensitivity Test and Technological Outcomes

A model-based test allows the estimation of metabolic states and defects with a minimally invasive test protocol. This minimal test is achieved by compensating for the lack of measured data with physiologically accurate models and parameters. A much more accurate and complete assessment of the metabolic system is thus possible, than just analysing the raw data. A short clinical test that was specifically developed by integrating practical clinical aspects and modelling techniques has not yet been presented.

The PK and PD models presented in this thesis were developed specifically to account for the key metabolic dynamics in glucose, insulin and C-peptide in such a short test protocol. Paired with the identification methods presented, physiologically valid parameters can be obtained that enable accurate metabolic assessment. The identification methods combine a-priori knowledge, parallels between C-peptide and insulin, and a novel convex integral-based fitting method to enable unique physiological model identification on limited clinical data.

The models represent simplified descriptions of the much more complex underlying system. When fitting them to clinically obtained data, unmodelled dynamics can affect the fitting process negatively and result in over- or underestimation of key model parameters. By identifying these unmodelled dynamics, the fitting algorithm was optimised to only account for relevant sections of the sampled data, thus improving robustness and repeatability of the overall method.

Model parameters that cannot be identified reliably on these short test data were fixed to mean population values. These assumptions allow observed variability to be captured by only one key parameter, insulin sensitivity S_I. This approach also matches the assumptions of the gold standard euglycaemic clamp test, which attributes all glucose uptake to insulin-dependent effects. The models are thus designed to correlate well to the gold standard test, as variability in insulin uptake is only attributed to insulin. In fact, validating the models on n=146 clamp trials, very good correlations of $r = 0.92$ and $r = 0.99$ were found in transient and steady states, respectively.

The proposed test protocol was specifically designed with practical clinical aspects in mind and was simulated in a Monte Carlo analysis prior to clinical pilot

testing. The Monte Carlo results show expected repeatability accuracies close to the clamp test. Clinical pilot testing confirmed these results. Mean repeatability accuracy was close to that assessed in simulation and showed good resolution in capturing different levels of insulin sensitivity. By including C-peptide sampling to estimate pancreatic insulin secretion, a full picture of basal and first phase β-cell response can be obtained. The fitting algorithm proved robust and fast in fitting all test subjects, without any prior data analysis and intervention.

Finally, optimised test protocols were proposed by systematically re-analysing sample reduced data sets of the pilot test data. Overall test time could be reduced to only 30 minutes and the number of samples reduced to 5 or less, without significantly reducing accuracy in repeatability in all metrics. The result is a suite of test protocols, suitable for a wide range of clinical and research settings, and providing a means of assessing overall metabolic state of insulin sensitivity and β-cell function with just one short and cost efficient test.

This outcome is a substantial contribution to modelling knowledge and clinical engineering, as the modelling work and protocol design were intentionally guided by practical clinical requirements and aspects. The result is thus a very robust test that can be performed by clinical staff without the need of prior data analysis or modelling expertise. A similarly good test performance has not been achieved previously in this field.

Clinical Outcomes

The clinical and diagnostic value of current clinical tests to assess IR is very limited. Their resolution is crude, usually only allowing the use of a threshold value as a cut-off point for the diagnosis. Due to this low resolution, a diagnosis of IR and β-cell dysfunction is only possible once the pathology is well advanced and significant irreversible damage has been occurred. Thus, current diagnostic tests do not offer the opportunity to intervene before significant future complications and costs become inevitable.

The insulin sensitivity test proposed in this thesis has the high resolution and repeatability necessary to detect declining insulin sensitivity and β-cell function. Furthermore, it could be used to monitor these metabolic markers in identified

high risk populations and intervene early to prevent or delay the onset of further complications. Hence, it offers this opportunity to prevent future complications and costs.

The metabolic information that can be obtained from the proposed test is very broad. Besides insulin sensitivity, S_I, the test yields information about various β-cell performance metrics. These metrics are important markers to assess the progression of the disorder. The test thus draws a complete picture of the extent and progression of metabolic disorder.

The intensity of the test is very low compared to similarly accurate tests. Nonetheless, its intensity could be considered high for wider clinical settings in which the lower resolution OGTT is the current test of choice. This slight increase in intensity is the compromise required for the vast increase in accuracy, resolution and metabolic information.

To simplify the test and broaden its field of application, different sample reduction steps were proposed, minimising the clinical intensity without a significant loss in accuracy. Possible alternatives include a shortened version that requires 30 minutes and five samples, or an option that only requires one full venous sample and three additional capillary blood samples over the next 30 minutes.

Overall, a range of possible protocols were presented for different clinical requirements and target settings. All tests measure the same effects and thus yield comparable outcomes, a feature not available in any other test so far. Direct comparisons between clinical and research study results are thus made possible.

Finally, the proposed insulin sensitivity test is repeatable and robust, yet simple, short and cost efficient. The broad metabolic information obtainable from the test can help the clinician to improve the diagnosis and thus improve treatment. High risk populations can be diagnosed much earlier and the onset of complications thus delayed, improving overall healthcare, saving lives and cost.

The contribution of this research outcome to the field of diabetes management and diagnosis is substantial. Diagnostic outcomes previously only available with complicated and intense tests in a research setting can now be made available to routinely diagnose at risk individuals. This greatly improved diagnostic capability can significantly change the way diabetes is currently managed.

Chapter 11

Future Work

The insulin sensitivity test presented in this thesis performs well and is practical enough for use in a clinical setting. Nonetheless, research and validation have to be continued to fully validate the test performance and its comparison against gold standard methods on different subgroups of individuals. Additional optimisation can also be implemented to improve the practical aspects of all the protocols presented and further simplify their eventual use.

11.1 Further Clinical Validation

11.1.1 Test Performance

Clinical validation is important to create confidence and credibility in the test. Additional test validation is also necessary to strengthen the results in repeatability and the effect of dosing obtained from the first pilot study presented in this thesis. Particular important aspects include:

Effect of dosing: This aspect was tested in the pilot study presented in this thesis, but the test and subject numbers were not large enough to obtain a significant result. With a more controlled test environment and a larger number of test subjects, the impact of outliers can be reduced and a more clear result obtained.

Repeatability: This aspect was also tested in the pilot study and showed very good results. Some outliers were still evident, and a validation with larger

subject numbers and possibly more tests on each subject could yield more confidence in the results. More controlled environmental aspects, such as time of day, state of health, previous exercise, or stage of menstrual cycle in female subjects should be observed to minimise variability in insulin sensitivity introduced by a natural cause.

Wider range of insulin sensitivities: To obtain a comparable scale of expected insulin sensitivities, and an idea of at-risk ranges, a large number of subjects from various subgroups and risk populations would need to be tested. With a complete range of expected sensitivities, results obtained in each subject could be classified in comparison to that scale.

11.1.2 Validation Against Gold Standard Tests

Clinical validation against gold standard tests is important to validate the test metrics against established methods. The model has shown good performance when fitted to euglycaemic clamp trial data, but a clinical validation, in which each test is performed on the same individual will provide final confirmation of the comparability of both test metrics. Additional validation should be performed on further research tests, such as the IVGTT, or clinical tests, such as the OGTT.

These comparisons against established methods will create comparability scales of each test metric for better inter-study comparison of results. This ability to easily compare results is important, as many tests effectively measure slightly different effects of insulin sensitivity and yield different direct or surrogate metrics.

11.2 Practical Clinical Improvements

11.2.1 Combined Administration of Glucose and Insulin

A simplification of the test in terms of time, protocol and intensity could be achieved by combining the administration of glucose and insulin into one syringe. This change would require only one injection and thus significantly simplify the

protocol for medical personnel, and also reduce the intensity for the subject. Mixing of insulin with glucose does not affect the stability of insulin [NovoNordisk, 2002], and is, in fact, common practice in some critical care units (Dr. Geoff Shaw, personal communication).

One drawback from this approach is that assessment of the first phase response of the β-cells would not be possible, as the simultaneous increase in both concentrations would inhibit endogenous insulin secretion. Hence, assessment of β-cell function would not be possible. The protocol could still be attractive, if the focus is on only assessing insulin sensitivity, or the reduced test time is important. Validation of this protocol variation would have to be carried out to assess any difference it might introduce to the outcome metric.

11.2.2 Reduce Fasting State Requirement

The fasting state requirement is a common aspect of all insulin sensitivity tests and is necessary to eliminate the impact of any remaining postprandial glucose or insulin appearance in plasma that could alter the results. This requirement is a limiting factor for a more frequent application of the test, as all tests need to be performed in the morning after an overnight fast. Further testing in the afternoon is not possible, unless the subject fasts all day. This aspect could increase the flexibility of a test, as it could be performed during any doctor's visit without significant prior preparation.

With the knowledge of the appearance rate of endogenous glucose and insulin, the fasting state requirement could be eliminated. Insulin secretion by the pancreas is not a problem, as it can be estimated well by sampling C-peptide. Glucose appearance from the gut cannot be measured easily and would have to be estimated. With moderate knowledge about the subject's last meal or snack, the appearance rate can be estimated within a reasonable range. By also knowing the starting glucose value at the beginning of the test, the introduced variability can be limited to the appearance rate.

This approach needs to be assessed in Monte Carlo simulation studies as well as in clinical validation. It could be possible that a partial fasting of 2-4 hours is still required, as by that time the postprandial glucose peak is mostly over and

the glucose appearance from the gut is more stable and can be estimated more robustly. Nonetheless, even a reduction to 2-4 hours of fasting would enable the test to be performed at various times during the day, such as in the morning, before lunch and later in the afternoon. This added flexibility could further enhance the applicability of the test in a wider clinical or screening setting.

11.2.3 Practical Protocol Aspects

The practical aspects of the test, such as the technique to sample blood, administer the injections of glucose and insulin, and the timing of samples need to be very robust to be reliable in a widely used clinical application. Market research of possible blood sampling devices and methods has to be carried out to find the least painful and most robust approach. One variation could be to use venous punctures instead of a cannula. Multiple venous punctures could be more painful for the subject than inserting a cannula once, but would also reduce potential contamination and blood clotting. The sampling routine could also be simplified, as no flushing of the cannula would be required.

The timing of samples and injections, which is critical for the computational portion of the test, needs to be easy to accurately manage by a sole clinician or nurse performing the test. One possible solution would be an electronic timing device, similar to a calculator, with customised buttons to press after each sampling step. A beeping signal could also be implemented to anticipate the next sample. The timing of samples could be stored by the device and eliminate any manual time keeping and logging, thus increasing reliability of this aspect of the test.

11.3 Outlook - Potential Additional Applications

Besides providing a clinical diagnostic test for early diagnosis of IR and β-cell dysfunction, this test offers opportunities for drug development and optimising current therapeutics. Potential applications could be to test the effect of insulin sensitisers [Gerstein et al., 2006; Kahn et al., 2006a], combination therapies, or drugs promoting insulin release [Larsen et al., 2007]. By directly assessing the

metabolic disorders being treated, instead of waiting for surrogate markers, such as fasting glucose, to indicate a significant effect, dosing and drug selection of hypoglycaemic agents could be improved.

Finally, the test developed in this thesis offers great potential for improved diagnosis and treatment of the risk of type 2 diabetes and the disease itself. Validation against currently accepted methods will provide confidence in the test's performance, and further optimisations in practical aspects can improve its widespread clinical use. Additional potential applications of this test, such as optimisation of dosing of medication and other treatments can further improve overall diabetes care.

Appendix A

Current Insulin Sensitivity Tests

This appendix includes three tables summarising aspects of current insulin sensitivity tests described in Chapter 2. The three tables on the following pages are separated into intravenous, oral and fasting tests, respectively.

Table A.1 Intravenous insulin sensitivity tests.

Test	Time	Samples	Input	Calculation	Repeatability	Use	Notes
Clamp	Subject: 180-300 min Staff: 180-300 min	G: 12-24+ I: 4-8+ CP: opt.	I infusion 40-1200 mU/min/m^2 var. G infusion	spreadsheet	CV=6-10 %[1]	research only	+gold standard +very repeatable -supra-physiological -special equipment -trained personnel
IVGTT K_g	Subject: 60 min Staff: 60 min	G: 13 I: 13	∼20 g G bolus	spreadsheet	CV=21 %[2]	mainly research/ limited clinical	+short, simple -inaccurate in IR/ diabetes
IVGTT-MM	Subject: 240 min Staff: 240 min	G: 12-30 I: 12-30 CP: opt.	∼20-30 g G bolus ∼2-4 U I bolus optional tolbutamide	computer software	CV=14-82 %[3]	mainly research/ limited clinical	+repeatability +broad information -many samples -long duration
ITT	Subject: 90 min Staff: 90 min	G: 7	∼8 U I bolus	spreadsheet	CV=7-31 %[4]	mainly research/ limited clinical	+short, simple -high hypo risk
CIGMA	Subject: 90 min Staff: 90 min	G: 5 I: 5 CP: 5	∼0.4 g/min G infusion for 60 min	computer software	CV=17-21 %[5]	mainly research/ limited clinical	+safe +physiological model -inaccurate in IR/ diabetes

[1] [DeFronzo et al., 1979; Mari et al., 2001; Monzillo and Hamdy, 2003]
[2] [Galvin et al., 1992]
[3] [Ferrannini and Mari, 1998; Mari and Valerio, 1997; Monzillo and Hamdy, 2003; Scheen et al., 1994]
[4] [Gelding et al., 1994; Monzillo and Hamdy, 2003]
[5] [Hosker et al., 1985; Nijpels et al., 1994]

Table A.2 Oral insulin sensitivity tests.

Test	Time	Samples	Input	Calculation	Repeatability	Use	Notes
OGTT 2-h	Subject: 120 min Staff: 20 min	G: 1	75 g oral G	-	CV=15-40 %[1] change in status in 30-60 % of cases after repeat test[2]	clinical ADA recommended	+simple, safe +low cost -low repeatability -surrogate metric -lumped effects
OGTT Matsuda Stumvoll Cederholm Gutt, etc.	Subject: 120-180 min Staff: 20-100 min	G: 1-7 I: 1-7	75 g oral G	spreadsheet	CV=7-15 %[3]	clinical/ limited research	+simple, safe +more reliable than OGTT 2-h -low repeatability -inaccurate in IR/ diabetes
OMM	Subject: 240 min Staff: 240 min	G: 20-25 I: 20-25 CP: 20-25 (opt.)	75 g oral G/ or meal	computer software	CV=12-15 %[4]	research/ limited clinical	+physiological admin +broad information +repeatability -long duration -many samples -costly

[1] [Levy et al., 1999; McDonald et al., 1965]
[2] [Ganda et al., 1978; Levy et al., 1999; Riccardi et al., 1985]
[3] [Breda et al., 2001; Mari et al., 2001]
[4] [Breda et al., 2001]

Table A.3 Fasting insulin sensitivity tests.

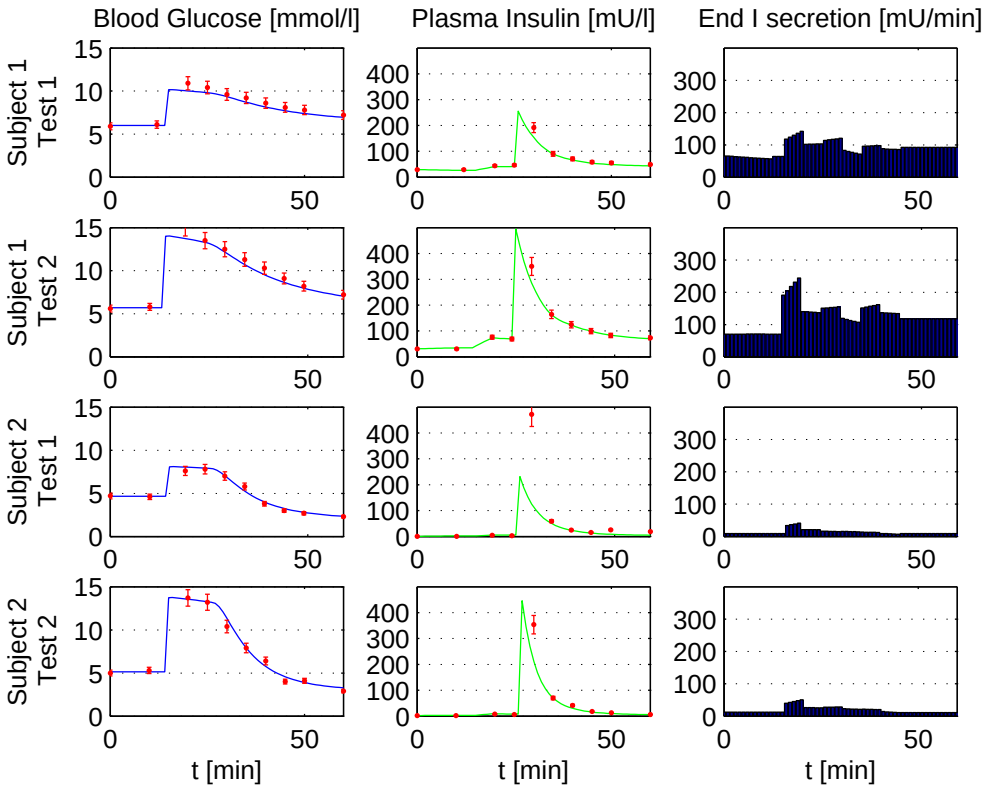
Test	Time	Samples	Input	Calculation	Repeatability	Use	Notes
FPI	Subject: 5 min Staff: 10 min	I: 1	-	-	CV=20 %[1]	clinical	+simple, safe +good indication of IR, even in normoglycaemic -surrogate metric -not feasible in IR/diabetes
FPG	Subject: 5 min Staff: 10 min	G: 1	-	-	-	clinical ADA recommended	+simple, safe +recommended by ADA -diagnosis of IR too late -surrogate metric
HOMA-IR	Subject: 5 min Staff: 10 min	G: 1 I: 1	-	spreadsheet	CV=10-30 %[2]	clinical/ limited research	+simple, safe +includes insulin and glucose +many reference studies -surrogate metric -low resolution/repeatability
HbA_{1C}	Subject: 5 min Staff: 10 min 10 min	HbA_{1C}: 1	-	-	CV=2-4 %[3]	clinical	+not affected by concentration fluctuations +indicative of mean glucose over 2-3 months +no fasting requirement -diagnosis of IR too late -surrogate metric

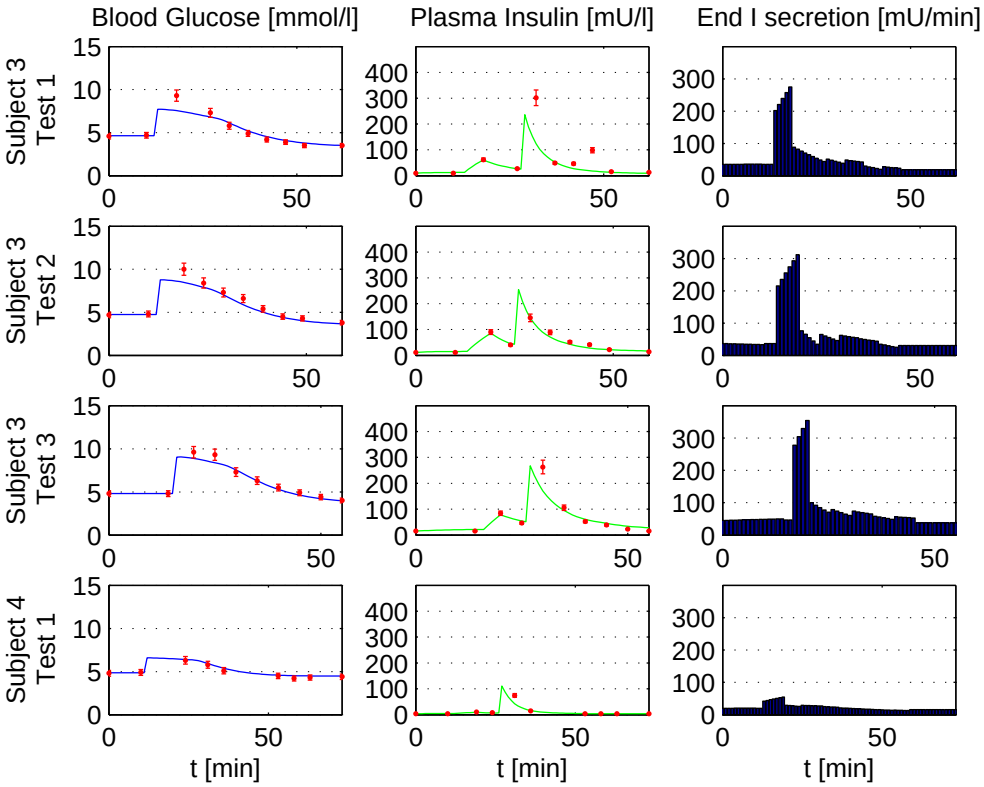
[1] [Mather et al., 2001]
[2] [Bonora et al., 2000; Matthews et al., 1985; Wallace et al., 2004b]
[3] [Barr et al., 2002]

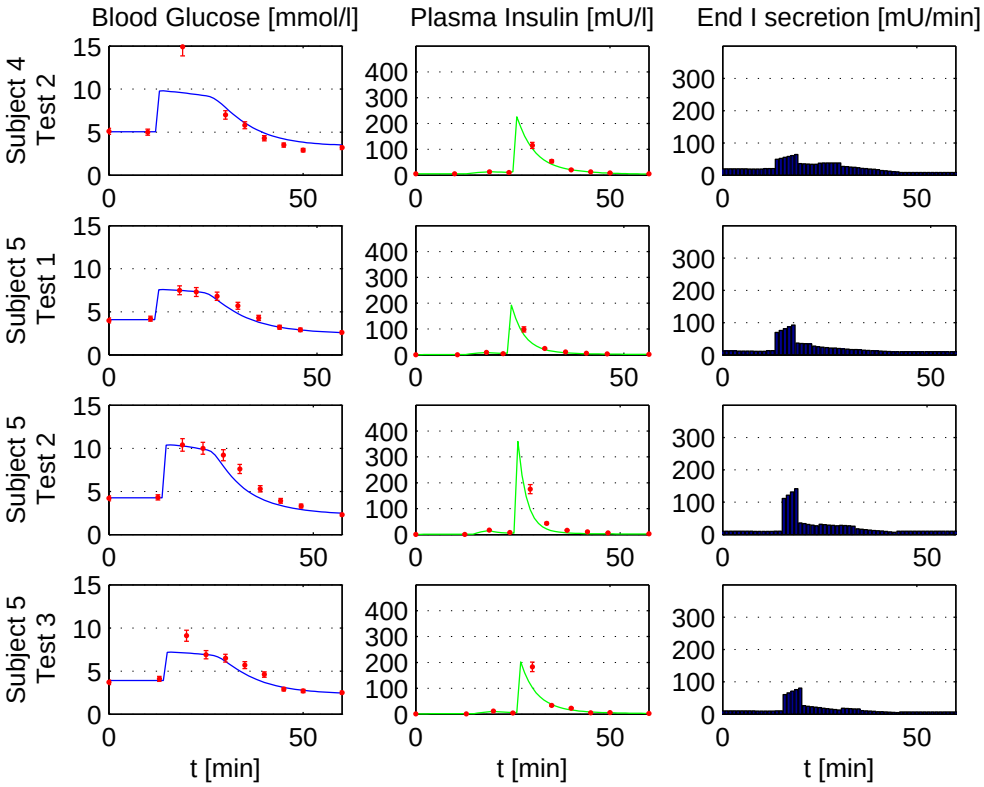
Appendix B

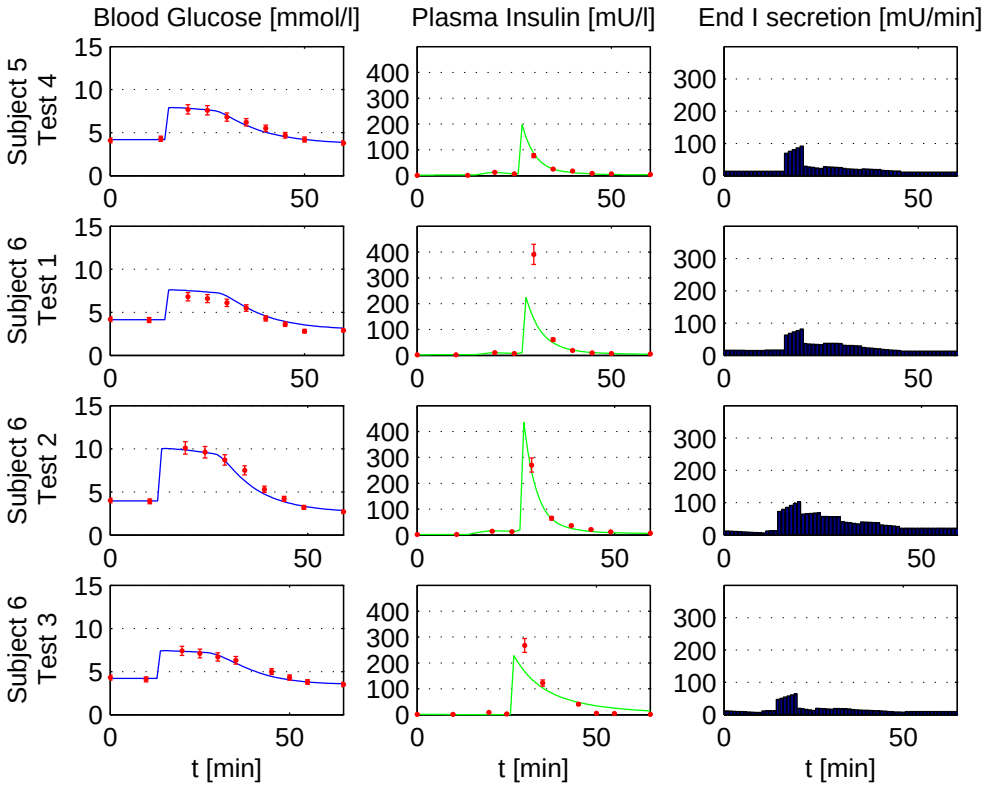
Pilot Test Model Fits

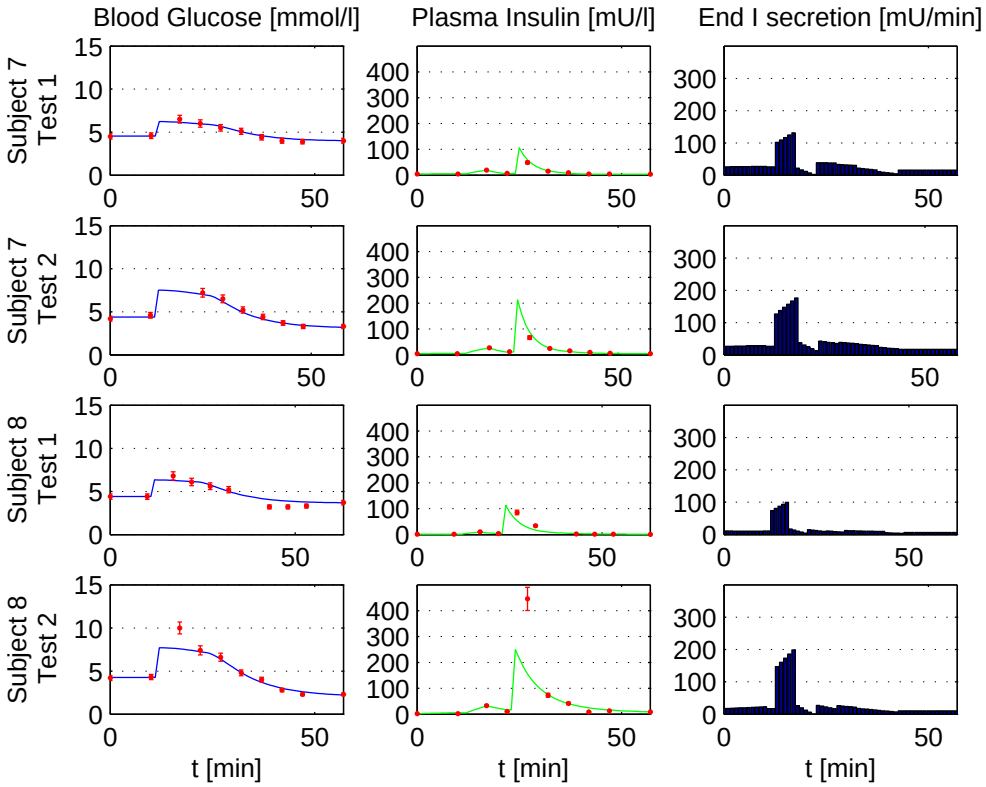
The 43 pilot tests of the proposed insulin sensitivity test are shown here. Shown are glucose and insulin concentrations with the respective model fits, and the estimated endogenous insulin secretion rate, obtained from the C-peptide concentrations.

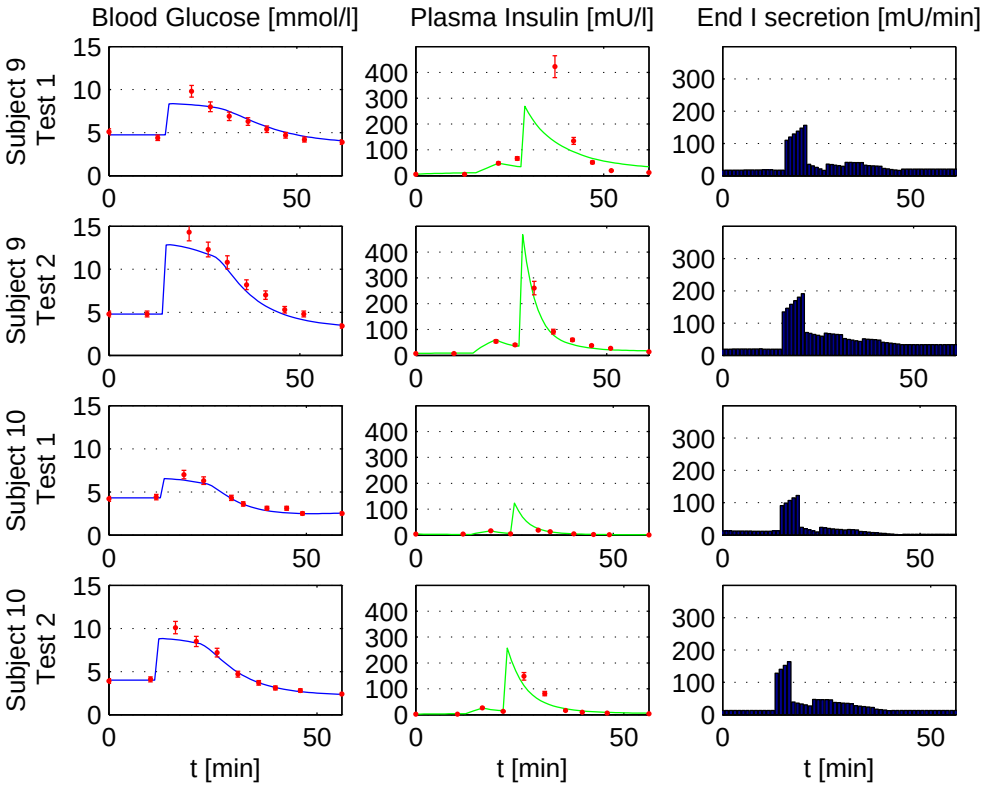


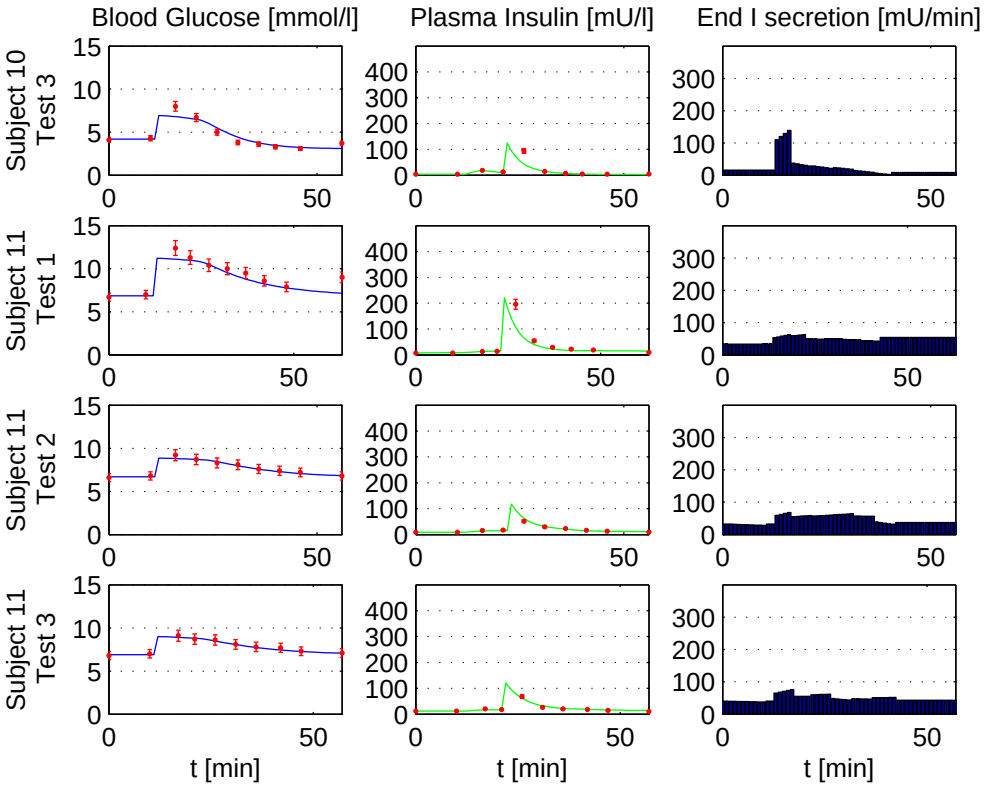

Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 3 Test 1
Subject 3 Test 2
Subject 3 Test 3
Subject 4 Test 1
t [min]

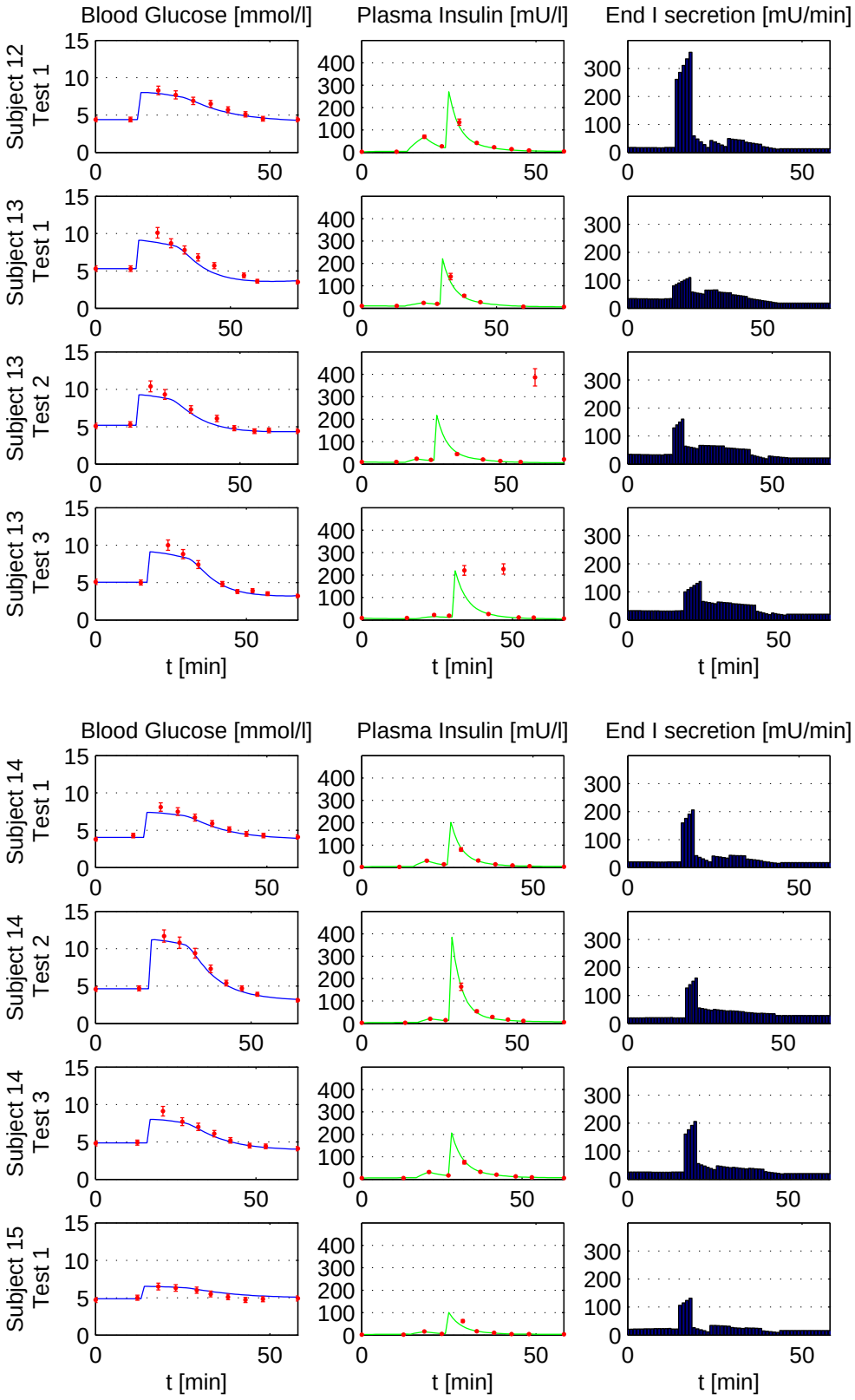

Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 4 Test 2
Subject 5 Test 1
Subject 5 Test 2
Subject 5 Test 3
t [min]

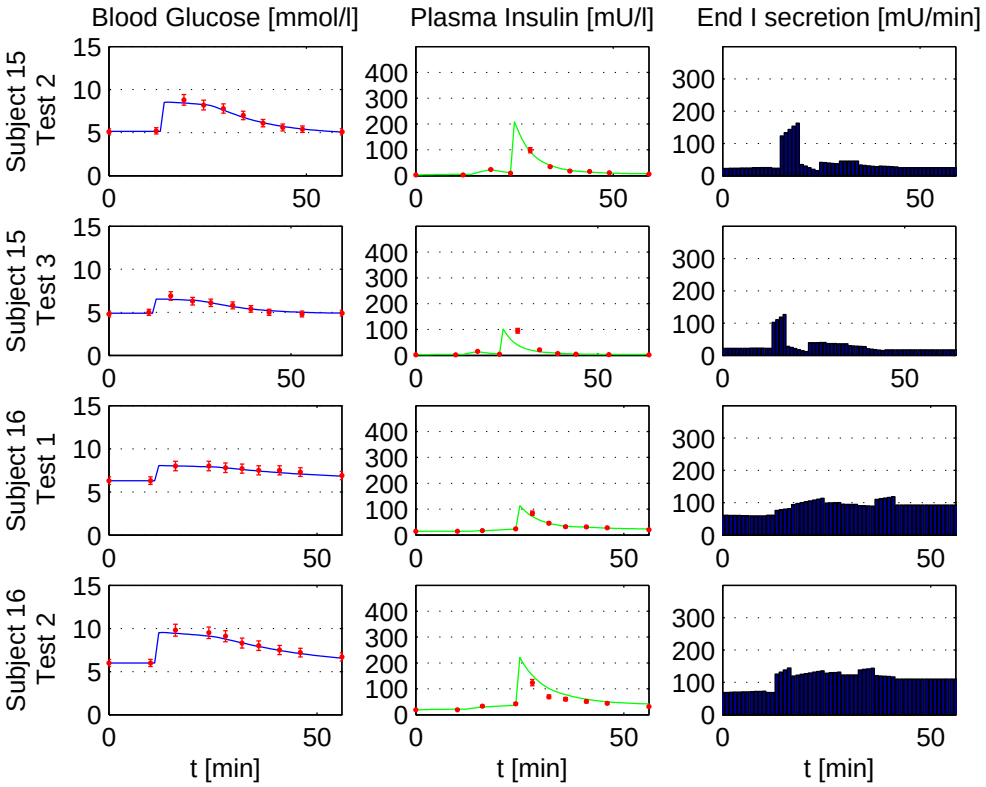

Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 5 Test 4
Subject 6 Test 1
Subject 6 Test 2
Subject 6 Test 3
t [min]

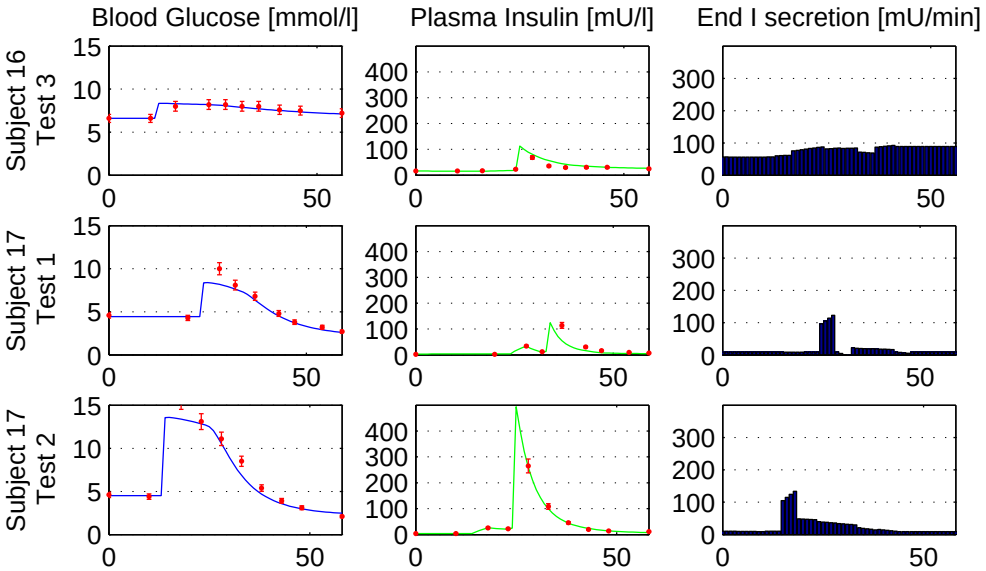

Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 7 Test 1
Subject 7 Test 2
Subject 8 Test 1
Subject 8 Test 2
t [min]


Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 9 Test 1
Subject 9 Test 2
Subject 10 Test 1
Subject 10 Test 2
t [min]


Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 10 Test 3
Subject 11 Test 1
Subject 11 Test 2
Subject 11 Test 3
t [min]


Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 12 Test 1
Subject 13 Test 1
Subject 13 Test 2
Subject 13 Test 3
Subject 14 Test 1
Subject 14 Test 2
Subject 14 Test 3
Subject 15 Test 1
t [min]


Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 15 Test 2
Subject 15 Test 3
Subject 16 Test 1
Subject 16 Test 2
t [min]


Blood Glucose [mmol/l]
Plasma Insulin [mU/l]
End I secretion [mU/min]
Subject 16 Test 3
Subject 17 Test 1
Subject 17 Test 2

References

ADA (1998). Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. *Diabetes Care*, 21(2):310–4.

ADA (2006). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 29(S1):S43–8.

Ader, M. and Bergman, R. N. (1987). Insulin sensitivity in the intact organism. *Baillieres Clin Endocrinol Metab*, 1(4):879–910.

Ader, M., Ni, T. C., and Bergman, R. N. (1997). Glucose effectiveness assessed under dynamic and steady state conditions. Comparability of uptake versus production components. *J Clin Invest*, 99(6):1187–99.

Andres, R., Swerdloff, R., Pozefsky, T., and Coleman, D. (1966). Manual feedback technique for control of blood glucose concentration. *Automation in Analytical Chemistry*, pages 486–91.

Argoud, G. M., Schade, D. S., and Eaton, R. P. (1987). Insulin suppresses its own secretion in vivo. *Diabetes*, 36(8):959–62.

Arleth, T., Andreassen, S., Federici, M. O., and Benedetti, M. M. (2000). A model of the endogenous glucose balance incorporating the characteristics of glucose transporters. *Comput Methods Programs Biomed*, 62(3):219–34.

Avignon, A., Boegner, C., Mariano-Goulart, D., Colette, C., and Monnier, L. (1999). Assessment of insulin sensitivity from plasma insulin and glucose in the fasting or post oral glucose-load state. *Int J Obes Relat Metab Disord*, 23(5):512–7.

Banting, F., Best, C., Collip, J., Campbell, W., and Fletcher, A. (1922). Pancreatic extracts in the treatment of diabetes mellitus. *Canadian Medical Association Journal*, 12:141–46.

Baron, A. D., Brechtel, G., Wallace, P., and Edelman, S. V. (1988). Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol*, 255(6 Pt 1):E769–74.

Barr, R. G., Nathan, D. M., Meigs, J. B., and Singer, D. E. (2002). Tests of glycemia for the diagnosis of type 2 diabetes mellitus. *Ann Intern Med*, 137(4):263–72.

Beard, J. C., Bergman, R. N., Ward, W. K., and Porte, D. J. (1986). The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values. *Diabetes*, 35:362–9.

Belfiore, F., Iannello, S., Camuto, M., Fagone, S., and Cavaleri, A. (2001). Insulin sensitivity of blood glucose versus insulin sensitivity of blood free fatty acids in normal, obese, and obese-diabetic subjects. *Metabolism*, 50(5):573–82.

Belfiore, F., Iannello, S., and Volpicelli, G. (1998). Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose, and FFA levels. *Mol Genet Metab*, 63(2):134–41.

Bennett, C. M., Guo, M., and Dharmage, S. C. (2007). HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review. *Diabet Med*.

Bergman, R. N., Finegood, D. T., and Ader, M. (1985). Assessment of insulin sensitivity in vivo. *Endocr Rev*, 6(1):45–86.

Bergman, R. N., Ider, Y. Z., Bowden, C. R., and Cobelli, C. (1979). Quantitative estimation of insulin sensitivity. *Am J Physiol*, 236(6):E667–77.

Bergman, R. N., Phillips, L. S., and Cobelli, C. (1981). Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest*, 68(6):1456–1467.

Bergman, R. N., Prager, R., Volund, A., and Olefsky, J. M. (1987). Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest*, 79(3):790–800.

Best, J. D., Kahn, S. E., Ader, M., Watanabe, R. M., Ni, T. C., and Bergman, R. N. (1996). Role of glucose effectiveness in the determination of glucose tolerance. *Diabetes Care*, 19(9):1018–30.

Best, J. D., Taborsky, G. J., J., Halter, J. B., and Porte, D., J. (1981). Glucose disposal is not proportional to plasma glucose level in man. *Diabetes*, 30(10):847–50.

Bolli, G. B. (1988). The Dawn Phenomenon - Its Origin and Contribution to Early Morning Hyperglycemia in Diabetes-Mellitus. *Diabetes and Metabolism*, 14(6):675–686.

Bonora, E., Moghetti, P., Zancanaro, C., Cigolini, M., Querena, M., Cacciatori, V., Corgnati, A., and Muggeo, M. (1989). Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. *J Clin Endocrinol Metab*, 68(2):374–8.

Bonora, E., Targher, G., Alberiche, M., Bonadonna, R. C., Saggiani, F., Zenere, M. B., Monauni, T., and Muggeo, M. (2000). Homeostasis Model Assessment Closely Mirrors the Glucose Clamp Technique in the Assessment of Insulin Sensitivity. *Diabetes Care*, 23(1):5763.

Boron, W. F. and Boulpaep, E. L. (2003). *Medical physiology : a cellular and molecular approach*. W.B. Saunders, Philadelphia, PA, 1st edition.

Breda, E., Cavaghan, M. K., Toffolo, G., Polonsky, K. S., and Cobelli, C. (2001). Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. *Diabetes*, 50(1):150–8.

Callegari, T., Caumo, A., and Cobelli, C. (2003). Bayesian two-compartment and classic single-compartment minimal models: comparison on insulin modified IVGTT and effect of experiment reduction. *IEEE Trans Biomed Eng*, 50(12):1301–9.

Camastra, S., Manco, M., Mari, A., Baldi, S., Gastaldelli, A., Greco, A. V., Mingrone, G., and Ferrannini, E. (2005). beta-cell function in morbidly obese subjects during free living: long-term effects of weight loss. *Diabetes*, 54(8):2382–9.

Carson, E. R. and Cobelli, C. (2001). *Modelling methodology for physiology and medicine*. Academic Press Series in Biomedical Engineering. Academic Press, San Diego.

Castillo, C., Bogardus, C., Bergman, R., Thuillez, P., and Lillioja, S. (1994). Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. *J Clin Invest*, 93(1):10–6.

Caumo, A., Bergman, R. N., and Cobelli, C. (2000). Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. *J Clin Endocrinol Metab*, 85(11):4396–402.

Caumo, A. and Cobelli, C. (1993). Hepatic glucose production during the labeled IVGTT: estimation by deconvolution with a new minimal model. *Am J Physiol*, 264(5 Pt 1):E829–41.

Caumo, A., Vicini, P., and Cobelli, C. (1996). Is the minimal model too minimal? *Diabetologia*, 39(8):997–1000.

Caumo, A., Vicini, P., Zachwieja, J. J., Avogaro, A., Yarasheski, K., Bier, D. M., and Cobelli, C. (1999). Undermodeling affects minimal model indexes: insights from a two-compartment model. *Am J Physiol*, 276(6 Pt 1):E1171–1193.

Cederholm, J. and Wibell, L. (1990). Insulin release and peripheral sensitivity at the oral glucose tolerance test. *Diabetes Res Clin Pract*, 10(2):167–75.

Chase, J., Shaw, G. M., Wong, X. W., Lotz, T., Lin, J., and Hann, C. E. (2006). Model-based Glycaemic Control in Critical Care - A review of the state of the possible. *Biomedical Signal Processing and Control*, 1(1):3–21.

Chase, J. G., Shaw, G. M., Lin, J., Doran, C. V., Bloomfield, M., Wake, G. C., Broughton, B., Hann, C., and Lotz, T. (2004). Impact of Insulin-Stimulated Glucose Removal Saturation on Dynamic Modelling and Control of Hyperglycaemia. *International Journal of Intelligent Systems Technologies and Applications (IJISTA)*, 1(1/2):79–94.

Chase, J. G., Shaw, G. M., Lin, J., Doran, C. V., Hann, C., Lotz, T., Wake, G. C., and Broughton, B. (2005a). Targeted glycemic reduction in critical care using closed-loop control. *Diabetes Technol Ther*, 7(2):274–82.

Chase, J. G., Shaw, G. M., Lin, J., Doran, C. V., Hann, C., Robertson, M. B., Browne, P. M., Lotz, T., Wake, G. C., and Broughton, B. (2005b). Adaptive bolus-based targeted glucose regulation of hyperglycaemia in critical care. *Med Eng Phys*, 27(1):1–11.

Chee, F., Fernando, T. L., Savkin, A. V., and van Heeden, V. (2003). Expert PID control system for blood glucose control in critically ill patients. *IEEE Trans Inf Technol Biomed*, 7(4):419–25.

Chevenne, D., Trivin, F., and Porquet, D. (1999). Insulin assays and reference values. *Diabetes Metab*, 25(6):459–76.

Chiu, K. C., Chuang, L. M., and Yoon, C. (2001). Comparison of measured and estimated indices of insulin sensitivity and beta cell function: impact of ethnicity on insulin sensitivity and beta cell function in glucose-tolerant and normotensive subjects. *J Clin Endocrinol Metab*, 86(4):1620–5.

Clark, P. M. (1999). Assays for insulin, proinsulin(s) and C-peptide. *Ann Clin Biochem*, 36 (Pt 5):541–64.

Coates, P. A., Luzio, S. D., Brunel, P., and Owens, D. R. (1995). Comparison of estimates of insulin sensitivity from minimal model analysis of the insulin-modified frequently sampled intravenous glucose tolerance test and the isoglycemic hyperinsulinemic clamp in subjects with NIDDM. *Diabetes*, 44(6):631–5.

Cobelli, C., Bettini, F., Caumo, A., and Quon, M. J. (1998). Overestimation of minimal model glucose effectiveness in presence of insulin response is due to undermodeling. *Am J Physiol*, 275(6 Pt 1):E1031–1036.

Cobelli, C., Caumo, A., and Omenetto, M. (1999). Minimal model SG overestimation and SI underestimation: improved accuracy by a Bayesian two-compartment model. *Am J Physiol*, 277(3 Pt 1):E481–488.

Cobelli, C., Pacini, G., Toffolo, G., and Sacca, L. (1986). Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT. *Am J Physiol*, 250(5 Pt 1):E591–8.

Cobelli, C., Toffolo, G., and Ferrannini, E. (1984). A model of glucose kinetics and their control by insulin, compartmental and noncompartmental approaches. *Mathematical Biosciences*, 72(2):291–315.

Conn, P. M. and Goodman, H. M. (1998). *The endocrine system*. Published for the American Physiological Society by Oxford University Press, New York ; Oxford.

Dalla Man, C., Caumo, A., Basu, R., Rizza, R., Toffolo, G., and Cobelli, C. (2004). Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. *American Journal of Physiology-Endocrinology and Metabolism*, 287(4):E637–E643.

Dalla Man, C., Caumo, A., Basu, R., Rizza, R., Toffolo, G., and Cobelli, C. (2005a). Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. *Am J Physiol Endocrinol Metab*, 289(5):E909–14.

Dalla Man, C., Caumo, A., and Cobelli, C. (2002). The oral glucose minimal model: Estimation of insulin sensitivity from a meal test. *Ieee Transactions on Biomedical Engineering*, 49(5):419–29.

Dalla Man, C., Yarasheski, K. E., Caumo, A., Robertson, H., Toffolo, G., Polonsky, K. S., and Cobelli, C. (2005b). Insulin sensitivity by oral glucose minimal models: validation against clamp. *Am J Physiol Endocrinol Metab*, 289(6):E954–9.

Dandona, P., Chaudhuri, A., Ghanim, H., and Mohanty, P. (2006). Anti-inflammatory effects of insulin and pro-inflammatory effects of glucose: relevance to the management of acute myocardial infarction and other acute coronary syndromes. *Rev Cardiovasc Med*, 7 Suppl 2:S25–34.

Davies, M. J., Rayman, G., Grenfell, A., Gray, I. P., Day, J. L., and Hales, C. N. (1994). Loss of the first phase insulin response to intravenous glucose in subjects with persistent impaired glucose tolerance. *Diabet Med*, 11(5):432–6.

DeFronzo, R. A., Tobin, J. D., and Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*, 237(3):E214–23.

Del Prato, S., Marchetti, P., and Bonadonna, R. C. (2002). Phasic insulin release and metabolic regulation in type 2 diabetes. *Diabetes*, 51 Suppl 1:S109–116.

Del Prato, S., Matsuda, M., Simonson, D. C., Groop, L. C., Sheehan, P., Leonetti, F., Bonadonna, R. C., and DeFronzo, R. A. (1997). Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. *Diabetologia*, 40(6):687–97.

Despopoulos, A. and Silbernagl, S. (2003). *Color atlas of physiology*. Thieme flexibook. G. Thieme Thieme Medical Publishers, Stuttgart New York, 5th edition.

Dobson, M. (1776). Nature of the urine in diabetes. *Med Obs Inqu*, 5:298–310.

Donner, C. C., Fraze, E., Chen, Y. D., Hollenbeck, C. B., Foley, J. E., and Reaven, G. M. (1985). Presentation of a new method for specific measurement of in vivo insulin-stimulated glucose disposal in humans: comparison of this approach with the insulin clamp and minimal model techniques. *J Clin Endocrinol Metab*, 60(4):723–6.

Droumaguet, C., Balkau, B., Simon, D., Caces, E., Tichet, J., Charles, M. A., and Eschwege, E. (2006). Use of HbA1c in predicting progression to diabetes in French men and women: data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care*, 29(7):1619–25.

Duckworth, W. C., Bennett, R. G., and Hamel, F. G. (1998). Insulin degradation: progress and potential. *Endocr Rev*, 19(5):608–24.

Duckworth, W. C., Hamel, F. G., and Peavy, D. E. (1988). Hepatic metabolism of insulin. *Am J Med*, 85(5A):71–6.

Duncan, G. E., Perri, M. G., Theriaque, D. W., Hutson, A. D., Eckel, R. H., and Stacpoole, P. W. (2003). Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. *Diabetes Care*, 26(3):557–562.

Eaton, R. P., Allen, R. C., Schade, D. S., Erickson, K. M., and Standefer, J. (1980). Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J Clin Endocrinol Metab*, 51(3):520–8.

Eaton, R. P., Friedman, N., Allen, R. C., and Schade, D. S. (1984). Insulin removal in man: in vivo evidence for a receptor-mediated process. *J Clin Endocrinol Metab*, 58(3):555–9.

Efron, B. and Tibshirani, R. (1993). *An introduction to the bootstrap*. Monographs on statistics and applied probability ; 57. Chapman and Hall, New York.

Ellemann, K., Thorsteinsson, B., Fugleberg, S., Feldt-Rasmussen, B., Andersen, O. O., Gronbaek, P., and Binder, C. (1987). Kinetics of insulin disappearance from plasma in cortisone-treated normal subjects. *Clin Endocrinol (Oxf)*, 26(5):623–628.

Erichsen, L., Agbaje, O. F., Luzio, S. D., Owens, D. R., and Hovorka, R. (2004). Population and individual minimal modeling of the frequently sampled insulin-modified intravenous glucose tolerance test. *Metabolism*, 53(10):1349–54.

Faber, O. K., Hagen, C., Binder, C., Markussen, J., Naithani, V. K., Blix, P. M., Kuzuya, H., Horwitz, D. L., Rubenstein, A. H., and Rossing, N. (1978). Kinetics of human connecting peptide in normal and diabetic subjects. *J Clin Invest*, 62(1):197–203.

Ferrannini, E. (1997). Insulin resistance is central to the burden of diabetes. *Diabetes Metab Rev*, 13(2):81–6.

Ferrannini, E. and Cobelli, C. (1987a). The kinetics of insulin in man. I. General aspects. *Diabetes Metab Rev*, 3(2):335–63.

Ferrannini, E. and Cobelli, C. (1987b). The kinetics of insulin in man. II. Role of the liver. *Diabetes Metab Rev*, 3(2):365–97.

Ferrannini, E., Gastaldelli, A., Miyazaki, Y., Matsuda, M., Mari, A., and DeFronzo, R. A. (2005). beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab*, 90(1):493–500.

Ferrannini, E. and Mari, A. (1998). How to measure insulin sensitivity. *J Hypertens*, 16(7):895–906.

Ferrannini, E. and Mari, A. (2004). Beta cell function and its relation to insulin action in humans: a critical appraisal. *Diabetologia*, 47(5):943–56.

Ferrannini, E., Natali, A., Bell, P., Cavallo-Perin, P., Lalic, N., and Mingrone, G. (1997). Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J Clin Invest*, 100(5):1166–73.

Ferrannini, E., Wahren, J., Faber, O. K., Felig, P., Binder, C., and DeFronzo, R. A. (1983). Splanchnic and renal metabolism of insulin in human subjects: a dose-response study. *Am J Physiol*, 244(6):E517–27.

Finegood, D. T., Hramiak, I. M., and Dupre, J. (1990). A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. *J Clin Endocrinol Metab*, 70(6):1538–49.

Finegood, D. T., Pacini, G., and Bergman, R. N. (1984). The insulin sensitivity index. Correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp. *Diabetes*, 33(4):362–8.

Finegood, D. T. and Tzur, D. (1996). Reduced glucose effectiveness associated with reduced insulin release: an artifact of the minimal-model method. *Am J Physiol*, 271(3 Pt 1):E485–95.

Foley, J. E., Chen, Y. D., Lardinois, C. K., Hollenbeck, C. B., Liu, G. C., and Reaven, G. M. (1985). Estimates of in vivo insulin action in humans: comparison of the insulin clamp and the minimal model techniques. *Horm Metab Res*, 17(8):406–9.

Frost, D. P., Srivastava, M. C., Jones, R. H., Nabarro, J. D., and Sonksen, P. H. (1973). The kinetics of insulin metabolism in diabetes mellitus. *Postgrad Med J*, 49:Suppl 7:949–54.

Galvin, P., Ward, G., Walters, J., Pestell, R., Koschmann, M., Vaag, A., Martin, I., Best, J. D., and Alford, F. (1992). A simple method for quantitation of insulin sensitivity and insulin release from an intravenous glucose tolerance test. *Diabet Med*, 9(10):921–8.

Ganda, O. P., Day, J. L., Soeldner, J. S., Connon, J. J., and Gleason, R. E. (1978). Reproducibility and comparative analysis of repeated intravenous and oral glucose tolerance tests. *Diabetes*, 27(7):715–25.

Gastaldelli, A., Ferrannini, E., Miyazaki, Y., Matsuda, M., and DeFronzo, R. A. (2004). Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. *Diabetologia*, 47(1):31–9.

Gelding, S. V., Robinson, S., Lowe, S., Niththyananthan, R., and Johnston, D. G. (1994). Validation of the low dose short insulin tolerance test for evaluation of insulin sensitivity. *Clin Endocrinol (Oxf)*, 40(5):611–5.

Gerstein, H. C., Yusuf, S., Bosch, J., Pogue, J., Sheridan, P., Dinccag, N., Hanefeld, M., Hoogwerf, B., Laakso, M., Mohan, V., Shaw, J., Zinman, B., and Holman, R. R. (2006). Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet*, 368(9541):1096–105.

Godsland, I. F., Felton, C., and Wynn, V. (1993). Venous blood arterialization and parameters from the minimal models of glucose disappearance and post-hepatic insulin delivery. *Clin Sci (Lond)*, 85(1):89–95.

Greville, G. D. (1943). The intravenous glucose tolerance equation. *Biochem J*, 37(1):17–24.

Gudbjornsdottir, S., Sjostrand, M., Strindberg, L., Wahren, J., and Lonnroth, P. (2003). Direct measurements of the permeability surface area for insulin and glucose in human skeletal muscle. *J Clin Endocrinol Metab*, 88(10):4559–64.

Gutt, M., Davis, C. L., Spitzer, S. B., Llabre, M. M., Kumar, M., Czarnecki, E. M., Schneiderman, N., Skyler, J. S., and Marks, J. B. (2000). Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract*, 47(3):177–84.

Guyton, A. C. and Hall, J. E. (2000). *Textbook of medical physiology*. Saunders, Philadelphia ; London, 10th edition.

Hanley, A. J., Williams, K., Gonzalez, C., D'Agostino, R. B., J., Wagenknecht, L. E., Stern, M. P., and Haffner, S. M. (2003). Prediction of type 2 diabetes using simple measures of insulin resistance: combined results from the San Antonio Heart Study, the Mexico City Diabetes Study, and the Insulin Resistance Atherosclerosis Study. *Diabetes*, 52(2):463–469.

Hann, C., Chase, J., Andreassen, S., Smith, B., and Shaw, G. (2005a). Diagnosis Using a Minimal Cardiac Model Including Reflex Actions. *Intensive Care Med*, 31(S1):S18.

Hann, C., Chase, J., and Shaw, G. (2006). Integral-based Identification of Patient Specific Parameters for a Minimal Cardiac Model. *Computer Methods and Programs in Biomedicine*, 81(2):181–192.

Hann, C. E., Chase, J. G., Lin, J., Lotz, T., Doran, C. V., and Shaw, G. M. (2005b). Integral-based parameter identification for long-term dynamic verification of a glucose-insulin system model. *Comput Methods Programs Biomed*, 77(3):259–270.

Hansen, T. K., Thiel, S., Wouters, P. J., Christiansen, J. S., and Van den Berghe, G. (2003). Intensive insulin therapy exerts antiinflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. *The Journal of clinical endocrinology and metabolism*, 88(3):1082–8.

Hanson, R. L., Pratley, R. E., Bogardus, C., Narayan, K. M., Roumain, J. M., Imperatore, G., Fagot-Campagna, A., Pettitt, D. J., Bennett, P. H., and Knowler,

W. C. (2000). Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol*, 151(2):190–198.

Herder, C., Peltonen, M., Koenig, W., Kraft, I., Muller-Scholze, S., Martin, S., Lakka, T., Ilanne-Parikka, P., Eriksson, J. G., Hamalainen, H., Keinanen-Kiukaanniemi, S., Valle, T. T., Uusitupa, M., Lindstrom, J., Kolb, H., and Tuomilehto, J. (2006). Systemic immune mediators and lifestyle changes in the prevention of type 2 diabetes: results from the Finnish Diabetes Prevention Study. *Diabetes*, 55(8):2340–6.

Himsworth, H. P. (1936). Diabetes Mellitus : Its Differentiation into Insulin-sensitive and Insulin-insensitive types. *The Lancet*, 227(5864):127–130.

Hogan, P., Dall, T., and Nikolov, P. (2003). Economic costs of diabetes in the US in 2002. *Diabetes Care*, 26(3):917–32.

Hollenbeck, C. and Reaven, G. M. (1987). Variations in insulin-stimulated glucose uptake in healthy individuals with normal glucose tolerance. *J Clin Endocrinol Metab*, 64(6):1169–73.

Horgaard, A. and Thayssen, T. (1929). Clinical investigation into the effect of the intravenous injection of insulin. *Acta Med Scand*, 72:92–5.

Hosker, J. P., Matthews, D. R., Rudenski, A. S., Burnett, M. A., Darling, P., Bown, E. G., and Turner, R. C. (1985). Continuous infusion of glucose with model assessment: measurement of insulin resistance and beta-cell function in man. *Diabetologia*, 28(7):401–11.

Hossain, P., Kawar, B., and El Nahas, M. (2007). Obesity and diabetes in the developing world–a growing challenge. *N Engl J Med*, 356(3):213–5.

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, 444(7121):860–7.

Hovorka, R., Koukkou, E., Southerden, D., Powrie, J. K., and Young, M. A. (1998). Measuring pre-hepatic insulin secretion using a population model of C-peptide kinetics: accuracy and required sampling schedule. *Diabetologia*, 41(5):548–54.

Hovorka, R., Powrie, J. K., Smith, G. D., Sonksen, P. H., Carson, E. R., and Jones, R. H. (1993). Five-compartment model of insulin kinetics and its use to

investigate action of chloroquine in NIDDM. *Am J Physiol*, 265(1 Pt 1):E162–75.

Hovorka, R., Shojaee-Moradie, F., Carroll, P. V., Chassin, L. J., Gowrie, I. J., Jackson, N. C., Tudor, R. S., Umpleby, A. M., and Jones, R. H. (2002). Partitioning glucose distribution/transport, disposal, and endogenous production during IVGTT. *Am J Physiol Endocrinol Metab*, 282(5):E992–1007.

Hovorka, R., Soons, P. A., and Young, M. A. (1996). ISEC: a program to calculate insulin secretion. *Comput Methods Programs Biomed*, 50(3):253–64.

Inchiostro, S. (2005). Measurement of insulin sensitivity in Type 2 diabetes mellitus: comparison between K and HOMA-with the components of the insulin resistance syndrome. *Diabet Med*, 22(1):39–44.

Insel, P. A., Kramer, K. J., Sherwin, R. S., Liljenquist, J. E., Tobin, J. D., Andres, R., and Berman, M. (1974). Modeling the insulin-glucose system in man. *Fed Proc*, 33(7):1865–8.

Jacquez, J. A. (1992). Theory of production rate calculations in steady and nonsteady states and its application to glucose metabolism. *Am J Physiol*, 262(6 Pt 1):E779–90.

Jefferson, L. S. and Cherrington, A. (2001). *The endocrine pancreas and regulation of metabolism*, volume 2 of *Handbook of physiology - The endocrine system*. Oxford University Press, Oxford.

Johnson, R. N. and Baker, J. R. (1999). Analytical error of home glucose monitors: a comparison of 18 systems. *Ann Clin Biochem*, 36(Pt 1):72–9.

Jones, C. N., Pei, D., Staris, P., Polonsky, K. S., Chen, Y. D., and Reaven, G. M. (1997). Alterations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant individuals. *J Clin Endocrinol Metab*, 82(6):1834–8.

Jones, R. H., Sonksen, P. H., Boroujerdi, M. A., and Carson, E. R. (1984). Number and affinity of insulin receptors in intact human subjects. *Diabetologia*, 27(2):207–11.

Kahn, S. E., Haffner, S. M., Heise, M. A., Herman, W. H., Holman, R. R., Jones, N. P., Kravitz, B. G., Lachin, J. M., O'Neill, M. C., Zinman, B., and Vib-

erti, G. (2006a). Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med*, 355(23):2427–43.

Kahn, S. E., Hull, R. L., and Utzschneider, K. M. (2006b). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, 444(7121):840–6.

Kanauchi, M. (2002). A new index of insulin sensitivity obtained from the oral glucose tolerance test applicable to advanced type 2 diabetes. *Diabetes Care*, 25(10):1891–2.

Kang, E. S., Yun, Y. S., Park, S. W., Kim, H. J., Ahn, C. W., Song, Y. D., Cha, B. S., Lim, S. K., Kim, K. R., and Lee, H. C. (2005). Limitation of the validity of the homeostasis model assessment as an index of insulin resistance in Korea. *Metabolism*, 54(2):206–11.

Katz, A., Nambi, S. S., Mather, K., Baron, A. D., Follmann, D. A., Sullivan, G., and Quon, M. J. (2000). Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*, 85(7):2402–10.

Kirwan, J. P., Huston-Presley, L., Kalhan, S. C., and Catalano, P. M. (2001). Clinically useful estimates of insulin sensitivity during pregnancy: validation studies in women with normal glucose tolerance and gestational diabetes mellitus. *Diabetes Care*, 24(9):1602–7.

Kleinfield, N. R. (2006). Diabetes and Its Awful Toll Quietly Emerge as a Crisis.

Ko, G. T., Chan, J. C., Woo, J., Lau, E., Yeung, V. T., Chow, C. C., and Cockram, C. S. (1998). The reproducibility and usefulness of the oral glucose tolerance test in screening for diabetes and other cardiovascular risk factors. *Ann Clin Biochem*, 35 (Pt 1):62–7.

Krudys, K. M., Kahn, S. E., and Vicini, P. (2006). Population approaches to estimate minimal model indexes of insulin sensitivity and glucose effectiveness using full and reduced sampling schedules. *Am J Physiol Endocrinol Metab*, 291(4):E716–23.

Larsen, C. M., Faulenbach, M., Vaag, A., Volund, A., Ehses, J. A., Seifert, B., Mandrup-Poulsen, T., and Donath, M. Y. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med*, 356(15):1517–26.

Lehmann, E. D. and Deutsch, T. (1992). Insulin dosage adjustment in diabetes. *J Biomech Eng*, 14(3):243–249.

Levy, J., Morris, R., Hammersley, M., and Turner, R. (1999). Discrimination, adjusted correlation, and equivalence of imprecise tests: application to glucose tolerance. *Am J Physiol*, 276(2 Pt 1):E365–75.

Levy, J. C., Matthews, D. R., and Hermans, M. P. (1998). Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*, 21(12):2191–2.

Lewanczuk, R. Z., Paty, B. W., and Toth, E. L. (2004). Comparison of the [13C]glucose breath test to the hyperinsulinemic-euglycemic clamp when determining insulin resistance. *Diabetes Care*, 27(2):441–7.

Li, C., Ford, E. S., McGuire, L. C., Mokdad, A. H., Little, R. R., and Reaven, G. M. (2006). Trends in hyperinsulinemia among nondiabetic adults in the U.S. *Diabetes Care*, 29(11):2396–402.

Limpert, E., Stahel, W. A., and Abbt, M. (2001). Log-normal distributions across the sciences: Keys and clues. *Bioscience*, 51(5):341–352.

Lindheim, S. R., Buchanan, T. A., Duffy, D. M., Vijod, M. A., Kojima, T., Stanczyk, F. Z., and Lobo, R. A. (1994). Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. *J Soc Gynecol Investig*, 1(2):150–4.

Lotz, T., Chase, J. G., Lin, J., Wong, X. W., Hann, C. E., McAuley, K. A., and Andreassen, S. (2006a). Integral-Based Identification of a Physiological Insulin and Glucose Model on Euglycaemic Clamp Trials. In *14th IFAC Symposium on System Identification (SYSID 2006)*, pages 463–8, Newcastle, Australia. IFAC.

Lotz, T. F., Chase, J. G., Andreassen, S., Hann, C. E., Lin, J., Wong, J., and McAuley, K. A. (2005a). A fully identifiable physiological model of insulin kinetics for clinical applications. In *12th International Conf on Biomedical Engineering (ICBME)*, pages 4–pages, Singapore.

Lotz, T. F., Chase, J. G., McAuley, K. A., Lee, D. S., Lin, J., Hann, C. E., and Mann, J. I. (2006b). Transient and steady-state euglycemic clamp validation of a model for glycemic control and insulin sensitivity testing. *Diabetes Technol Ther*, 8(3):338–46.

Lotz, T. F., Chase, J. G., McAuley, K. A., Shaw, G. M., Hann, C. E., and Mann, J. I. (2005b). A highly correlated method to assess insulin resistance in broad populations. In *12th International Conf on Biomedical Engineering (ICBME)*, Singapore.

Lozner, E. L., Winkler, A. W., Taylor, F. H., and Peters, J. P. (1941). The Intravenous Glucose Tolerance Test. *J Clin Invest*, 20(5):507–15.

Mari, A. (1997). Assessment of insulin sensitivity with minimal model: role of model assumptions. *Am J Physiol*, 272(5 Pt 1):E925–34.

Mari, A. (1998). Assessment of insulin sensitivity and secretion with the labelled intravenous glucose tolerance test: improved modelling analysis. *Diabetologia*, 41(9):1029–39.

Mari, A., Pacini, G., Brazzale, A. R., and Ahren, B. (2005). Comparative evaluation of simple insulin sensitivity methods based on the oral glucose tolerance test. *Diabetologia*, 48(4):748–51.

Mari, A., Pacini, G., Murphy, E., Ludvik, B., and Nolan, J. J. (2001). A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care*, 24(3):539–548.

Mari, A. and Valerio, A. (1997). A Circulatory Model for the Estimation of Insulin Sensitivity. *Control Eng Practice*, 5(12):1747–1752.

Martin, B. C., Warram, J. H., Krolewski, A. S., Bergman, R. N., Soeldner, J. S., and Kahn, C. R. (1992). Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet*, 340(8825):925–9.

Mather, K. J., Hunt, A. E., Steinberg, H. O., Paradisi, G., Hook, G., Katz, A., Quon, M. J., and Baron, A. D. (2001). Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab*, 86(11):5457–5464.

Matsuda, M. and DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*, 22(9):1462–70.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. (1985). Homeostasis model assessment: insulin resistance

and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28(7):412–9.

McAuley, K. A., Williams, S. M., Mann, J. I., Goulding, A., Chisholm, A., Wilson, N., Story, G., McLay, R. T., Harper, M. J., and Jones, I. E. (2002). Intensive lifestyle changes are necessary to improve insulin sensitivity: a randomized controlled trial. *Diabetes Care*, 25(3):445–52.

McAuley, K. A., Williams, S. M., Mann, J. I., Walker, R. J., Lewis-Barned, N. J., Temple, L. A., and Duncan, A. W. (2001). Diagnosing insulin resistance in the general population. *Diabetes Care*, 24(3):460–4.

McDonald, G. W., Fisher, G. F., and Burnham, C. (1965). Reproducibility of the Oral Glucose Tolerance Test. *Diabetes*, 14:473–80.

McGuire, E. A., Tobin, J. D., Berman, M., and Andres, R. (1979). Kinetics of native insulin in diabetic, obese, and aged men. *Diabetes*, 28(2):110–20.

McLaughlin, T., Abbasi, F., Lamendola, C., and Reaven, G. (2007). Heterogeneity in the prevalence of risk factors for cardiovascular disease and type 2 diabetes mellitus in obese individuals: effect of differences in insulin sensitivity. *Arch Intern Med*, 167(7):642–8.

Meier, J. J., Veldhuis, J. D., and Butler, P. C. (2005). Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. *Diabetes*, 54(6):1649–56.

Miles, P. D., Levisetti, M., Reichart, D., Khoursheed, M., Moossa, A. R., and Olefsky, J. M. (1995). Kinetics of insulin action in vivo. Identification of rate-limiting steps. *Diabetes*, 44(8):947–53.

Monzillo, L. U. and Hamdy, O. (2003). Evaluation of insulin sensitivity in clinical practice and in research settings. *Nutr Rev*, 61(12):397–412.

Nagasaka, S., Tokuyama, K., Kusaka, I., Hayashi, H., Rokkaku, K., Nakamura, T., Kawakami, A., Higashiyama, M., Ishikawa, S., and Saito, T. (1999). Endogenous glucose production and glucose effectiveness in type 2 diabetic subjects derived from stable-labeled minimal model approach. *Diabetes*, 48(5):1054–60.

Natali, A., Gastaldelli, A., Camastra, S., Sironi, A. M., Toschi, E., Masoni, A., Ferrannini, E., and Mari, A. (2000). Dose-response characteristics of insulin

action on glucose metabolism: a non-steady-state approach. *Am J Physiol Endocrinol Metab*, 278(5):E794–801.

Nijpels, G., van der Wal, P. S., Bouter, L. M., and Heine, R. J. (1994). Comparison of three methods for the quantification of beta-cell function and insulin sensitivity. *Diabetes Res Clin Pract*, 26(3):189–95.

Nishida, Y., Tokuyama, K., Nagasaka, S., Higaki, Y., Fujimi, K., Kiyonaga, A., Shindo, M., Kusaka, I., Nakamura, T., Ishikawa, S. E., Saito, T., Nakamura, O., Sato, Y., and Tanaka, H. (2002). S(G), S(I), and EGP of exercise-trained middle-aged men estimated by a two-compartment labeled minimal model. *Am J Physiol Endocrinol Metab*, 283(4):E809–16.

Nishida, Y., Tokuyama, K., Nagasaka, S., Higaki, Y., Shirai, Y., Kiyonaga, A., Shindo, M., Kusaka, I., Nakamura, T., Ishibashi, S., and Tanaka, H. (2004). Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartment-labeled minimal model. *Diabetes*, 53(2):315–320.

NovoNordisk (2002). The compatibility of insulin in intravenous infusions. Technical report, Novo Nordisk UK Medical Information.

O'Gorman D, J., Karlsson, H. K., McQuaid, S., Yousif, O., Rahman, Y., Gasparro, D., Glund, S., Chibalin, A. V., Zierath, J. R., and Nolan, J. J. (2006). Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. *Diabetologia*.

Orr-Ewing, J. (1931). The tolerance of normal rabbits to intravenous injection of glucose. *J Physiol*, 73(4):365–70.

Oterdoom, L. H., de Vries, A. P., van Son, W. J., van der Heide, J. J., Ploeg, R. J., Gansevoort, R. T., de Jong, P. E., Gans, R. O., and Bakker, S. J. (2005). Validation of insulin resistance indexes in a stable renal transplant population. *Diabetes Care*, 28(10):2424–9.

Overkamp, D., Gautier, J. F., Renn, W., Pickert, A., Scheen, A. J., Schmulling, R. M., Eggstein, M., and Lefebvre, P. J. (1997). Glucose turnover in humans in the basal state and after intravenous glucose: a comparison of two models. *Am J Physiol*, 273(2 Pt 1):E284–96.

Pacini, G. and Bergman, R. N. (1986). MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed*, 23(2):113–22.

Pacini, G. and Mari, A. (2003). Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Pract Res Clin Endocrinol Metab*, 17(3):305–22.

Peters, A. L., Davidson, M. B., Schriger, D. L., and Hasselblad, V. (1996). A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated hemoglobin levels. Meta-analysis Research Group on the Diagnosis of Diabetes Using Glycated Hemoglobin Levels. *Jama*, 276(15):1246–1252.

Petersen, K. F. and Shulman, G. I. (2006). Etiology of insulin resistance. *Am J Med*, 119(5 Suppl 1):S10–6.

Petrie, A. and Sabin, C. (2005). *Medical statistics at a glance*. Blackwell, Malden, Mass., 2nd edition.

Pillonetto, G., Sparacino, G., Magni, P., Bellazzi, R., and Cobelli, C. (2002). Minimal model S(I)=0 problem in NIDDM subjects: nonzero Bayesian estimates with credible confidence intervals. *Am J Physiol Endocrinol Metab*, 282(3):E564–573.

Polonsky, K. S., Given, B. D., Pugh, W., Licinio-Paixao, J., Thompson, J. E., Karrison, T., and Rubenstein, A. H. (1986a). Calculation of the systemic delivery rate of insulin in normal man. *J Clin Endocrinol Metab*, 63(1):113–8.

Polonsky, K. S., Licinio-Paixao, J., Given, B. D., Pugh, W., Rue, P., Galloway, J., Karrison, T., and Frank, B. (1986b). Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *J Clin Invest*, 77(1):98–105.

PriceWaterhouseCoopers (2001). Type 2 Diabetes, Managing for Better Health Outcomes. Technical report, Diabetes New Zealand Inc.

Prigeon, R. L., Roder, M. E., Porte, D., J., and Kahn, S. E. (1996). The effect of insulin dose on the measurement of insulin sensitivity by the minimal model technique. Evidence for saturable insulin transport in humans. *J Clin Invest*, 97(2):501–7.

Quon, M. J., Cochran, C., Taylor, S. I., and Eastman, R. C. (1994a). Direct comparison of standard and insulin modified protocols for minimal model estimation of insulin sensitivity in normal subjects. *Diabetes Res*, 25(4):139–49.

Quon, M. J., Cochran, C., Taylor, S. I., and Eastman, R. C. (1994b). Non-insulin-mediated glucose disappearance in subjects with IDDM. Discordance between experimental results and minimal model analysis. *Diabetes*, 43(7):890–6.

Rabkin, R., Ryan, M. P., and Duckworth, W. C. (1984). The renal metabolism of insulin. *Diabetologia*, 27(3):351–7.

Radziuk, J. (2000). Insulin sensitivity and its measurement: structural commonalities among the methods. *J Clin Endocrinol Metab*, 85(12):4426–33.

Radziuk, J., Norwich, K. H., and Vranic, M. (1978). Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol*, 234(1):E84–93.

Rasio, E. A., Hampers, C. L., Soeldner, J. S., and Cahill, G. F., J. (1967). Diffusion of glucose, insulin, inulin, and Evans blue protein into thoracic duct lymph of man. *J Clin Invest*, 46(6):903–10.

Regittnig, W., Ellmerer, M., Fauler, G., Sendlhofer, G., Trajanoski, Z., Leis, H. J., Schaupp, L., Wach, P., and Pieber, T. R. (2003). Assessment of transcapillary glucose exchange in human skeletal muscle and adipose tissue. *Am J Physiol Endocrinol Metab*, 285(2):E241–51.

Regittnig, W., Trajanoski, Z., Leis, H. J., Ellmerer, M., Wutte, A., Sendlhofer, G., Schaupp, L., Brunner, G. A., Wach, P., and Pieber, T. R. (1999). Plasma and interstitial glucose dynamics after intravenous glucose injection: evaluation of the single-compartment glucose distribution assumption in the minimal models. *Diabetes*, 48(5):1070–81.

Riccardi, G., Vaccaro, O., Rivellese, A., Pignalosa, S., Tutino, L., and Mancini, M. (1985). Reproducibility of the new diagnostic criteria for impaired glucose tolerance. *Am J Epidemiol*, 121(3):422–9.

Robbins, D. C., Andersen, L., Bowsher, R., Chance, R., Dinesen, B., Frank, B., Gingerich, R., Goldstein, D., Widemeyer, H. M., Haffner, S., Hales, C. N., Jarett, L., Polonsky, K., Porte, D., Skyler, J., Webb, G., and Gallagher, K. (1996). Report of the American Diabetes Association's Task Force on standardization of the insulin assay. *Diabetes*, 45(2):242–56.

Roche (2004). Data Sheet - Insulin Immunoassay, Elecsys 1010/2010/Modular Analytics E170. Technical Report 12017547 122, Roche Diagnostics, Mannheim, Germany.

Roche (2005). Data Sheet - C-Peptide Immunoassay, Elecsys 1010/2010/Modular Analytics E170. Technical Report 03184897 190, Roche Diagnostics, Mannheim, Germany.

Rubenstein, A. H., Clark, J. L., Melani, F., and F., S. D. (1969). Secretion of proinsulin C-peptide by pancreatic beta cells and its circulation in blood. *Nature*, 224:697–699.

Ruige, J. B., Mertens, I. L., Bartholomeeusen, E., Dirinck, E., Ferrannini, E., and Van Gaal, L. F. (2006). Fasting-based estimates of insulin sensitivity in overweight and obesity: a critical appraisal. *Obesity (Silver Spring)*, 14(7):1250–6.

Saad, M. F., Anderson, R. L., Laws, A., Watanabe, R. M., Kades, W. W., D., C. Y., Sands, R. E., Pei, D., Savage, P. J., and Bergman, R. N. (1994). A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Diabetes*, 43:1114–21.

Saad, M. F., Steil, G. M., Kades, W. W., Ayad, M. F., Elsewafy, W. A., Boyadjian, R., Jinagouda, S. D., and Bergman, R. N. (1997). Differences between the tolbutamide-boosted and the insulin-modified minimal model protocols. *Diabetes*, 46(7):1167–71.

Salkind, N. J. and Rasmussen, K. (2007). *Encyclopedia of measurement and statistics*. SAGE Publications, Thousand Oaks, Calif.

Samnegard, B. and Brundin, T. (2001). Renal extraction of insulin and C-peptide in man before and after a glucose meal. *Clin Physiol*, 21(2):164–71.

Sapin, R. (2003). Insulin assays: previously known and new analytical features. *Clin Lab*, 49(3-4):113–21.

Scheen, A. J., Paquot, N., Castillo, M. J., and Lefebvre, P. J. (1994). How to measure insulin action in vivo. *Diabetes Metab Rev*, 10(2):151–88.

Schinner, S., Scherbaum, W. A., Bornstein, S. R., and Barthel, A. (2005). Molecular mechanisms of insulin resistance. *Diabet Med*, 22(6):674–82.

Shen, S. W., Reaven, G. M., and Farquhar, J. W. (1970). Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. *J Clin Invest*, 49(12):2151–60.

Sherwin, R. S., Kramer, K. J., Tobin, J. D., Insel, P. A., Liljenquist, J. E., Berman, M., and Andres, R. (1974). A model of the kinetics of insulin in man. *J Clin Invest*, 53(5):1481–92.

Shoelson, S. E., Lee, J., and Goldfine, A. B. (2006). Inflammation and insulin resistance. *J Clin Invest*, 116(7):1793–801.

Silvers, A., Swenson, R. S., Farquhar, J. W., and Reaven, G. M. (1969). Derivation of a three compartment model describing disappearance of plasma insulin-131-I in man. *J Clin Invest*, 48(8):1461–9.

Sjostrand, M., Gudbjornsdottir, S., Strindberg, L., and Lonnroth, P. (2005a). Delayed transcapillary delivery of insulin to muscle interstitial fluid after oral glucose load in obese subjects. *Diabetes*, 54(1):152–7.

Sjostrand, M., Gudbjornsdottir, S., Strindberg, L., and Lonnroth, P. (2005b). Retraction - Delayed transcapillary delivery of insulin to muscle interstitial fluid after oral glucose load in obese subjects. *Diabetes*, 54(7):2266.

Sjostrand, M., Holmang, A., and Lonnroth, P. (1999). Measurement of interstitial insulin in human muscle. *Am J Physiol*, 276(1 Pt 1):E151–4.

Sjostrand, M., Holmang, A., Strindberg, L., and Lonnroth, P. (2000). Estimations of muscle interstitial insulin, glucose, and lactate in type 2 diabetic subjects. *Am J Physiol Endocrinol Metab*, 279(5):E1097–103.

Song, S. H., McIntyre, S. S., Shah, H., Veldhuis, J. D., Hayes, P. C., and Butler, P. C. (2000). Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *J Clin Endocrinol Metab*, 85(12):4491–9.

Soonthornpun, S., Setasuban, W., Thamprasit, A., Chayanunnukul, W., Rattarasarn, C., and Geater, A. (2003). Novel insulin sensitivity index derived from oral glucose tolerance test. *J Clin Endocrinol Metab*, 88(3):1019–23.

Steil, G. M., Ader, M., Moore, D. M., Rebrin, K., and Bergman, R. N. (1996). Transendothelial insulin transport is not saturable in vivo. No evidence for a receptor-mediated process. *J Clin Invest*, 97(6):1497–503.

Steil, G. M., Rebrin, K., Darwin, C., Hariri, F., and Saad, M. F. (2006). Feasibility of automating insulin delivery for the treatment of type 1 diabetes. *Diabetes*, 55(12):3344–50.

Stumvoll, M., Mitrakou, A., Pimenta, W., Jenssen, T., Yki-Jarvinen, H., Van Haeften, T., Renn, W., and Gerich, J. (2000). Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care*, 23(3):295–301.

Sturgeon, C., Sam, A. D., n., and Law, W. R. (1998). Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. *J Appl Physiol*, 84(6):2154–62.

Thorsteinsson, B. (1990). Kinetic models for insulin disappearance from plasma in man. *Dan Med Bull*, 37(2):143–53.

Thorsteinsson, B., Fugleberg, S., Feldt-Rasmussen, B., Ellemann, K., and Binder, C. (1987). Kinetics Models for Insulin Disappearance from Plasma in Type 1 Diabetic Patients. *Pharmacol Toxicol*, 60(2):90–95.

Toffolo, G., Bergman, R. N., Finegood, D. T., Bowden, C. R., and Cobelli, C. (1980). Quantitative estimation of beta cell sensitivity to glucose in the intact organism: a minimal model of insulin kinetics in the dog. *Diabetes*, 29(12):979–90.

Toffolo, G., Campioni, M., Basu, R., Rizza, R. A., and Cobelli, C. (2006). A minimal model of insulin secretion and kinetics to assess hepatic insulin extraction. *Am J Physiol Endocrinol Metab*, 290(1):E169–76.

Toffolo, G., De Grandi, F., and Cobelli, C. (1995). Estimation of beta-cell sensitivity from intravenous glucose tolerance test C-peptide data. Knowledge of the kinetics avoids errors in modeling the secretion. *Diabetes*, 44(7):845–54.

Tranberg, K. G. and Dencker, H. (1978). Modeling of plasma disappearance of unlabeled insulin in man. *Am J Physiol*, 235(6):E577–85.

Trout, K. K., Rickels, M. R., Schutta, M. H., Petrova, M., Freeman, E. W., Tkacs, N. C., and Teff, K. L. (2007). Menstrual cycle effects on insulin sensitivity in women with type 1 diabetes: A pilot study. *Diabetes Technol Ther*, 9(2):176–182.

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M., Salminen, V., and Uusitupa, M. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med*, 344(18):1343–50.

Turnheim, K. and Waldhausl, W. K. (1988). Essentials of insulin pharmacokinetics. *Wien Klin Wochenschr*, 100(3):65–72.

Valera Mora, M. E., Scarfone, A., Calvani, M., Greco, A. V., and Mingrone, G. (2003). Insulin clearance in obesity. *J Am Coll Nutr*, 22(6):487–93.

Van Cauter, E., Mestrez, F., Sturis, J., and Polonsky, K. S. (1992). Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes*, 41(3):368–77.

Van Cauter, E., Polonsky, K. S., and Scheen, A. J. (1997). Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocr Rev*, 18(5):716–38.

Van den Berghe, G., Wilmer, A., Hermans, G., Meersseman, W., Wouters, P. J., Milants, I., Van Wijngaerden, E., Bobbaers, H., and Bouillon, R. (2006). Intensive Insulin Therapy in the Medical ICU. *N Engl J Med*, 354(5):449–61.

Vella, A., Reed, A. S., Charkoudian, N., Shah, P., Basu, R., Basu, A., Joyner, M. J., and Rizza, R. A. (2003). Glucose-induced suppression of endogenous glucose production: dynamic response to differing glucose profiles. *Am J Physiol Endocrinol Metab*, 285(1):E25–30.

Vicini, P. and Cobelli, C. (2001). The iterative two-stage population approach to IVGTT minimal modeling: improved precision with reduced sampling. Intravenous glucose tolerance test. *Am J Physiol Endocrinol Metab*, 280(1):E179–86.

Vicini, P., Zachwieja, J. J., Yarasheski, K. E., Bier, D. M., Caumo, A., and Cobelli, C. (1999). Glucose production during an IVGTT by deconvolution: validation with the tracer-to-tracee clamp technique. *Am J Physiol*, 276(2 Pt 1):E285–94.

Wahren, J. (2004). C-peptide: new findings and therapeutic implications in diabetes. *Clin Physiol Funct Imaging*, 24(4):180–9.

Wahren, J., Efendic, S., Luft, R., Hagenfeldt, L., Bjorkman, O., and Felig, P. (1977). Influence of somatostatin on splanchnic glucose metabolism in postabsorptive and 60-hour fasted humans. *J Clin Invest*, 59(2):299–307.

Wallace, T. M., Levy, J. C., and Matthews, D. R. (2004a). An increase in insulin sensitivity and basal beta-cell function in diabetic subjects treated with pioglitazone in a placebo-controlled randomized study. *Diabet Med*, 21(6):568–76.

Wallace, T. M., Levy, J. C., and Matthews, D. R. (2004b). Use and abuse of HOMA modeling. *Diabetes Care*, 27(6):1487–95.

Watanabe, R. M., Volund, A., Roy, S., and Bergman, R. N. (1989). Prehepatic beta-cell secretion during the intravenous glucose tolerance test in humans: application of a combined model of insulin and C-peptide kinetics. *J Clin Endocrinol Metab*, 69(4):790–7.

Waterhouse, C. and Keilson, J. (1972). Transfer times across the human body. *Bull Math Biophys*, 34(1):33–44.

Wild, S., Roglic, G., Green, A., Sicree, R., and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27(5):1047–53.

Windhager, E. (1992). *Renal physiology*. Handbook of Physiology. Oxford University Press, New York.

Wong, X. W., Chase, J. G., Shaw, G. M., Hann, C. E., Lotz, T., Lin, J., Singh-Levett, I., Hollingsworth, L. J., Wong, O. S., and Andreassen, S. (2006a). Model predictive glycaemic regulation in critical illness using insulin and nutrition input: a pilot study. *Med Eng Phys*, 28(7):665–81.

Wong, X. W., Chase, J. G., Shaw, G. M., Lin, J., Lotz, T., and Hann, C. E. (2005). Clinical trials of active and adaptive insulin and nutrition control to control hyperglycaemia in critically ill patients. In *Canterbury Health Research Conference*, pages 1–page, Christchurch.

Wong, X. W., Singh-Levett, I., Hollingsworth, L. J., Shaw, G. M., Hann, C. E., Lotz, T., Lin, J., Wong, O. S., and Chase, J. G. (2006b). A novel, model-based insulin and nutrition delivery controller for glycemic regulation in critically ill patients. *Diabetes Technol Ther*, 8(2):174–90.

Yang, Y. J., Hope, I. D., Ader, M., and Bergman, R. N. (1989). Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest*, 84(5):1620–8.

Yang, Y. J., Youn, J. H., and Bergman, R. N. (1987). Modified protocols improve insulin sensitivity estimation using the minimal model. *Am J Physiol*, 253(6 Pt 1):E595–602.

Zavaroni, I., Deferrari, G., Lugari, R., Bonora, E., Garibotto, G., Dall'Aglio, E., Robaudo, C., and Gnudi, A. (1987). Renal metabolism of C-peptide in man. *J Clin Endocrinol Metab*, 65(3):494–8.

Zierler, K. (1999). Whole body glucose metabolism. *Am J Physiol*, 276(3 Pt 1):E409–426.