Modelling Methodologies to Assess Insulin Resistance and Insulin Secretion in Type 2 Diabetes Subjects

by

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Thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Measurement and Information in Medicine

City University

Year 2003

Abstract

The development of type 2 diabetes (T2D) is a gradual process, and the relative roles of impaired insulin secretion and resistance in aetiology of the disease remain controversial. However, it is widely recognised that both defects are present in all overt subjects with T2D.

The current work provides new knowledge on both methodological and pathophysiological levels related to T2D. On the methodological level, a population-based approach, Bayesian hierarchical analysis (BAY), developed and was used to estimate parameters of the minimal model of glucose kinetics using data collected in newly presenting T2D subjects to investigate insulin sensitivity and glucose effectiveness. BAY results were compared with the standard two-stage approach, which employs the non-linear regression analysis. BAY was also employed to derive and compare estimates of insulin sensitivity and glucose effectiveness with a full sample scheme (30 points) with those estimated with 12 and 13 sample schemes.

The results demonstrated that BAY, besides avoiding parameter estimation failures, gives a smaller unbiased estimate of the population variance for both insulin sensitivity and glucose effectiveness. The analysis of reduced sampling schemes with BAY suggested that the adoption of the 13 sample scheme is preferable to that of the 12 sample scheme.

On the pathophysiological level, BAY was employed in combination with other techniques to increase understanding of early progression of T2D. The research investigated the progression of insulin sensitivity and insulin secretion over 2 years after diagnosis of T2D and relationships with clinical measures of glucose control.

The results demonstrated that β-cell function can be ameliorated for at least two years by adequate conventional treatments after diagnosis of T2D. The improvement in glycated haemoglobin (HbA_{1C}) in the earlier years of T2D are associated with improved fasting and postprandial insulin pancreatic responsiveness. The results showed that the ability of indices of insulin sensitivity and pancreatic β-cell responsiveness to explain inter-individual variability of measures of glucose control in newly presenting T2D decrease over time for clinical measures of glucose control, and measures of glucose and insulin responses to the meal tolerance test.

Glossary

IVGTT	Intravenous glucose tolerance test
MTT	Meal tolerance test
HOMA	Homeostatic model assessment
CIGMA	Constant glucose infusion with model assessment
MCMC	Markov chain Monte Carlo
CV	Coefficient of variation
FPI	Fasting plasma insulin
FPG	Fasting plasma glucose
AUC	Area under curve
BMI	Body mass index
CODA	Convergence Diagnostic and Output Analysis (software for Gibbs sampling output)
BAY	(as superscript) Derived by the Bayesian analysis
NLR	(as superscript) Derived by the non-linear regression analysis
p_2	Parameter of the minimal model
S_G	Glucose effectiveness
S_I	Insulin sensitivity
V	Volume of distribution
ADA	American Diabetes Association
UKPDS	United Kingdom Prospective Diabetes Study
DCCT	Diabetes Control and Complicatins Trial
STS	Standard two-stage
CI	Confidence interval or Credible interval (for the Beyesian output)

Acknowledgement

I would like to express my sincere appreciation to Dr. Roman Hovorka for his guidance, advice, support, and for being always there to help throughout the course of this study and during the preparation of this thesis.

I am deeply grateful to Professor David Owens and Dr. Stephen Luzio for their support, invaluable advice, and for sharing their clinical and experimental expertise in the field of Type 2 diabetes.

This work was supported by CASE EPSRC studentship award (99802790).

Many thanks to my children Olamide, Jide, Jimi, and Femi for their love, understanding, and support.

Finally, I am very grateful to Adefolakemi for her love and constant support.

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Chapter 1

Introduction and Thesis Structure

1.1 Background and Motivation

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in both insulin secretion and insulin action [36, 37, 73]. The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made [73, 75].

The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [60].

Recently compiled data show that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025 [138]. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity, and sedentary lifestyles. By 2025, while most

people with diabetes in developed countries will be aged 65 years or more, in developing countries most will be in the 45-64 year age bracket and affected in their most productive years [6].

Diabetes mellitus is classified into four types: type 1, type 2, "other specific types", and gestational diabetes. Each of the types extends across a clinical continuum of hyperglycaemia and insulinaemia. Type 2 diabetes mellitus (T2D) is characterised by insulin resistance in the peripheral tissues and an insulin secretory defect of the β-cell [97]. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, age, obesity, and lack of exercise. It accounts for about 85-95% of all cases of diabetes [6]. Insulin resistance and hyperinsulinaemia eventually lead to impaired glucose tolerance. Defective β-cells become exhausted, further fuelling the cycle of glucose intolerance and hyperglycaemia. The aetiology of T2D is multifactorial and probably genetically based, but it also has strong behavioural components.

Several pathogenetic processes are involved in the development of T2D. These include processes which destroy the β-cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat, and protein metabolisms are due to deficient action of insulin on target tissues resulting from insulin sensitivity or lack of insulin. The development of T2D is a gradual process, and the relative roles of impaired insulin secretion and resistance in aetiology of the disease remain controversial. However, it is widely recognised that both defects are usually present in all overt T2D patients [37, 36, 109, 25].

In response to the on growing burden on T2D, the increasing prevalence of T2D reaching epidemic proportions worldwide, and the need to improve understanding of the relationships between insulin secretion and insulin resistance, substantial resources are being allocated to assess quantitatively and adequately insulin secretion and action. These activities have resulted in the successful introduction of models of glucose and insulin kinetics for quantifying both insulin secretion and insulin action in subjects with T2D [32, 29, 129, 127, 16, 15, ?, 69].

Methods of population kinetics are indicated when kinetic data of different individuals have to be combined and when the average behaviour of a population is used to predict individual kinetics. The naive method of calculating weighted mean has been abandoned in favour of mixed/random effects models and Bayesian methods. A three-stage hierarchical Bayesian approach uses the individual time-concentration relationship, the distributional form for the kinetic parameters, optionally covariate information, and a prior distribution of parameters of the second-stage and the intra-individual variability [132].

It is now recognised that an individual approach to parameter estimation is at times inadequate, and it may be more appropriate to describe parameters by a probability distribution. This is due to the fact that probability-based parameters could be more appropriate in explaining significant variability in human population. Racine-Poon et al [106] demonstrated that collective information from modelling of individuals could provide a basis for learning about a form of mean underlying population characteristics. They showed, from the statistical inference and the modelling perspective, that it is of a paramount importance and of a great benefit to incorporate both a model for the measurement process of individual profiles and also a model of the variation of these individual profiles around a mean population profile, whether the main interest of study is in an individual or population or both.

Bearing in mind the relationships between insulin secretion and insulin resistance and the need to assess adequately these indices, we examined these relationships using a population-based Bayesian hierarchical analysis of the minimal model data to investigate insulin sensitivity and glucose effectiveness in T2D. We also examined these relationships over two years to investigate the pathophysiological changes.

The conceptual framework for the population-based approach will be based on the minimal model of glucose kinetics, mathematical and statistical procedures involving compartmental and random effects models, respectively. The random effects model will be in an hierarchical framework of the Bayesian approach. The approach will enable to examine critically the inter-subject variability of model parameters.

The structure of the population-based Bayesian hierarchical model is complex and involves a joint probability distribution for model parameters. Handling such very complex models is difficult, and exact, analytic tools fail to be practicable and only approximation methods work. The Markov chain Monte Carlo (MCMC) method is a general method for sampling from stochastic multivariate distributions and computing expectations. MCMC is particularly useful when the correlation structure is complex and the probability density function only known up to a proportionality constant, and presently, it is the only approach that is feasible for such problems.

1.2 Aims

The general aim of this thesis is to develop and evaluate stochastic modelling and apply this in combination with existing deterministic approaches to advance our knowledge of insulin sensitivity and insulin secretion in subjects with T2D.

1.3 Objectives

The objectives can be divided into methodological and pathophysiological. The former relate to the development and validation of new methods, the latter to the adoption of newly developed methods in combination with other techniques to obtain new knowledge about progression of T2D.

1.3.1 Methodological Objectives

The objectives are:

1. To adopt the Bayesian analysis in combination with hierarchical (population) modelling to estimate simultaneously population and individual insulin sensitivity S_I and glucose effectiveness S_G from data collected during IVGTT;
2. To compare the results of the Bayesian hierarchical approach with the standard two-stage approach, which employs non-linear regression analysis;
3. To employ the Bayesian hierarchical modelling approach to derive and compare estimates of S_I and S_G from the full sampling scheme (30 points) with those estimated from the previously suggested 12 and 13 points sampling schemes in subjects with newly presenting T2D.

1.3.2 Pathophysiological Objectives

In subjects with newly presenting T2D the objectives are:

1. To investigate the progression of insulin secretion and insulin resistance over two years;

2. To relate a change in HbA_{1C} to a change in metabolic indices (insulin resistance and insulin secretion) and clinical measures of glucose control;

3. To investigate the ability of insulin resistance and insulin secretion to explain inter-subject variability of clinical measures of glucose control in T2D over two years.

1.4 Thesis Structure

Chapter 2 reviews the literature. It contains a background review of glucose metabolism, complications, and management of T2D. This is followed by an outline of models for the assessment of insulin resistance and insulin secretion.

Chapter 3 outlines problems with the minimal model of glucose kinetics, that is, the problem of failure rates, insulin sensitivity value equal to zero, in highly insulin-deficient patients and the problem of parameter estimation using the non-linear regression analysis. The chapter focuses on the development of the Bayesian hierarchical analysis of the minimal model of glucose kinetics and its implementation. Last, we discuss the advantages and disadvantages of the Bayesian hierarchical analysis in relation to the non-linear regression approach.

Chapter 4, compares the parameter estimation capabilities of the minimal model of glucose kinetics using the Bayesian hierarchical analysis and the standard two-stage approach employing the non-linear regression analysis.

Chapter 5 employs the Bayesian hierarchical analysis to investigate the effect of reduced sampling schemes with IVGTT in T2D subjects. We compare the reduced sampling schemes with the full sampling scheme in terms of the bias of parameter estimates and the precision of the estimates.

Chapter 6 evaluates progression of insulin secretion, insulin resistance, and clinical measures of glucose and insulin using IVGTT and MTT data in T2D patients over two years. The metabolic indices investigated in this chapter are divided into clinical measures of glucose control from IVGTT, postprandial glucose and insulin responses to MTT, and metabolic indices.

Chapter 7 investigates the ability of metabolic indices at the time of diagnosis to predict the change in HbA_{1C} over time. In addition, we investigated the relationship between

the change in HbA_{1C} and the change in metabolic settings (insulin sensitivity and insulin secretion) over time.

Chapter 8 assesses the ability of insulin secretion and insulin resistance to explain the inter-individual variability of clinical measures of glucose control in T2D over two years.

Chapter 9 provides an overall discussion of the thesis and summarises thesis achievements.

The overall conclusions of the thesis and recommendations for future work are given in Chapter 10.

Chapter 2

Literature Review

2.1 Glucose Metabolism

Glucose is liberated from dietary carbohydrate such as starch or sucrose by hydrolysis within the small intestine and is then absorbed into the blood. Elevated concentrations of glucose in blood stimulate release of insulin, and insulin acts on cells throughout the body to stimulate uptake, utilisation, and storage of glucose. It is important that the blood glucose level is maintained within a normal range (3.6-6.1 $mmol \cdot L^{-1}$), deviation of blood glucose from this normal range can result in diabetes symptoms, and if not treated or controlled it can result in diabetes complications and possibly death.

2.1.1 Normal Regulation of Blood Glucose

Glucose regulation and homeostasis is maintained, in part, by insulin, a pancreatic hormone. Insulin is formed, stored, and released by the β-cells in the pancreas. Insulin binds to receptor sites on cell membranes and stimulates glucose transport proteins within the cell to bind with glucose and carry it into the cell, effectively reducing the amount of glucose in the blood. Uptake of glucose in most, but not all, body cells is facilitated by insulin. The tissues that utilise insulin for glucose transport or storage are the liver, adipose tissue, and muscle. The brain and nerve tissue are non-insulin dependent, that is, they do not require insulin to use glucose.

Several other hormones help to regulate blood glucose in addition to insulin. Glucagon, another pancreatic hormone, has an effect on blood glucose that is opposite to that of insulin. In fact, insulin and glucagon are the primary hormones that regulate daily blood glucose fluctuations. Adrenalin, a hormone from the adrenal medulla, will raise

blood glucose within minutes but is reserved by the body for more emergency situations. This hormone can cause uncomfortable side effects such as shakiness, tremors, diaphoresis, and feelings of anxiety [135].

2.1.2 Pancreas

Pancreas Anatomy and Physiology

The pancreas is a fish-shaped, grayish-pink gland about 12.7 cm long that stretches across the back of the abdomen, behind the stomach. It releases insulin, glucagon, and some enzymes of digestion. With a lumpy surface, the pancreas is divided into a head, a body, and a tail. Small ducts from the releasing cells empty into the main duct that runs the length of the organ. The main duct empties into the intestine at the same spot as the exit of the common bile duct. About 1 million cell units (islets of Langerhans) are buried in the pancreas. β-cells of the islets release insulin, which helps control the body's use of carbohydrate. α-cells of the islets release glucagon, which counters the action of insulin. Other units of the pancreas release enzymes that help digest fats and proteins [135, 79].

Cell Types in Pancreatic Islets

Pancreatic islets house three major cell types, each of which produces a different endocrine product:

- Alpha cells (α-cells) secrete the hormone glucagon;
- Beta cells (β-cells) produce insulin and are the most abundant of the islet cells;
- Delta cells (δ-cells) secrete the hormone somatostatin, which is also produced by a number of other endocrine cells in the body.

Interestingly, the different cell types within an islet are not randomly distributed - β-cells occupy the central portion of the islet and are surrounded by a "rind" of α and $\delta-$cells. Aside from the insulin, glucagon, and somatostatin, a number of other "minor" hormones have been identified as products of pancreatic islets cells.

Islets are richly vascularised, allowing their secreted hormones ready access to the circulation. Although islets comprise only 1-2% of the mass of the pancreas, they

receive about 10 to 15% of the pancreatic blood flow. Additionally, they are innervated by parasympathetic and sympathetic neurons, and nervous signals clearly modulate secretion of insulin and glucagon.

2.1.3 Pathology of Glucose Metabolism

Insulin Resistance

Insulin resistance occurs when the normal amount of insulin secreted by the pancreas is not able to promote the glucose transport to cells. To maintain a normal blood glucose, the pancreas secretes additional insulin. In some cases (about 1/3 of the people with insulin resistance), when the body cells resist or do not respond to even high levels of insulin, glucose builds up in the blood resulting in high blood glucose or type 2 diabetes. Even people with diabetes who take oral medication or require insulin injections to control their blood glucose levels can have higher than normal blood insulin levels due to insulin resistance.

Insulin resistance can be defined as an impaired biological response to either exogenous or endogenous insulin. The measured biological responses could reflect, in theory, metabolic processes (changes in carbohydrate, lipid or protein metabolism) as well as mitogenic processes (alterations in growth, differentiation, DNA synthesis, regulation of gene transcription). In vivo biological responses to insulin vary according to insulin concentration, exposure time, tissue delivery, and pulsatility [113, 110, 109].

Even though the glucose-insulin relationship is clinically pertinent, it is also important to recognise that, conceptually, insulin resistance does not have to be confined just to parameters of glucose metabolism. The concept of insulin resistance should apply to any of the biological actions of insulin, and might include its effects on lipid and protein metabolism, vascular endothelial function, and gene expression.

Researches have shown that obesity and physical inactivity aggravate insulin resistance [84]. People who are insulin resistant typically have an imbalance in their blood lipids. They have an increased level of triglycerides (blood fat) and a decreased level of HDL cholesterol. Imbalances in triglycerides and HDL cholesterol increase the risk of heart disease. These findings have heightened awareness of insulin resistance and its impact on health.

Insulin Deficiency

Persons with type 2 diabetes have a relative insulin deficiency rather than an absolute deficiency as seen in type 1 diabetes. This relative insulin deficiency appears to be due to a limitation in the pancreatic β-islet cell response to hyperglycaemia [80]. As much as 50% of the β-islet cell mass is lost in people with type 2 diabetes when compared to people without diabetes. The hyperglycaemia associated with T2D would be expected to produce hypertrophy and hyperfunction in the β-islet cell population, but this is not the case. Autoantibodies are not present, as is true in most people with type 1 diabetes where autoantibodies are responsible for destroying the β-islet cell population. The exact aetiology of this reduced β-islet cell population in type 2 diabetes is unknown.

In a person without diabetes, insulin is secreted in a biphasic manner to a glucose stimulus. The first phase occurs as stored insulin is released as a bolus. This initial surge of insulin serves to prime the target tissues, primarily the liver. The second phase in response to a glucose load is the continuous secretion of newly manufactured insulin, which continues until a normal glucose level is restored [36]. In a person with T2D, circulating insulin levels may appear normal or even elevated, but the intrinsic patterns of insulin secretion are altered. People with type 2 diabetes lose the acute-phase or pulsatile release of insulin in response to increased blood glucose concentrations. This has been referred to as "glucose toxicity." β-islet cells exposed continuously to hyperglycaemia become progressively less capable of responding to ensuing glucose challenges [108]. The large bolus of insulin release that usually follows a meal is lost, and it takes much longer to absorb prandial glucose.

The relative insulin deficiency is a defect in either the compensatory response of the β-islet cell population to hyperglycaemia and/or an abnormality in the pattern of insulin secretion. In either case, this limitation in β-islet cell function is the obligatory factor in the pathogenesis of type 2 diabetes [80].

2.1.4 Diabetes

Types of Diabetes

Type 1 Diabetes. Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease. An autoimmune disease results when the body's system for fighting infection (the immune system) turns against a part of the body. In diabetes, the immune system attacks the insulin-producing β-cells in the pancreas and destroys

them. The pancreas then produces little or no insulin. Someone with type 1 diabetes needs to take insulin daily to live.

Type 1 diabetes develops most often in children and young adults, but the disorder can appear at any age. Symptoms of type 1 diabetes usually develop over a short period, although β-cell destruction can begin years earlier.

Symptoms include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. If not diagnosed and treated with insulin, a person can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis [102].

Type 2 Diabetes. The most common form of diabetes is type 2 or non-insulin-dependent diabetes mellitus (NIDDM). About 90 to 95 percent of people with diabetes have type 2. This form of diabetes usually develops in adults, age 40 and older, and is most common in adults over age 55. About 80 percent of people with type 2 diabetes are overweight. Type 2 diabetes is often part of a metabolic syndrome that includes obesity, elevated blood pressure, and high levels of blood lipids. Unfortunately, as more children become overweight, type 2 diabetes is becoming more common in young people [102, 79].

When type 2 diabetes is diagnosed, the pancreas is usually producing enough insulin, but, for unknown reasons, the body cannot use the insulin effectively, a condition called insulin resistance. Glucose builds up in the blood and the body cannot make efficient use of its main source of fuel [102, 135].

The symptoms of type 2 diabetes develop gradually. They are not as sudden in onset as in type 1 diabetes. Some people have no symptoms. Symptoms may include fatigue or nausea, frequent urination, unusual thirst, weight loss, blurred vision, frequent infections, and slow healing of wounds or sores.

People with impaired glucose metabolism, a state between "normal" and "diabetes," are at risk for developing diabetes, heart attacks, and strokes. There are two forms of impaired glucose metabolism, impaired fasting glucose and impaired glucose tolerance.

Impaired Fasting Glucose (IFG). A person has impaired fasting glucose when fasting plasma glucose is 6.1 to 6.9 $mmol \cdot L^{-1}$. This level is higher than normal but less than the level indicating a diagnosis of diabetes.

Impaired Glucose Tolerance (IGT). Impaired glucose tolerance means that blood glucose during the oral glucose tolerance test is higher than normal but not high enough for a diagnosis of diabetes. IGT is diagnosed when the glucose level is 7.8 to 11.1 $mmol \cdot L^{-1}$ 2 hours after a person is given a drink containing 75 grams of glucose.

Gestational diabetes. Gestational diabetes develops only during pregnancy. Though it usually disappears after delivery, the mother is at increased risk of getting type 2 diabetes later in life.

2.1.5 Screening for and Diagnosing Diabetes

American Diabetes Association Criteria for Screening for and Diagnosing Diabetes Mellitus

Screening Recommendations. Testing for diabetes should be considered in all individuals at age 45 years and above and, if normal, it should be repeated at 3-year intervals.

Testing should be confirmed at a younger age or be carried out more frequently in individuals who:

- Are obese ($\geq$ 120% desirable body weight or a body mass index $\geq$ 27 kg/m^2);
- Have a first-degree relative with diabetes;
- Are members of a high-risk ethnic population (e.g., African-American, Hispanic-American, native American, Asian-American, Pacific Islander);
- Have delivered a baby weighing >9 lb or have been diagnosed with gestational diabetes mellitus;
- Are hypertensive (blood pressure $\geq$ 140/90) mmHg;
- Have a high-density lipoprotein cholesterol level $\geq$ 0.90 $mmol \cdot L^{-1}$ and/or triglyceride level $\geq$ 2.82 $mmol \cdot L^{-1}$;
- On previous testing, had impaired glucose tolerance or impaired fasting glucose.

Diagnostic Criteria.

- Symptoms of diabetes plus casual plasma glucose concentration $\geq$ 11.1 mmol·L^{-1}. Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss;
- Fasting plasma glucose (FPG) $\geq$ 7.0 mmol·L^{-1}. Fasting is defined as no caloric intake for at least 8 hours;
- 2-hour postload glucose $>$ 11.1 mmol·L^{-1} during an oral glucose tolerance test (OGTT). The test should be performed as described by the World Health Organisation using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycaemia with acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day. The OGTT or FPG test may be used to diagnose diabetes; however, in clinical settings the FPG test is greatly preferred because of ease of administration, convenience, acceptability to patients, and lower cost. The OGTT is not recommended for routine clinical use.[1]

2.2 Type 2 Diabetes

2.2.1 Introduction

Type 2 diabetes (T2D) or mature-onset diabetes, is a chronic metabolic disorder associated with significant morbidity and mortality. T2D usually starts in middle age or in the elderly. T2D is more common, constituting about 80% of diabetes in most European countries and North America. It is thought to be due to both impaired insulin secretion and resistance to the action of insulin at its target cells. About 80% of T2D patients are obese [59].

2.2.2 Epidemiology and Aetiology of Type 2 Diabetes

T2D is the commonest type of diabetes. Various clinical factors which are associated with the disease, such as obesity, increasing age, family history of diabetes, ethnic and

[1] Adapted from [98].

geographical variations in its frequency, give clues to the aetiology and pathophysiology of T2D [40].

The prevalence of T2D increases with age and affects about 10-20% of subjects over the age of 65 years in many Western countries. Most subjects are diagnosed after the age of 40 years, the peak age of onset being 60 years [40].

There is a large variation in the frequency of T2D in different countries. The highest rates (50%) are found in some native American tribes, notably the Pima Indians of Arizona and in the South Pacific island of Nauru. Low prevalence (<1%) is found in a poorly developed rural communities such as in part of Chile and China.

There are two widely advocated hypotheses about the primary etiologic factor in T2D. The first holds that a primary β-cell defect causes insufficient insulin secretion, resulting in hyperglycaemia. The peripheral tissues (muscle and liver) are normally insulin-responsive at first, but may become insulin-resistant in response to ongoing hyperglycaemia. The alternate hypothesis proposes that the basic underlying abnormality is insulin resistance in the peripheral tissues, occurring first in the muscle tissue and later in the liver. The β-cells initially compensate to maintain normal glucose metabolism by increasing the amount of insulin that is secreted. However, in time, the demand exceeds the ability to compensate. This ultimately leads to pancreatic exhaustion [80].

Considerable evidence exists for both theories. The first theory is supported by several studies showing abnormalities in insulin secretion and normal insulin action in patients with T2D diabetes [112]. The second is supported by other studies that find insulin resistance (but normal glucose metabolism) in first-degree relatives of patients with T2D [77]. Whether diminished insulin secretion or insulin resistance is the primary defect in T2D is the subject of continuing debate. Many individuals who are obese and have marked insulin resistance do not develop T2D and yet it appears that the combination of the two abnormalities is necessary for the development of T2D .

2.2.3 Complications of Type 2 Diabetes

Short-term Complications (Acute Complications)

Acute complications may develop over hours or days, and often resolve completely with appropriate treatment. The recognition and management are important to preserve the well being of T2D patients. The acute complications for diabetes can be classified as:

- hypoglycaemia;
- persistent hyperglycaemia;
- hyperosmolar coma;
- diabetic ketoacidosis.

Long-term Complications (Chronic Complication)

The long-term complications affect patients with T2D. These complications are responsible for most of the increased morbidity, mortality, and costs associated with diabetes. The long-term complications are generally divided into two main categories: microvascular and macrovascular complications. Poor glycaemic control is associated with development of microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular disease) complications.

Microvascular Complications. Microvascular complications include retinopathy, nephropathy, and neuropathy. Diabetic retinopathy is the leading cause of blindness for persons aged 20 to 74 years in the United States, and at least 8,000 new cases are diagnosed each year [72, 4]. Because of the delay in diagnosing type 2 diabetes, presence of retinopathy at the time of diagnosis is not uncommon.

Diabetic neuropathies occur with approximately the same prevalence in both type 1 and type 2 diabetes, and about 8% of people with type 2 diabetes have some degree of neuropathy at diagnosis [104]. The disorder is more prevalent with severe diabetes and diabetes of long duration. Most neuropathies are distal, symmetric, cause a loss of sensation, and may be accompanied by autonomic neuropathies (e.g., gastroparesis) as well [53].

Macrovascular Complications. The three types of macrovascular complications are

- ischaemic heart disease;
- stroke;

- peripheral vascular disease.

Ischaemic heart disease occurs earlier and more often in people with diabetes, and diabetes causes women to lose their estrogen-related protection. Ischaemic heart disease accounts for the majority of deaths in people with diabetes (up to 60%), especially the type 2 population [97]. This high rate of cardiovascular mortality may be partially explained by the high rate of cardiovascular risk factors found in the T2D population, i.e.

- hyperglycaemia;
- hypertension;
- dyslipidaemia;
- obesity;
- smoking.

Specific data related to differences in occurrence rates between people with type 1 and type 2 diabetes are lacking, but overall the mortality rates for strokes are three to five times greater in people with diabetes when compared to cohorts without diabetes [87].

Complications occur with both type 1 and type 2 diabetes and contribute significantly to the disease's morbidity and mortality. Resource utilisation is greatly affected by these complications. Ischaemic heart disease and peripheral vascular disease account for 26% of hospital days in people with diabetes [1]. Approximately 20% of the direct health care expenditures related to diabetes are due to these two macrovascular complications, and ischaemic heart disease and strokes account for 58% of the mortality costs caused by premature death in those with diabetes [1]. The Diabetes Control and Complications Trial (DCCT) [33] demonstrated that tight glycaemic control can prevent or delay progression of some of the chronic complications associated with type 1 diabetes, no conclusive outcomes data existed for those with type 2 diabetes until recently, when the UKPDS demonstrated that tight glycaemic control with oral agents and insulin can reduce the risk of complications [57, 54].

2.2.4 Control and Management of Type 2 Diabetes

A variety of evidence has shown the benefit of tight glycaemic control in patients with diabetes. The relationship between improved glycaemic control and the delay or prevention of complications has been reported not only in the Diabetes Control and Complications Trial (DCCT), but also in the United Kingdom Prospective Diabetes Study (UKPDS) and a smaller clinical trials performed in T2D [55, 96]. Data from the UKPDS show that improved blood glucose control in patients with T2D decreases the progression of microvascular complications by about 25 percent [57, 54].

The DCCT was a significant trial that first demonstrated the benefits of tight glycaemic control [33]. The goal of the DCCT was to determine if intensive insulin therapy, aimed at achieving near-normal levels of glucose control, would decrease the onset and progression of diabetes complications. Over approximately 10 years, 1,441 carefully selected people with type 1 diabetes were randomised into two groups. The treatment group received intensive therapy while the control group was treated using a standard or conventional insulin regimen. Intensive therapy was defined as control using an external insulin pump or the use of three or more daily injections combined with frequent blood glucose monitoring for the purpose of guiding dosing changes. Conventional therapy was defined as one or two daily injections with less frequent blood glucose monitoring.

In the treatment group, glycated haemoglobin (HbA_{1C}) values, a marker of long-term glucose control, showed a significant reduction, about 1.8%, compared to the control group. The average HbA_{1C} for the treatment group was 7.2% compared to a value of 9.0% in the control group. This decrease in HbA_{1C} values resulted in a decreased risk of developing microvascular complications by 50% to 75%. Unfortunately, there were no T2D patients included in this trial and many practitioners had difficulty in extrapolating the findings to the vast majority of people, especially those with T2D.

A smaller trial performed in T2D patients, the Kumomoto study, showed that tight glycaemic control achieved through intensive insulin therapy can delay the onset and progression of diabetic retinopathy, nephropathy, and neuropathy [96]. Comparable to the DCCT, decreases in risks of developing microvascular complications were established. The small study population in this trial, 110 patients, coupled with generally non-obese Japanese patients made it difficult to generalise and extrapolate the outcomes of this study to a wider population where the majority of T2D patients are obese and on oral agents, not insulin. In addition, neither study provided data to suggest that improved glycaemic control will affect the increased incidence of macrovascular complications experienced by the T2D population. The UKPDS, the largest and longest

study of type 2 diabetes, was designed to specifically address whether intensive therapy with oral agents or insulin is beneficial.

The UKPDS was a multicentre, randomised, controlled trial designed to determine if improved glycaemic control to near normal levels in newly diagnosed T2D patients would reduce the risk of diabetes-related complications, both cardiovascular and microvascular. A secondary goal was to ascertain if any one agent (chlorpropamide, glyburide, insulin, or metformin) was more advantageous than diet alone [57, 54]. In addition, the recruited patients who had hypertension (39%) as well as T2D were randomised to "tight" or "less tight" blood pressure control to determine the value of lowering blood pressure. Like the blood glucose study design, a secondary aim was to determine whether the use of the ACE inhibitor captopril or the beta-blocker atenolol was more advantageous than the other [58, 56]. A total of 5102 newly diagnosed type 2 patients were recruited to participate in the study between 1977 and 1991. The main question of the study was whether reducing blood glucose was beneficial in the type 2 diabetes population.

The study showed that over time all patients experienced a progressive increase in blood glucose. This progressive deterioration in glucose control is thought to be due to the progressive loss of β-islet cell function in people with type 2 diabetes [55]. However, intensive therapy produced a median HbA_{1C} value of 7.0% compared to 7.9% in the control group over the 10-year study period. This reduction in HbA_{1C} values reduced the risk of developing:

- any diabetes-related (microvascular and macrovascular complications and cataract extraction) endpoint by 12% (P =0.029);
- microvascular endpoints by 25% (P =0.0099);
- myocardial infarction by 16% (P =0.052);
- cataract extraction by 24% (P =0.046);
- retinopathy by 21% (P =0.015);
- albuminuria by 33% (P =0.000054).

The UKPDS outcomes also indicated that, similar to type 1 diabetes, microvascular complications in type 2 diabetes are caused by hyperglycaemia and a continuous relationship exists between blood glucose and the risks of these complications. The UKPDS results demonstrated that by reducing the HbA_{1C} value by one percentage point (1%),

there is a 25% reduction in the risk of microvascular complications. Further, the risks can be lowered significantly even when HbA_{1C} values are <8.0%. No glycaemic threshold exists for microvascular complications—the lower the HbA_{1C} value (above a normal of 6.2%), the lower the risk of microvascular complications.

Although approaching borderline (P = 0.052) statistical significance, there was no difference in cardiovascular complications. This implies that lowering blood glucose alone is insufficient in reducing cardiovascular morbidity and mortality in the type 2 population. However, a continuous relationship exists between blood glucose and the risks of cardiovascular complications. The UKPDS results demonstrated that a one percentage point (1%) reduction in the HbA_{1C} value results in:

- a reduction in diabetes-related deaths by 25%;
- a reduction in all-cause mortality by 7%;
- a reduction in myocardial infarction by 18%.

No glycaemic threshold exists for cardiovascular complications—the lower the HbA_{1C} value (above a normal of 6.2%), the lower the risk of these complications.

The UKPDS found no differences between the pharmacological agents used. All produced the same effect on lowering HbA_{1C} values, and all were equally effective in reducing the risks of major clinical outcomes. The UKPDS found no increase in the incidence of cardiovascular death, myocardial infarction, or sudden death in patients treated with these agents when compared to controls. Therefore, these agents are safe and effective in the treatment of type 2 diabetes. When comparing sulphonylureas, insulin, and metformin, both sulphonylureas and insulin were associated with hypoglycaemia and weight gain. Metformin caused no weight gain and had little risk of hypoglycaemia. Also, metformin may significantly decrease the risks of cardiovascular disease through these same beneficial effects (e.g. no weight gain), but the data are less certain due to the smaller numbers receiving metformin and the multiple crossovers between treatments. The UKPDS indicates that metformin may be the preferred agent for obese type 2 patients.

A total of 1,148 patients with both type 2 diabetes and hypertension were randomised to two groups: the treatment group striving for tight blood pressure control as the goal, and the control group striving for less tight control. Both groups were followed for a median of 8.4 years. The tight-control group achieved a mean blood pressure of 144/82 mm Hg compared to a blood pressure of 154/87 mm Hg in the less tight group. This statistically significant reduction in blood pressure reduced the risk of developing:

- any diabetes-related endpoint by 24% (P =0.0046);
- diabetes-related deaths by 32% (P =0.019);
- strokes by 44% (P =0.013);
- microvascular complications by 37% (P =0.0092);
- heart failure by 56% (P =0.0043);
- retinopathy progression by 34% (P =0.0038);
- deterioration of vision by 47% (P =0.0036).

Again, no threshold exists for these complications—the lower the systolic value (above a normal of 130 mm Hg), the lower the risk of these complications. Both captopril and atenolol were equally efficacious in reducing the risks of both microvascular and macrovascular complications. This implies, like blood glucose control, that lowering blood pressure (or blood glucose) is more important than the agent used. However, the data indicated that lowering blood pressure in patients with type 2 diabetes may be difficult, and that up to one-third of the patients will require three or more agents to achieve the desired level of control.

2.2.5 Treatment of Type 2 Diabetes

The aims of treatment are to relieve acute symptoms, improve quality of life and prevent long-term complications without precipitating hypoglycaemia. Good glycaemic control is essential to reduce the risk of microvascular disease [57, 54]. It is estimated that at the time a T2D diagnosis is made, 20% of patients already have diagnosable coronary artery disease and retinopathy. The usual first step in treatment of T2D involves non-pharmacological therapies. These therapies are instituted in an effort to improve both good glycaemic control and to begin the process of helping patients make healthy life style changes.

Non-Pharmacological Treatment

Modification of the nutrition is the first task that is undertaken. Treatment goals of the nutrition therapy include maintenance of normal blood glucose, optimum blood lipid level, and weight loss. Self blood glucose monitoring (SBGM) is taught, an exercise programme within the patient's limitations is strongly encouraged and smoking

is discouraged. Adoption of these lifestyle changes will improve insulin sensitivity and lower blood glucose with favourable effects on blood pressure and lipid profile. These recommendations should be reinforced regularly.

Pharmacological Treatment

If treatment goals are not achieved after a trial of dietary and lifestyle changes, an oral hypoglycaemic should be prescribed. In the UKPDS only 23% of patients allocated to diet alone attained fasting plasma glucose (FPG) levels below 7.8mmol·L^{-1} [57, 54] Oral hypoglycaemics currently available include the sulphonylureas, metformin and acarbose. Choice of drug will depend on body weight and clinical status. Metformin is the drug of choice in obese patients, otherwise a sulphonylurea is prescribed. These medications are begun alone and if glucose control is not achieved, another class of drug is added.

Sulphonylureas. The sulphonylureas have been the mainstay of T2D therapy over 40 years. There are currently six available on the market:

- chlorpropamide and tolbutamide (first generation) and
- glibenclamide, glipizide, gliclazide and glimepiride (second generation).

Sulphonylureas stimulate insulin secretion by acting directly on β-cells [101]. They bind to a cell surface receptor that induces the closure of adenosine triphosphate-dependent potassium (K_{ATP}) channels, leading to membrane depolarisation and influx of extracellular calcium. The increase in intracellular calcium prompts insulin secretion. At maximum dosages, sulphonylureas can lower fasting glucose concentrations by 3.3-3.9 mmol·L^{-1} and reduce HbA_{1C} concentrations by 1.5-2% [82]. Because of their insulin-stimulating action, these agents are associated with an increased risk of hypoglycaemia, especially in the elderly. Sulphonylureas may also produce weight gain [57]

Metformin. Metformin, a biguanide, improves insulin sensitivity in the liver (reducing hepatic glucose output) and in the peripheral tissues (promoting glucose uptake) [11]. When used at maximum dosage as monotherapy, metformin has a comparable efficacy to sulphonylurea in reducing FPG (2.2-3.3 mmol·L^{-1} reduction) and HbA_{1C}

concentrations (1-2% reduction) [39, 45]. This agent has beneficial effects on lipid profiles and may even promote some weight loss [39, 45]. Its most common side effect is gastrointestinal disturbances. Metformin is also associated with lactic acidosis [61], and it should not be used in patients with impaired renal or hepatic function.

Combination Therapy. The natural history of type 2 diabetes includes progressive decline in the β-cell function and concomitantly increased hyperglycaemia, which invariably leads to failure to achieve an adequate glycaemic control with monotherapy [123]. The complementary mechanism of action of available insulin secretagogues and insulin-sensitising agents has demonstrated an additive, and possibly even synergistic effects when used in combination.

Treatment with metformin and a second-generation sulphonylurea has been the most widely used combination. Monotherapy with metformin or glyburide produce comparable levels of glycaemic control in patients with sulphonylurea failure, whereas combination therapy achieved additional reductions in FPG (3.3-3.9mmol$\cdot$L^{-1}) and HbA_{1C} (2%) over the effects of ongoing sulphonylurea treatment [39]. Moreover, combination therapy attenuated the weight gain associated with sulphonylurea treatment and lowered low density lipoprotein (LDL) cholesterol and triglyceride concentrations [39]. Similar effects are seen with combination therapy using repaglinide and metformin [93].

Acarbose. Acarbose slows the absorption of carbohydrates through the inhibition of intestinal alpha-glucosidase, thus reducing postprandial hyperglycaemia. Up to two-thirds of patients experience significant gastrointestinal flatulence, abdominal pain, and diarrhoea. A slow dose escalation may limit these adverse effects, but they also reduce patient acceptance and limit its use as a primary therapeutic drug.

Repaglinide. Repaglinide works similarly to the sulphonylureas in that it increases β-islet release of insulin. The drug can be given just before meals and still improves the cellular uptake of carbohydrates consumed.

2.3 Mathematical Models to Assess Insulin Sensitivity and Insulin Secretion

2.3.1 Minimal Model of Glucose Kinetics

Introduction

There is a general consensus that the glucose clamp technique, particularly in its euglycaemic version, is the best available reference for the measurement of insulin action. However, the clamp technique has a number of limitations. The disadvantages of the clamp method are its requirements (two intravenous lines, calibrated pumps, and online plasma glucose level determination) and the need for trained personnel. The complexity in the methodology, the cost, the number of doses needed to assess a full spectrum of insulin resistance and the problem of achieving a steady-state limit its use to research laboratories. The clamp techniques cannot be adapted for the assessment of insulin resistance in the clinical setting.

In an attempt to develop a more practical method of measuring insulin resistance that could be applied to larger populations, Bergman et al developed the minimal model [16].

The Minimal Model Analysis

The minimal model is a development of the intravenous glucose tolerance test (IVGTT), a time-dependent approach for the assessment of insulin sensitivity. The minimal model proposed by Bergman et al [16] accounts for both insulin and glucose concentrations during IVGTT by using a simple mathematical representation of the glucose-insulin relationships.

The minimal model describes the glucose disappearance curve with two differential equations. One equation represents glucose kinetics, thus assuming a single-compartment model for glucose distribution, the other equation describes the insulin effect, which is assumed to take place in the compartment remote from plasma (the effect compartment). The insulin sensitivity index of the minimal model represents the link between insulin levels in the effect compartment and glucose disappearance from the glucose compartment. The fractional disappearance rate (min^{-1}) from the glucose compartment is assumed to be a linear function (S_G+S_IX) where X is the increment over the

basal insulin concentration in the effect compartment. S_I (min^{-1}·per·pmol·L^{-1}) is the insulin sensitivity index and S_G (min^{-1}) is the glucose effectiveness.

By using the measured insulin concentration as the input to the model, insulin sensitivity and glucose effectiveness are estimated by least squares fitting of the IVGTT glucose concentration profile.

An inherent limitation of the minimal model analysis of the IVGTT is that it requires a discrete insulin response, that is, insulin concentrations that rise detectably and consistently above basal. As a result the minimal model analysis of the IVGTT fails for insulin-deficient subjects, for no effect of insulin on glucose disappearance rate is seen. In this situation, the IVGTT protocol is modified to include an intravenous bolus of tolbutamide (to stimulate endogenous insulin secretion) or a brief exogenous insulin infusion, both administered 20 minutes after the injection of the glucose bolus.

The so-called one compartmental minimal model (1CMM) of glucose kinetics during IVGTT (Bergman et al [16]; Bergman et al [14]), is shown in Figure 2.1. The key features of this model are

- glucose kinetics are described by a single-compartmental model;
- glucose inhibition of production and simulation of utilisation is proportional to glucose plasma concentration;
- insulin inhibition of glucose production and simulation of glucose utilisation is proportional to insulin concentration in a compartment remote from plasma.

The model does assume that during an IVGTT the inhibition of glucose production and the stimulation of glucose utilisation have similar functional descriptions. This allows one to combine the controls exerted by insulin and glucose on glucose production and utilisation and make the model uniquely identifiable.

The one-compartmental model in its uniquely identifiable parameterisation form is given by

$$\dot{Q}(t) = -[p_1 + X(t)]Q(t) + p_1 Q_b \qquad Q(0) = Q_b + D_{iv} \tag{2.1}$$

$$\dot{X}(t) = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \tag{2.2}$$

$$G(t) = \frac{Q(t)}{V} \tag{2.3}$$

where Q is the glucose mass ($mg \cdot kg^{-1}$), with Q_b denoting its basal (end-test) steady state value; D_{iv} the glucose dose ($mg \cdot kg^{-1}$); X is a variable related to insulin concentration (derived from basal) in a compartment remote from plasma, $X(t) = (k_4+k_6)I'(t)$, where k_4 and k_6 are rate parameters (min^{-1}); I(t) is the plasma insulin concentration ($\mu U \cdot ml^{-1}$), with I_b denoting its basal value; G is the plasma glucose concentration, with G_b denoting its basal (end-test) value ($\mu mol \cdot L^{-1}$); V is the distribution volume per unit body weight ($ml.kg^{-1}$); and $p_1=k_1+k_5$, $p_2=k_3$ and $p_3=k_2(k_4+k_6)$ are rate parameters expressed in min^{-1}, min^{-1}, and min^{-2} per $\mu U^{-1}ml$, respectively. Clearly one has $Q_b=G_bV$.

From the above 1CMM, we derive the indices of glucose effectiveness, S_G, at basal insulin, and insulin sensitivity, S_I.

The glucose effectiveness, S_G, is given as

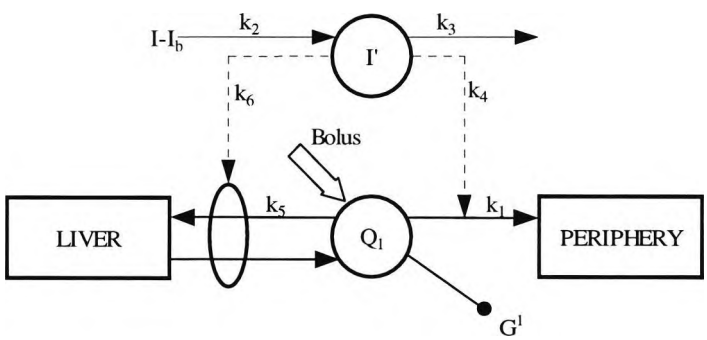
$$S_G = [-\frac{\partial \dot{Q}(t)}{\partial\, G(t)}]_{ss} = p_1 V = S'_G V \qquad (ml \cdot min^{-1} \cdot kg^{-1}) \tag{2.4}$$

and insulin sensitivity, S_I, is given as

$$S_I = [-\frac{\partial \dot{Q}(t)}{\partial\, I(t) \partial\, G(t)}]_{ss} = \frac{p_3}{p_2} V = S'_I V \qquad (ml \cdot min^{-1} \cdot kg^{-1} \cdot (\mu U \cdot ml^{-1})^{-1}) \tag{2.5}$$

where $S'_G = p_1$ and $S'_I = \frac{p_3}{p_2}$.

Figure 2.1: The classical one-compartment minimal model (1CMM).



The controversy [23] with this model is whether the adoption of a one-compartmental model is quite adequate for estimating the metabolic indices, S_I and S_G in glucose kinetics during an IVGTT. It is being argued that one-compartmental minimal model tends to overestimate S_G and underestimate S_I when its results are compared with that of the analogous glucose clamp-based indices. This has recently been investigated by Cobelli et al using a two-compartmental minimal model [29].

Application of the Minimal Model

The classical minimal model of glucose kinetics [16] is commonly used to estimate metabolic indices of glucose effectiveness (S_G) and insulin sensitivity (S_I) in vivo in both normal and pathophysiological conditions. Its application is widespread, and the model has been used to examine alterations in S_I in a variety of physiological and pathophysiological states such as in lean and obese subjects [9, 78], examining the dynamic interaction between glucose metabolism and endogenous insulin release in hypertension [95], examining the influence of positive family history of T2D on aspects of insulin resistance in prepubertal children [52], and in subjects with myocardial infarction and the associated insulin resistance before the age of 40 years [24].

The classical minimal model of glucose kinetics is simple and has provided valuable epidemiological data While the test is useful for a broader number of studies in clinical settings, it still not suitable for population studies. This is because of the complexity and large number of samples, the duration of the test, the complicated data analysis, and the cost of the test.

Sampling Schemes

Minimal model quantitative assessment of whole body glucose metabolism during IVGTT involves blood sampling to estimate insulin sensitivity and glucose effectiveness. Usually, subjects are studied after an overnight fast for 12 hours. To adequately and accurately define the glucose/insulin-time relationships in the body, sampling with 30 blood samples (full sampling scheme; FSS) is used. The use of the full sampling has limited the use of the minimal model of glucose kinetics in large population studies.

Many studies have advocated [28, 117] the use of reduced sampling schemes (RSS) in order to give the minimal model approach the wider application area it deserves.

2.3.2 Insulin Secretion Model

Introduction

The insulin secretion model, see Figure 2.2, is based on the analysis of glucose and C-peptide secretion time-concentration profiles during the meal tolerance test [68]. The model gives two mechanistic β-cell indices. The first index represents fasting C-peptide secretion normalised (divided) by fasting glucose (fasting β-cell responsiveness M_O).

The second index represents the ability of postprandial glucose to stimulate C-peptide secretion and corresponds to the C-peptide increment in secretion per unit elevation of postprandial glucose (postprandial β-cell responsiveness M_I).

Insulin Secretion Model

The model assumes that C-peptide secretion is linearly related to the plasma glucose concentration. The linear relationship is imposed from the time of meal ingestion until the time plasma glucose returns to its fasting concentration. The model is described by a set of differential equations

$$\frac{dc_1(t)}{dt} = -(k_{01} + k_{21})c_1(t) + k_{12}c_2(t) + u(t) \qquad c_1(0) = \frac{u(0)}{k_{01}} \tag{2.6}$$

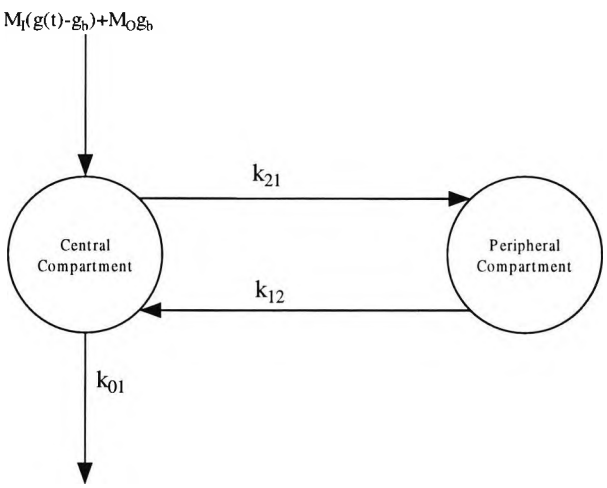
$$\frac{dc_2(t)}{dt} = k_{21}c_1(t) - k_{12}c_2(t) \qquad c_2(0) = \frac{c_1(0)k_{21}}{k_{12}} \tag{2.7}$$

$$u(t) = \max(0, M_I(g(t) - g_b) + M_O g_b) \tag{2.8}$$
$$0 \leq t \leq t_{\max}$$

where $c_1(t)$ is C-peptide concentration in the central (plasma) compartment (mmol·L^{-1}), $c_2(t)$ is equivalent concentration in the peripheral compartment (mmol·L^{-1}), i.e. the amount of the C-peptide in the peripheral compartment per unit volume of the central compartment, k_{ij} are transfer rate constants (min^{-1}), g(t) is plasma glucose concentration, u(t) is the secretion rate of C-peptide per unit volume of the central compartment and is constrained to non-negativity value, t_{max} is either 180 min or the time when plasma glucose returns to its fasting value, g_b is the fasting glucose concentration. M_I (postprandial sensitivity index) represents the ability of postprandial glucose to stimulate β-cells. A change in plasma glucose by 1 mmol·L^{-1} results in a change in the C-peptide secretion rate by M_I pmol·L^{-1}·min. M_O (fasting sensitivity index) is the ability of fasting glucose to stimulate β-cells. M_O is numerically equal to the fasting C-peptide concentration divided by the fasting plasma glucose concentration.

The population model of C-peptide kinetics determines parameters k_{21}, k_{12}, and k_{01} from a subject's demographic data using a regression model and avoiding the need to assess C-peptide kinetics on an individual basis. The model parameters M_I and M_O are estimated using weighted regression analysis. The plasma glucose concentration

Figure 2.2: Insulin secretion model.



is taken as the model input while the C-peptide concentration is taken as the model output.

Application of the Insulin Secretion Model

The insulin secretion model was validated in healthy subjects and subjects with T2D [68], M_O and M_I indices have been investigated to assess in vivo pancreatic β-cell responsiveness in man to facilitate further understanding of β-cell dysfunction and its relationships with other variables [68, 70, 67, 5].

2.3.3 Other Methods

Assessment of Insulin Sensitivity

Glucose Clamp. The most accepted research method or 'gold standard' is the euglycaemic glucose clamp technique originally developed by Andres et al [8] and widely studied by DeFronzo et al [38]. With the euglycaemic (or insulin) clamp technique, exogenous insulin is administered as a priming dose followed by a constant infusion at a rate designed to maintain a pre-set hyperinsulinaemic plateau; simultaneously, the plasma glucose concentration is clamped at the normal fasting or pre-existing level by means of an exogenous infusion of glucose. By doing this, insulin action is measured

in vivo under comparable conditions of stimulus (the plasma insulin concentration) and substrate (the plasma glucose concentration). When a steady state is attained, the exogenous glucose infusion rate equals the amount of glucose disposed of by all the tissues in the body and thus provides an estimate of overall insulin sensitivity. The more glucose that has to be infused per unit time, then the more sensitive the patient is to insulin. Conversely, the insulin-resistance patient requires much less glucose to maintain basal plasma glucose levels.

The advantage of this test is that the effect of insulin on fuel metabolism can be assessed in the absence of the confounding effects of hypoglycaemic counterregulation, endogenous insulin secretion, or variable levels of hyperglycaemia. Another advantage is that multiple insulin actions can be assessed by using isotopes, including regulation of glucose uptake and production, inhibition of lipolysis, and changes in protein metabolism.

The clamp technique has a number of limitations. To assess the full spectrum of insulin resistance, several doses of insulin may be needed, and a steady state must be achieved for each dose. The test does not reproduce physiological conditions, in which both plasma glucose and insulin change. Most importantly, the complexity and cost of the procedure limits its use to research laboratories, in which scientific questions can be addressed in limited numbers of subjects.

Two-Compartmental Minimal Model (2CMM). The drawback of the minimal (one-compartmental) model of glucose kinetics has been suggested to be overcome by an improved version, the two-compartmental minimal model, when a second compartment is appended to the accessible one and a Bayesian approach is used to incorporate a priori knowledge on the exchange rate parameters, k_{21} and k_{12}, between the accessible and the nonaccessible compartments. The results in normal subjects showed that this approach provides estimates of S_G and S_I that are, respectively, 60% lower and 35% higher [29] than the corresponding indices, S_G and S_I, of one-compartmental minimal model.

The two-compartmental minimal model structure is defined by the following first order differential equations

$$\dot{Q}_1(t) = -[p_1 + k_{21} + X(t)]Q_1(t) + k_{12}Q_2(t) + p_1Q_{1b} \qquad Q_1(0) = Q_{1b} + D_{iv} \tag{2.9}$$

$$\dot{Q}_2(t) = k_{21}Q_1(t) - k_{12}Q_2(t) \qquad Q_2(0) = Q_{2b} \tag{2.10}$$

$$\dot{X}(t) = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \tag{2.11}$$

$$G^1(t) = \frac{Q_1(t)}{V} \tag{2.12}$$

where Q_1 and Q_2 ($mg \cdot kg^{-1}$) denote the glucose masses in the accessible and non-accessible compartments respectively, with suffix b denoting their basal steady-state values; V is the volume of the accessible compartment ($ml \cdot kg^{-1}$); k_{12}, k_{21} (min^{-1}) are the rates of parameters describing glucose kinetics; X is the variable related to insulin concentration (deviation from basal) in the compartment remote from plasma, $X(t) = (k_4+k_6)I'(t)$ where k_4, k_6 are parameters (min^{-1}); I(t) is the plasma insulin concentration ($\mu U \cdot ml^{-1}$) with I_b denoting basal value; G^1 is plasma glucose concentration with G_b denoting its basal value; $p_1=k_1+k_5$ (min^{-1}), $p_2=k_3(min^{-1})$, $p_3=k_2(k_4+k_6)(min^{-2} \cdot \mu U^{-1} \cdot ml)$ are rate parameters; $Q_{1b}=G_bV_1$, $Q_2(0)=\frac{k_{21}Q_{1b}}{k_{12}}$ from a steady-state constraint.

From the above 2CMM, we calculate the indices of glucose effectiveness, S_G, at basal insulin, and insulin sensitivity, S_I. The glucose effectiveness, S_G, is given as

$$S_G = [-\frac{\partial \dot{Q}_1(t)}{\partial\, G(t)}]_{ss} = p_1 V = S'_G V \qquad (ml \cdot min^{-1} \cdot kg^{-1}) \tag{2.13}$$

and insulin sensitivity, S_I, is given as

$$S_I = [\frac{\partial \dot{Q}_1(t)}{\partial\, I(t) \partial\, G(t)}]_{ss} = \frac{p_3}{p_2} V = S'_I V \qquad (ml \cdot min^{-1} \cdot kg^{-1} \cdot (\mu U \cdot ml^{-1})^{-1}) \tag{2.14}$$

where $S'_G = p_1$ and $S'_I = \frac{p_3}{p_2}$.

The 2CMM differs from the 1CMM only in allowing an exchange of glucose between the accessible and nonaccessible compartment.

Homeostatic Model Assessment (HOMA) and Constant Glucose Infusion with Model Assessment (CIGMA). With the homeostatic model assessment and constant infusion of glucose with model assessment approaches, insulin sensitivity is determined from the steady (or nearly-steady) glucose and insulin concentrations measured under basal conditions (HOMA) or after a standardised, 1 hour intravenous glucose infusion (CIGMA) [65] [90]. Insulin sensitivity is expressed as an index of relative insulin resistance, R (dimensionless or a percentage), which is calculated as a function of measured glucose and insulin levels.

In HOMA, the measurement of just basal glucose and insulin concentration is sufficient to detect insulin resistance on a quantitative basis. However, in general it cannot be

taken for granted that two subjects with the same value of glucose-insulin concentration product, that is the same R, have the same insulin sensitivity. Moreover, with HOMA the site of insulin resistance, hepatic versus peripheral, remains undetermined, whereas the standard clamp essentially assesses peripheral tissue insulin resistance. CIGMA, being a sort of simplified hyperglycaemia clamp, is more informative. However, the interpretation of R remains unclear, particularly when the insulin response is insufficient to stimulate glucose uptake, as occurs in insulin-deficient subjects. The important advantage of the HOMA and CIGMA tests is that the experiments are simple.

Quantitative Insulin Sensitivity Checked Index (QUICKI). QUICKI [76] is based on the steady-state (or quasi-steady-state) glucose and insulin concentrations that are achieved after an overnight fast. It defines insulin sensitivity as proportional to the inverse of the log of the product of fasting insulin and glucose concentrations.

The expression used as an index of insulin sensitivity is

$$QUICKI = \frac{1}{[\log(I_o)+\log(G_o)]}$$

where I_0 and G_0 are the fasting insulin and glucose. This was obtained by examining a variety of transformations of these fasting data and choosing the one that correlates best to S_{Iclamp}.

QUICKI has demonstrated good correlation with the "gold standard" method, or the hyperinsulinaemic euglycaemic clamp [76]. In this regard, it seems to compare favourably with the minimal model approach.

Insulin Concentration. Blood insulin circulation has been widely used as a surrogate for estimating insulin sensitivity. A predicted output of the insulin-glucose feedback is that the worse the insulin resistances the higher the plasma insulin concentration. Both fasting and post-glucose plasma insulin levels have been used: the product of fasting insulin and fasting plasma glucose levels in addition to their ratio have likewise been proposed as proxies for insulin action. When the insulin sensitivity estimates obtained from these procedures are tested against insulin sensitivity measured by euglycaemic clamp, all are found to have a low correlations with the euglycaemic clamp estimates, and no matter what transformation or manipulation of the indices of these surrogate estimates, the correlation values remains low. This result clearly arises from the fact that insulin levels, in addition to tissue insulin sensitivity, also depend on

secretion, distribution, and degradation of insulin. Glucose level on the other hand, is controlled by more factors than just insulin.

Insulin Tolerance Test. This is the first method developed to evaluate insulin sensitivity in vivo. It is based on the measurement of rate of decay of plasma glucose level after a bolus injection of regular insulin (0.1 U. kg^{-1} body weight). On plotting plasma glucose concentrations measured every 5 minutes from 10 to 40 minutes after the intravenous injection on a semi-logarithmic scale, a linear decline is observed in most cases. The slope of the line, given as K_{ITT}, can be calculated simply (0.693 divided by the plasma glucose half time) and used to rank insulin sensitivity, that is, the greater the slope the better the insulin sensitivity. The assumption behind this method is that the glucose system is a single compartment, from which insulin accelerates the net disappearance of the substrate both by promoting its uptake into target tissues and by shutting off the endogenous production. By also assuming a glucose distribution volume (usually, 200-250 ml . kg^{-1}), a clearance rate can be calculated by:

$$MCR = E \times flow\ rate = kV \tag{2.15}$$

where MCR is the metabolic clearance rate, k is the average disappearance rate, V is the glucose distribution volume, and E is the extraction ratio. Either the K_{ITT} index or the glucose clearance is found to correlated to clamp-derived estimates of insulin sensitivity [21]. K_{ITT} is dependent on the time interval over which it is calculated, because glucose disappearance is not a mono-exponential but rather a multi-exponential process.

The principal drawback of this test is hypoglycaemia, which in addition to be unpleasant, can cause neurological and cardiovascular side effects, particular in diabetic and elderly subjects with diffuse atherosclerotic disease. In addition, hypoglycaemic counter-regulation will antagonise the insulin effect, thereby contaminating the insulin sensitivity estimate.

Insulin-Suppression Test. The insulin-suppression test is a reverse clamp, by which the exogenous glucose infusion rate during infusion of insulin is constant while plasma glucose concentration is allowed to vary: at a steady state, the higher the level of hyperglycaemia attained the worse the insulin sensitivity. Because the experimentally induced hyperglycaemia will stimulate endogenous insulin release, two different approaches to suppress β-cell response have been taken, namely:

1. The quadruple-infusion technique devised by Shen et al [114]. The main problem with this method is that the biological effect of adrenaline on glucose metabolism may be incompletely or unpredictably blocked by propranolol. In addition, adrenaline can cause significant disturbances of cardiac rhythm even in the present of propranolol, the use of the quadruple-infusion technique in patients is somewhat hazardous.
2. The modification of insulin-suppression test, first introduced by Harano et al [63], which has virtually replaced the quadruple-infusion technique.

Problems with the insulin-suppression test are that plasma glucose concentration may not stabilize satisfactory over infusion period and, in very sensitive subjects, may occasionally drop below baseline.

Circulatory Model. Another method is based on the circulatory model by Mari et al [88]. Circulatory models represent the glucose system rather more realistically then compartmental models do. Glucose kinetics in the organ blocks encompassed by the circulatory loop are mathematically described using techniques derived from the so-called model-independent methods developed by Meier et al [91] and Zeirler et al [137]. Arbitrary assumptions such as those required by multi-compartmental analysis to specify model configuration are not needed. The circulatory model parameters reported for normal subjects and subjects with T2D agree with the respective literature values [89], although comparison with more established methods has yet to be made.

A comprehensive overview of insulin sensitivity measurement is provided in Ferrannini et al [41] and a short review is provided in [10].

Assessment of Insulin Secretion

Hyperglycaemic Clamp. The hyperglycaemic clamp [38] is the 'gold standard' for assessing pancreatic responsiveness. During the hyperglycaemic glucose clamp, plasma glucose in rapidly elevated by an exogenous bolus and maintained by a variable infusion of glucose to produce a desired circulating glucose level, thus stimulating the endogenous insulin secretion. The extent of the stimulation is regarded as an index of pancreatic β-cell responsiveness, and the response is usually evaluated in terms of plasma insulin concentrations. The exogenous glucose injection is followed by a frequent sampling schedule to enable the evolution of early pancreatic response. Samples are taken at basal states before the glucose administration.

Minimal Model of C-peptide Secretion during IVGTT. The model [30] accounts for the effect of glucose on C-peptide concentration during IVGTT. It provides the time course of C-peptide secretion CpS(t), the values of parameters Φ_{1Cp} and Φ_{2Cp} which represent the first and the second phase β-cell responsiveness to glucose respectively, and k_{01}, the C-peptide fractional clearance, i.e. the peptide disappearance in unit time per unit volume. CpS(t) equals β-cell insulin secretion because of the equimolarity between the two peptides. Basal insulin secretion, BSR, is computed as the product between clearance k_{01} and basal C-peptide concentration.

Minimal Model of Insulin Secretion during IVGTT. The model was conceived to account for the effect of glucose on the time course of plasma insulin [121]. It provides the time course of posthepatic insulin deliver IDR(t), and n, the peripheral fractional clearance rate of insulin. Basal posthepatic insulin delivery, BDR, is the product between n and the basal insulin concentration.

Combined Model of Insulin and C-peptide Secretion during IVGTT. The use of C-peptide and insulin models allows the reconstruction of the time course of the hepatic insulin extraction, H(t), which is computed as the difference of CpS(t)-IDR(t), normalised to CpS(t) [30].

2.4 Population Modelling

2.4.1 Introduction

Population modelling consists of several stages. A kinetic model describes the flow of glucose emphasising several parameters for each individual. A population model describes the distribution of the parameters in the population as a function of several population parameters, which in turn have a prior distribution based on existing knowledge. Lastly, the measurement model describes the distribution of deviations of the data from their expected values predicted by the glucose kinetic model.

2.4.2 Modelling Stages

Notation

In formulating a population model, we use the following notation. The observed responses corresponding to covariates x_{ij} will be denoted by y_{ij}. The number of individuals from the population will be denoted by N (so that i = 1,...,N) and the number of observations made on an individual will be denoted by n_i (so that j = 1,...,n_i). In general the number and values of covariates will differ from individual to individual, but in cases where the same sample design is used for each individual, and there are no missing observations, n_i and x_{ij} will not require subscript i.

Individual Measurement Model

We define the structure of the kinetic model for the i^{th} individual, i = 1,...,N. For each individual, we have the model:

$$y_{ij} = f(\boldsymbol{\theta}_i, t_{ij}) + e_{ij} \tag{2.16}$$

where y_{ij} is the j^{th} measurement, j=1,...n_i, for the i^{th} individual at time t_{ij}; f is the model function; $\boldsymbol{\theta}_i$ is the vector of random parameters of the kinetic model; and e_{ij} is the zero-mean measurement error. The measurement error is assumed independent from observation to observation, whose variance might either be constant for all observations [$V(e_{ij}) = \sigma^2$)] or constant within individuals but varying across individuals [$V(e_{ij}) = \sigma_i^2$] or functionally related to the mean value $f(\boldsymbol{\theta}_i,t_{ij})$ [$V(e_{ij}) = \sigma^2 f(\boldsymbol{\theta}_i,t_{ij})$].

Population Structure Model

From a sample of individuals identified as being from the same population, the same general form of functional relationship, f, is assumed to hold between the expected response and the covariates of each individual, but the parameters, $\boldsymbol{\theta}_i$, determining the precise shape of profile for each individual are assumed to vary across the population in a manner of a random sample. It is then often reasonable to assume the following reparameterisation of the individual components of the parameter vector, that

$$\boldsymbol{\theta}_i = \boldsymbol{\alpha} + \boldsymbol{\phi}_i$$

The θ_i is a known function of parameter α which does not vary across the population; it is known as a fixed effect, and of parameter ϕ_i which changes across the population; it is known as a random effect

$$\theta_i = g(\alpha, \phi_i) \tag{2.17}$$

For this reason, these models are called mixed effects models. Moreover, in the parametric approach, the assumption is usually made that the random effects ϕ_i are normally distributed with mean μ and covariance Σ

$$\phi_i \sim N(\mu, \Sigma) \tag{2.18}$$

this case, the population kinetic analysis problem is to estimate mean μ and covariance Σ based on the population data.

Two approaches are used to facilitate estimation of this population distribution

- the parametric approach and
- the nonparametric approach.

In the parametric approach, the population distribution is assumed to be known except for the population parameters, for example, the distribution can be assumed to be multivariate normal with unknown mean and covariance. Hence, the population analysis problem is then the estimation of both mean and the covariance. In the nonparametric approach, no assumption is made about the underlying population distribution, the entire distribution is estimated from the population data. A third approach that falls in-between the above mentioned approaches is called semi-nonparametric approach. We use only parametric approach in this study. Vicini et al [126] and a chapter on parameter estimation [23], provide an excellent review of population kinetic analysis and parameter estimation.

There are two basic classes of methods for obtaining estimates of mean μ and covariance Σ

- Maximum likelihood estimation (MLE)

- Bayesian methods.

Maximum likelihood methods are based on the maximisation of the likelihood function for the population problem, while the Bayesian methods use Bayesian inference approaches to estimate the full conditional population distribution.

2.4.3 Population Methods/Statistical Methods

Introduction

The discussion of population methods here will be focused only on two methods that provide estimates of population means and covariances, the standard two-stage approach, and the Bayesian approach. Therefore, both the naive averaged data approach, Steimer et al [118], and linearisation approach, the NONMEM [12], will not be discussed.

Two-Stage Approaches

Standard Two-Stage Approach. In the first stage, each individual's parameters are estimated. In the second stage, individual estimates in the first stage serve as input data for the second stage calculation of the corresponding population parameters $(\boldsymbol{\mu}, \boldsymbol{\Sigma})$. Analysis of dependencies between parameters and covariates using classical statistical approaches (linear regression, covariance analysis, cluster analysis) may be included in the second stage. These methods can be nonparametric, as in the Standard Two-Stage approach; or parametric, as in the global two-stage approach.

Iterative Two-Stage. The iterative two-stage (ITS) method is a parametric iterative method belonging to the family of expectation-maximisation (EM) algorithms, based in particular on the maximum a posteriori probability (MAP) empirical Bayes estimator. It was proposed as a possible computationally attractive alternative to the nonlinear mixed-effects modelling approach [118]. The ITS has been used in both pharmacokinetics and metabolic studies to estimate population parameter mean $(\boldsymbol{\mu})$ and variances $(\boldsymbol{\Sigma})$ from reduced data sets [128].

Bayesian Hierarchical Models

Bayesian Method. The Bayesian method was first introduced to the population kinetic analysis by Wakefield et al [132], in which the Gibbs sampler was used for estimating highly dimensional parameters. The Bayesian methods consider the complete population sample, rather then the individual as a unit of analysis. The population methods of analysis aim to estimate the distribution of the parameters and their relationships with covariates. The method uses individual data of the observational or experimental type, which may be unbalanced or fragmentary, in addition to or instead of conventional data from traditional studies characterised by rigid and extensive design. The Bayesian population kinetic analysis exploits the statistical framework of nonlinear hierarchical random effects model and provides estimates of population characteristics that define the population distribution of the population kinetic parameters. This is achieved by describing each hierarchical stage in a probability context, regarding every variable in the model as random and estimating directly the parameters of the population from the full set of individual concentration values. The individuality of the subject is maintained and accounted for, even when the data are sparse. See Davidian and Giltinan [35] for an excellent survey of two-stage methods and population kinetic analysis.

Hierarchical Model. Many statistical applications involve multiple parameters that can be regarded as related or connected in some way by the structure of the problem, implying that a joint probability model for these parameters should reflect the dependency among them. For example, estimating metabolic indices, S_I and S_G, using the glucose kinetics minimal model described above, Figure 2.1, with an i^{th} individual having an estimated parameter $\boldsymbol{\theta}_i$, it might be reasonable to expect that estimates $\hat{\theta}_i$, which represent a sample of individual, should be related to each other. This could be achieved by using an a priori distribution in which $\boldsymbol{\theta}_i$ are viewed as a sample from a common population distribution. A key feature of the hierarchical model is that the observed data y_{ij}, j = 1,...n_i and i = 1,...,N, as described in Section 2.16 can be used to estimate an aspect of the population distribution of $\boldsymbol{\theta}_i$ even though the values of $\boldsymbol{\theta}_i$ are not themselves observed.

A relevant Bayesian framework for simultaneously modelling of intra-individual and inter-individual variability requires a three-stage hierarchical model. At the first-stage, intra-individual variability is characterised by a linear or nonlinear regression model

with a model for the individual covariance structure. Inter-individual variability is represented in the second-stage through individual specific parameters, which may incorporate both systematic and random effects. A relevant model and statistical framework will be described in the next chapter, where we will consider a population approach to the minimal model of glucose kinetic in hierarchical form.

2.4.4 Methods of Statistical Inference

Introduction

In implementing the Bayesian methods, the need to evaluate integrals occurs at many stages. Calculating expectations, forming marginal distributions and predictive distributions, eliminating nuisance parameters, and so on, all require integrals to be evaluated. For relatively simple models, the integrals can frequently be determined analytically if prior information corresponds to a natural conjugate prior distribution. For more complex models, for example, where the conjugate distribution is inappropriate, numerical approximation methods are usually needed to estimate the integrals. One such method that is straightforward to implement and which has a wider application in non-linear hierarchical statistical modelling is Gibbs sampling.

Gibbs Sampling

Gibbs sampling is based on Markov chain Monte Carlo (MCMC) methods [49, 120, 115]. MCMC is a general method for sampling from stochastic multivariate processes and computing expectations. This idea was first used by Wakefield et al [132] for population PK models using a Gibbs sampler. The idea behind such methods is to construct a Markov chain whose stationary distribution is the required posterior distribution.

Metropolis-Hasting methods

A drawback of the Gibbs sampler is that conditional distributions must be known completely but, in most applications, these distributions are only known to within a constant of proportionality. Such is the case of our, non-standard, conditional distribution of the parameters that will be described in the model formulation and implementation section in Chapter 3, where we will not know the constant of proportionality. This prevents the use of a Gibbs sampler, but there are other methods that can still be

used. The best known of these methods is the Metropolis-Hastings algorithm, which we describe briefly below.

The Metropolis-Hastings algorithm was developed by Metropolis and subsequently generalised by Hastings [92, 64].

Let $q(\theta, \vartheta)$ be an arbitrary proposal density such that

$$\int q(\theta, \vartheta) d\vartheta = 1 \tag{2.19}$$

Also let U(0, 1) denote the uniform distribution over (0,1). Then, a general version of the Metropolis-Hastings algorithm for sampling from a posterior distribution $\pi(\theta|D)$ can be described as follows:

Metropolis-Hastings Algorithm.

Step 0. Choose an arbitrary starting point θ_0 and set $i = 0$.

Step 1. Generate a candidate point θ^* from $q(\theta_i, .)$ and u from U(0, 1).

Step 2. Set $\theta_{i+1} = \theta^*$ if $u \leq a(\theta_i, \theta_*)$ and $\theta_{i+1} = \theta$, otherwise, where the acceptance probability is given by

$$a(\theta, \vartheta) = \min \{ \frac{\pi(\theta|D) q(\theta, \vartheta)}{\pi(\theta|D) q(\vartheta, \theta)}, 1 \} \tag{2.20}$$

Step 3. Set $i = i + 1$ and go to Step 1.

The described Metropolis-Hastings algorithm is very general. The Gibbs sampler is obtained as a special case of the Metropolis-Hastings algorithm choosing an appropriate q(θ, ϑ) [47, 27]. The performance of the above algorithm depends on the choice and the spread of a proposed distribution density q of interest [27].

Part I

Methodological Development

Chapter 3

Bayesian Hierarchical Modelling of Minimal Model of IVGTT

3.1 Introduction

The homeostasis of glucose, involving the secretion of its controlling hormone insulin by the pancreas, has been the object of both mathematical and statistical models over the last fifty years [3, 26, 20]. As a result, numerous models have been proposed to quantify the whole-body glucose and insulin kinetics.

The minimal model, introduced in 1979 by Bergman et al [16] to estimate whole-body glucose and insulin kinetics from the intravenous glucose tolerance test (IVGTT) data, has become an invaluable method. The usefulness of the minimal model approach is demonstrated by the growing number of published studies, on both small and large scale [5, 94, 107].

In vivo glucose tolerance is determined by both insulin-dependent and non-insulin-dependent processes. Two important metabolic parameters related to these two processes are estimated by the minimal model - insulin sensitivity (S_I), which characterises insulin action on glucose kinetics, and glucose effectiveness (S_G), which characterises the ability of glucose to promote its own disposal at basal insulin.

Despite the potential advantages of the minimal model analysis in studying glucose metabolism, including its cost-effectiveness and being a single-test procedure, one reported problem with minimal model studies is that, in a number of subjects, especially those with T2D, insulin sensitivity S_I is calculated as $S_I = 0$. The occurrence of S_I values indistinguishable from zero ("zero S_I") in large clinical studies has not been fully understood and whether it represents a physiological relevant phenomenon or a

manifestation of modelling deficiency remains to be investigated. This is because the minimal model analysis of the IVGTT data requires a discrete insulin response (i.e. insulin concentration that rises detectably and consistently above basal). As a result, the minimal model analysis of the IVGTT fails for highly insulin-deficient subjects, where no effect of insulin on glucose disappearance rate is seen [50, 128].

It has been documented that the minimal model fails in up to 50% cases of subjects with highly insulin-deficient subjects [44]. Although with insulin modification, the inclusion of an intravenous bolus of tolbutamide (to stimulate endogenous insulin secretion) or a brief exogenous insulin infusion, both administered 20 min after the injection of the glucose bolus and careful data analysis, the failure rate is normally around 10%. Hence, the use of S_I for measuring insulin sensitivity in vivo is more widely accepted. Godsland and Walton [50] documented higher failure rates of 61% without modification, and showed the importance of basal glucose levels to improve the success rate.

The problem of zero S_I values from the minimal model analysis has been attributed to a possible manifestation of modelling deficiency. Nearly all the minimal model data analysis strategies employed in the literature use a non-linear regression analysis (NLR), employing a gradient-type estimation algorithm to obtain point estimates of insulin sensitivity and glucose effectiveness [100] and, by means of the Fisher information matrix, a measure of its uncertainty expressed as the standard deviation (SD) can be obtained. However, the NLR approach has difficulties in handing possible asymmetries in the probability distribution of the estimates, as a result, negative values are included in the confidence interval. The above interpretation difficulties of the "zero S_I" has generated a wider interest in finding more sophisticated parameter estimation techniques than NLR.

Recently, several others techniques have been proposed to improve the precision of parameter estimates from the minimal model, among them is the Bayesian analysis with the most widely used Markov chain Monte Carlo computational/simulation strategies [48]. The generalised Metropolis Hastings algorithm and the Gibbs sampler have made significant advances as a result of their efficient forms of parameter estimation procedures. These have been adopted to estimate insulin sensitivity and glucose effectiveness by analysing data on an individual basis [103] aiming to reduce the minimal model failures and to provide physiologically plausible confidence intervals (credible intervals within the context of the Bayesian analysis) of the estimates.

The present study extends these recent advances and reports on a population-based hierarchical Bayesian analysis of the minimal model data. The combination of the Bayesian methodology and the hierarchical analysis promised to be suitable to reduce/avoid the minimal model failures and extract correctly and in full all information

Table 3.1: Characteristics of subjects with newly presenting T2D (N = 65; mean ± SE).

Age (years)	54 ± 1
Gender	53/12 (m/f)
BMI ($kg \cdot m^{-2}$)	30.0 ± 0.7
FPG ($mmol \cdot L^{-1}$)	11.0 ± 0.4

such as inter-individual variability from the experimental data. The use of the population analysis is also for the first time investigated for T2D subjects.

3.2 Subject and Experimental Protocol

3.2.1 Subjects

Subjects with newly presenting T2D according to WHO criteria participated in the study, see Table 3.1. The study was approved by the Bro Taf Local Research Ethics Committee, Cardiff, UK.

3.2.2 Experimental Protocol

The subjects were admitted on the study day to the Diabetes Research Unit, Llandough Hospital (Penarth, UK). Subjects were studied after an overnight fast for 12 hours and underwent the insulin-modified intravenous glucose tolerance test (IVGTT) consisting of a $300mg \cdot kg^{-1}$ glucose bolus per body weight given at 0 minute over 2 minutes, followed by an insulin injection of $0.05U \cdot kg^{-1}$ at 20 minutes [99]. Blood samples were collected at -30, -15, -1, 0, 1, 2, 3, 4, 5, 6. 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30,40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 minutes for measurement of plasma glucose and insulin.

3.2.3 Analytical Techniques

Plasma glucose concentration was assayed using the glucose oxidase method (Yellow Spring Analyser, YSI 2300, USA; intra-assay CV $< 2\%$). Insulin was assayed using immunoassay utilising monoclonal antibodies (Dako Diagnostics, Ely, Cambs, UK; intra-assay CV $< 5\%$). Sample collected from the patients were sent to the laboratory

immediately. Blood samples were then centrifuged (2000g, 5mins) in a refrigerated (4^0C) centrifuge and the plasma put into aliquot and frozen at -20^0C immediately. Samples remained frozen until assayed.

3.3 Model Specification

3.3.1 Minimal Model of Glucose Kinetics

The one compartment minimal model (1CMM) of glucose kinetics, in Figure 3.1, defines insulin sensitivity (S_I, ability of insulin to enhance the net glucose disappearance from plasma) and glucose effectiveness (S_G, ability of glucose to promote its own disposal) [16, 15]. It is described in its uniquely identifiable parameterisation form by two differential equations

$$\dot{Q}(t) = -[p_1 + X(t)]Q(t) + p_1 Q_b \qquad Q(0) = Q_b + D_{iv} \tag{3.1}$$

$$\dot{X}(t) = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \tag{3.2}$$

$$g(t) = \frac{Q(t)}{V} \tag{3.3}$$

where Q is glucose mass (mg·kg^{-1}) with Q_b denoting its basal (end-test) steady state value; D_{iv} is the amount of the exogenous glucose dose (mg·kg^{-1}) injected at time 0 min; X is a variable related to insulin concentration in compartment remote from plasma, $X(t) = (k_4+k_6)I'(t)$, where k_4 and k_6 are rate parameters(min^{-1}); I(t) is plasma insulin concentration (μU·ml^{-1}) with I_b denoting its basal value; g is plasma glucose concentration with g_b denoting its basal (end-test) value; V is the distribution volume per unit body weight (ml·kg^{-1}); and $p_1 = k_1+k_5$, $p_2 = k_3$ and $p_3 = k_2(k_4+k_6)$ are rate parameters expressed in min^{-1}, min^{-1} and min$^{-2}\mu$U^{-1}ml, respectively. Clearly one has $Q_b = g_b V$.

From the above 1CMM, we derive glucose effectiveness S_G at basal insulin as

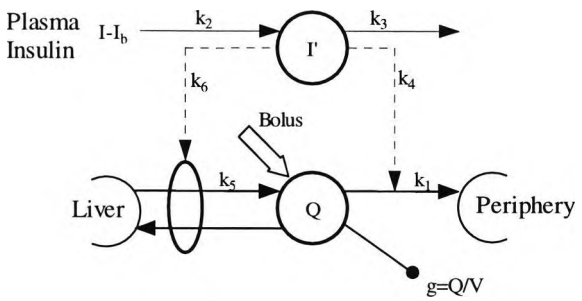
$$S_G = [-\frac{\partial \dot{Q}(t)}{\partial g(t)}]_{ss} = p_1 V = S'_G V \qquad (\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) \tag{3.4}$$

and insulin sensitivity S_I as

$$S_I = [-\frac{\partial \dot{Q}(t)}{\partial I(t) \partial g(t)}]_{ss} = \frac{p_3}{p_2} V = S'_I V \qquad (\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{per } \mu\text{U} \cdot \text{ml}^{-1}) \tag{3.5}$$

where $S_I' = \frac{p_3}{p_2}$ and $S_G' = p_1$. Glucose concentration from values 0 to 8 minutes are excluded from the parameter estimation process.

Figure 3.1: The classical one compartment minimal model (Bergman et al [16]).



3.3.2 Three-Stage Hierarchical Model

A three-stage hierarchical model will be postulated. It allows variability in the concentration to be separated into intra-individual and inter-individual components.

First stage model (intra-individual variation)

Let c_{ij} denote the plasma concentration of glucose during IVGTT of individual i at time t_j, i = 1,...,N and j = 1,...,n_i.

The concentration is obtained as

$$c_{ij} = g(\boldsymbol{\theta}_i, t_{ij}) + \epsilon_{ij} \quad \epsilon_{ij} \sim N(0, \frac{g^2}{\tau_c}) \tag{3.6}$$

where g is a solution to the minimal model Equations 3.1-3.3 (inputs such as glucose dose and plasma insulin were omitted for clarity); $\boldsymbol{\theta}_i$ denotes a vector of individual-specific parameters $\left\{\hat{\boldsymbol{\theta}}_i = p_{1i}, p_{2i}, p_{3i}, \text{and } V_i\right\}$; ϵ_{ij} is the random term representing the multiplicative measurement error, and the model specification error and other unaccounted variability, whose coefficient of variation is assumed constant within and between individuals; and τ_c^2 denotes intra-individual precision parameter for concentration of IVGTT data. The random term ϵ_{ij} is drawn from a normal distribution with a zero mean and an unknown variance $\frac{g^2}{\tau_c}$.

We denote the first stage distribution by

$$g(\mathbf{c}_i \mid \boldsymbol{\theta}_i, \tau_c) \tag{3.7}$$

where c_i is a vector of measurements, $c_i = (c_{i1},...c_{in_i})$. We write the likelihood function for IVGTT parameters of a particular individual as

$$l(\boldsymbol{\theta}_i, \tau_c) = g(\mathbf{c}_i \mid \boldsymbol{\theta}_i, \tau_c) \tag{3.8}$$

Second stage model (inter-individual variation)

The population approach assumes that the parameter vector is drawn from a parametric distribution

$\boldsymbol{\theta}_i \sim p_\theta(. \mid \boldsymbol{\phi}_\theta)$

In particular $p_\theta(. \mid \boldsymbol{\phi}_\theta)$ may represent multivariate normal in which case $\boldsymbol{\phi}_\theta = (\boldsymbol{\mu}_\theta, \boldsymbol{\Sigma}_\theta)$ where $\boldsymbol{\mu}_\theta$ represents a population mean parameter and $\boldsymbol{\Sigma}_\theta$ represents a population scale matrix and where $\boldsymbol{\mu}_\theta$ is a vector of length p_θ and $\boldsymbol{\Sigma}_\theta$ is a matrix of dimension $p_\theta \times p_\theta$.

In this study we assumed $p_\theta(. \mid \boldsymbol{\phi}_\theta)$ to arise from a multivariate log-normal distribution guaranteeing non-negativity of parameters and we also assumed no covariate relationships.

Third stage model

At this stage the vague priors representing "lack" of prior knowledge for the population parameters τ_c, μ_θ, and Σ_θ were specified.

The priors chosen for our analysis were

$$\tau_c \sim Ga(a_c, b_c) \tag{3.9}$$

$$\boldsymbol{\mu}_\theta \sim N(\mathbf{c}_\theta, \mathbf{C}_\theta) \tag{3.10}$$

$$\boldsymbol{\Sigma}_\theta^{-1} \sim W(r_\theta, (r_\theta \mathbf{R}_\theta)^{-1}) \tag{3.11}$$

where $Ga(a_c, b_c)$, $N(\mathbf{c}_\theta, \mathbf{C}_\theta)$, and $W(r_\theta, (r_\theta \mathbf{R}_\theta)^{-1})$ denote Gamma, normal, and Wishart distributions, respectively.

The values $\mathbf{c}_\theta$, $\mathbf{R}_\theta$, and a_c represent prior guesses for the relevant parameters and $\mathbf{C}_\theta$, r_θ, and b_c represent the precision of these estimates. The values of priors specified for these parameters are

$$\mu_\theta \sim Normal\left[\mathbf{0}, \begin{pmatrix} 10^6 & 0 & 0 & 0 \\ 0 & 10^6 & 0 & 0 \\ 0 & 0 & 10^6 & 0 \\ 0 & 0 & 0 & 10^6 \end{pmatrix}\right] \tag{3.12}$$

$$\Sigma_\theta \sim Whisart\left[4, \begin{pmatrix} 50 & 0 & 0 & 0 \\ 0 & 50 & 0 & 0 \\ 0 & 0 & 50 & 0 \\ 0 & 0 & 0 & 50 \end{pmatrix}\right] \tag{3.13}$$

$$\tau_c \sim Gamma(0.001, 0.001) \tag{3.14}$$

Figure 3.2 shows the model for IVGTT as a directed acyclic graph (DAG) [116]. The graph represents local relations among variables.

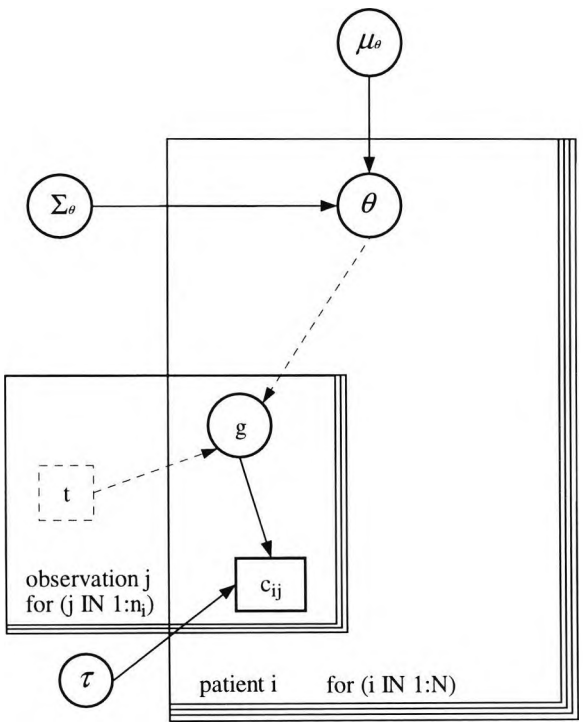
3.3.3 Statistical Considerations

In this section, we describe the statistical relationship associated with the Bayesian analysis of the minimal model. Inference with the Bayesian technique is performed mainly on the posterior distribution of the unknown parameters of the model.

Concentration data from IVGTT were collected in N individuals. We denote the data by $\mathbf{c}_i=(c_{i1},\ldots,c_{in_i})$. The notation represents the fact that there may be missing concentration data. The data were collected at time points t_{ij}, i = 1,...,N and j = 1,...,n_i. The individual unknown parameters are $\boldsymbol{\theta} = (\boldsymbol{\theta}_1, \ldots, \boldsymbol{\theta}_N)$.

We summarised the three stages of the hierarchy as follows.

Figure 3.2: A directed acyclic graph for the population analysis of the minimal model. Dashed squares denote fixed quantities, while solid squares denote observed quantities (concentrations). Solid arrows are probabilistic dependencies, while dashed arrows show functional (deterministic) relationships; t is measurement time, c_{ij} is glucose measurment during IVGTT, g is a nonlinear function to predicted glucose concentration during IVGTT, τ_θ, μ_θ and Σ_θ are prior population parameters.



Stage one

The likelihood function is given as

$$\prod_{i=1}^{N} l(\boldsymbol{\theta}_i, \tau_c) = \prod_{i=1}^{N} g(\mathbf{c}_i \mid \boldsymbol{\theta}_i, \tau_c) \tag{3.15}$$

Stage two

The likelihood function for the population parameters of N individuals is given as

$$\prod_{i=1}^{N} p_\theta(\boldsymbol{\theta}_i \mid \phi_\theta) \tag{3.16}$$

Stage three

The third stage specifies priors for some of the parameters and the joint distribution of the prior is assumed to be the product of independent prior distributions for each component

$$p(\phi_\theta(\boldsymbol{\mu}_\theta, \boldsymbol{\Sigma}_\theta)) \times p(\tau_c) \tag{3.17}$$

Taking the product of Equations 3.15, 3.16 and 3.17, we define the joint posterior distribution for all our parameters for N individuals as:

$$p(\boldsymbol{\theta}, \tau_c, \phi_\theta \mid \mathbf{c}) \propto \prod_{i=1}^{N} [g(\mathbf{c_i} \mid \boldsymbol{\theta}_i, \tau_c)] \tag{3.18}$$
$$\times \prod_{i=1}^{N} [p(\boldsymbol{\theta}_i \mid \phi_\theta)] \times p(\tau_c) \times p(\phi_\theta)$$

In Equation 3.18, we are interested in the marginal distributions which are obtained by integrating out all other parameters except the parameter of interest. Due to the nonlinear nature of the first stage of the above model, the integral of the marginal distribution of the parameters of interest is analytically intractable. Also the large dimensionality of the parameter space makes it impossible to use numerical integration.

For many such nonlinear, nonconjugate hierarchical models that arise in practice, more advanced computational methods, such as Markov chain Monte Carlo (MCMC) methods, are necessary. The methods conveniently summarise by random draws of the posterior distribution of model parameters. The exact number of simulation draws depends on the form of the posterior distribution, the estimates of interest, and the summaries required. The key to the Markov chain is to create a Markov process whose stationary distribution is specified, say $q(\boldsymbol{\theta} \mid c)$, and run the simulation long enough so that the distribution of the draws is close enough to the target distribution $p(\boldsymbol{\theta} \mid c)$.

3.4 Data Analysis

3.4.1 Solving for g(t)

In this section we describe the solution for the nonlinear function g(t). The nonlinear function was solved by both analytic and numerical methods. Equations 3.1-3.3 repre-

sent a first-order initial-value problem and were solved numerically using a fourth-order Runge Kutta method.

The solution for X(t), Equation 3.2, was obtained analytically and substituted into Equation 3.1 before solving the latter numerically.

Assume plasma insulin I(t) to be piecewise linear; the analytic solution for Equation 3.2 is obtained with the following algorithm

Step 0 Set working variable X0 = 0, i = 1

Step 1 Set slope S=$\frac{I_i - I_{i-1}}{t_i - t_{i-1}}$

Step 2 Solution on $[t_{i-1}, t_i]$ is obtained as

$$X(t) = \frac{p_3}{p_2}[S(t_i - t_{i-1}) + I_i - St_i - I_b] - \frac{p_3}{p_2^2}S + [X_0 - \frac{p_3}{p_2}(I_i - I_b) + \frac{p_3}{p_2^2}S]e^{-p_2(t_i - t_{i-1})} \quad (3.19)$$

Step 3 Set X0 = $X(t_i)$ and i = i + 1

Step 4 If i $\leq$ N goto Step 2 otherwise terminate

where I_i is the plasma insulin measurement at t_i and N+1 is the number of measurements.

Substituting X(t) into Equation 3.1, the numerical solution is obtained by using a fourth-order Runge Kutta method on

$$\begin{aligned} \dot{Q}(t) &= X_A Q(t) + p_1 Q_b \\ Q(0) &= Q_b + D_{iv} \end{aligned} \quad (3.20)$$

where $X_A = -[p_1 + X(t)]$.

The minimal model was parameterised in terms of $\boldsymbol{\theta} = [\log(p_1), \log(p_2), \log(\frac{p_3}{p_2}), \log(V)]$.

3.4.2 Model Implementation

We employed for the calculations the public domain WinBUGS program [85] extended by a purpose-made module implementing the numerical solution of Equations 3.1 and 3.2. The WinBUGS program adopted the Metropolis-Hastings algorithm [92] to calculate a single chain with 26,000 samples (with thinning of 4), from which the first 6,000 samples were discarded and the remaining 20,000 samples were used in further analyses.

The calculations were performed on a PC running the MS Windows 98 operating system with 512MB RAM and a single 650MHz Pentium processor. The generation of the chain with 26,000 samples took approximately 12 hours.

The WinBUGS extensibility code (the purpose-made module) for implementing the numerical solution is shown in Appendix A.

3.4.3 MCMC Convergence Monitoring

The diagnostics criteria in Convergence Diagnostics and Output Analysis Software (CODA) [17] described below were used to assess convergence of the MCMC chains.

- Geweke method: This was done by dividing the chain into "windows", containing the first 10% and the last 50% of the iterates. For stationary assessment, the mean of the values early and late in the sequence should be similar. The convergence diagnostic test Z is the difference between these 2 means divided by the asymptotic standard error of their difference. As the chain length $\rightarrow \infty$ the sampling distribution of Z$\rightarrow$ N(0,1) if the chain converged. The results for the chain provide no evidence against convergence. for all the parameters, see Appendix B, Table B.1 for the result of this test. This approach could also be used to assess whether thinning is necessary.

- Raftry and Lewis method: This was also used to assess convergence to the stationary distribution. The CODA output also reported the minimum number of updates that were needed to estimate the specified quartile to the desired precision. CODA also reported the total number updates for each variable. In the results in Appendix B, Table B.2, values of I (Dependence factor) much greater than 1.0 indicate high within-chain correlations probable convergence failure (Raftery and Lewis (1992a) suggest that I $>$ 5.0 often indicates problems). In this case reparameterisation of the model is advised.

- Heldelberger and Welch method: This method indicates 'passed' if the stationary test is passed. The method also reported the number of updates to keep and the number to discard at the initial stage. The results in Appendix B, Table B.3 suggest convergence was achieved for all parameters.

Convergence criteria of the chains were also assessed by monitoring parameter mixing history in WinBUGS, see Appendix B, Figure B.1.

3.5 Statistical Analysis

Summary statistics were in terms of posterior medians, credible intervals, and quartiles of the WinBUGS outputs.

3.6 Results

3.6.1 Plasma Glucose and Insulin

Individual measurements for plasma glucose and insulin concentration data are shown in Figure 3.3.

Figure 3.3: Plasma glucose (top panel) and plasma insulin (bottom panel) during insulin modified IVGTT in newly presenting T2D. Individual measurements are plotted (N = 65).

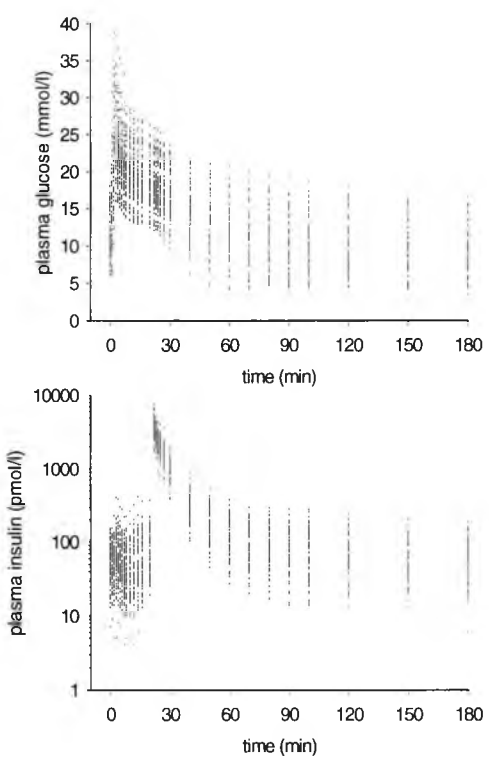
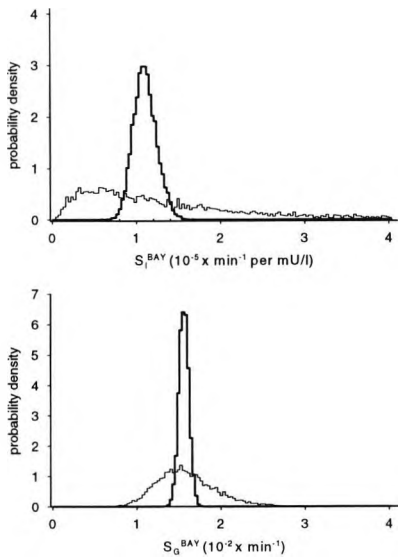


Figure 3.4: The posterior density of the population mean (thick line) and the posterior density of individual values (thin line) for insulin sensitivity (top panel) and glucose effectiveness (bottom panel) estimated with the Bayesian hierarchical analysis, see text for details.



3.6.2 Bayesian Hierarchical Analysis

Individual values of p_1, p_2, p_3 and V (obtained as posterior median estimates) of Bayesian hierarchical analysis of the minimal model are shown in Appendix C, Table C.1.

The Bayesian hierarchical analysis provided estimates of S_I^{BAY} and S_G^{BAY} in all subjects with good precision (16%, range: 3 - 56%; 14%, range: 7-18%). Individual values and precisions of S_I^{BAY} and S_G^{BAY} are given in Appendix C, Table C.2.

Population statistical summaries (posterior median estimates, credible intervals and inter-quartile ranges) of p_1, p_2, p_3, V, S_I^{BAY} and S_G^{BAY} are given in Table 3.2.

The posterior densities of the population mean of S_I^{BAY} and S_G^{BAY} are shown in Figure 3.4. The posterior densities of individual values of S_I^{BAY} and S_G^{BAY} are also shown.

The overall estimate of the measurement error is 3.0% (3.1%, 3.3%) (median, 95% credible interval).

The population densities in Figure. 3.4 (thick lines) are narrow and nearly symmetrical. This shows that the population means for the two parameters are well defined and

Table 3.2: Population characteristics of the minimal model with the Bayesian hierarchical analysis (S_I^{BAY} and S_G^{BAY}) during the insulin-modified IVGTT in subjects with newly presenting T2D.

	Median (95% CI)*	Interquartile range
p_1 (10^{-2} x min^{-1})	1.53 (1.41 - 1.64)	0.46
p_2 (10^{-2} x min^{-1})	5.35 (4.58 - 5.13)	3.43
p_3 ($10^{-5}\times$ min^{-2}pmol·L^{-1})	5.65 (4.07 - 7.56)	1.17
V (10^{-2} $\times$ L·kg^{-1})	138.4 (133.8 - 144.3)	3.27
S_G^{BAY} (10^{-2} x min^{-1})	1.53 (1.41 - 1.64)	0.46
S_I^{BAY} (10^{-5} x min^{-1} per pmol·L^{-1})	1.07 (0.82 - 1.36)	1.43

* posterior median estimate and 95% credible interval of mean

their credible intervals are symmetrical around the mean. The posterior density of the individual values of insulin sensitivity in Figure 3.4. (thin line, top panel) is skewed to the right specifying that individuals with newly presenting T2D diabetes have very low insulin sensitivity with a most likely value (the mode) of 0.2 x 10^{-5} min^{-1} per pmol L^{-1} but that there is also a non-negligible number of individuals with ten (and more) fold higher insulin sensitivity. Individual values of glucose effectiveness are nearly symmetrically distributed around the most likely value of about 1.5 x 10^{-2} min^{-1} with a small inter-individual variability (nearly no subject below 0.7 x 10^{-2} min^{-1} or above 2.7 x 10^{-2} min^{-1}.

3.7 Discussion

The results illustrate the use of the Bayesian hierarchical analysis to estimate individual insulin sensitivity and glucose effectiveness with the minimal model analysis of IVGTT. The method has been used in pharmacokinetics and also in metabolic studies [132, 130, 106] as a result of recent developments in Monte Carlo Bayesian statistical computing, which have alleviated some of the problems that could hamper the alliance between statistics and physiological modelling.

The main motivation for this study came from two major criticisms regarding the validity of S_I and S_G estimates from the minimal model analysis of IVGTT. First, S_G is suspected to be overestimated [29, 43, 105] and S_I underestimated [105, 44, 29] by the minimal model. Second, the occurrence of S_I values indistinguishable from zero that is common in large clinical studies and the uncertainty of its physiological meaning.

The first criticism is related to the validation of the minimal model-derived S_G and S_I, whose importance has recently been emphasised and investigated [29]. The other serious question about the validity of the minimal model analysis is the occurrence of zero S_I values [71, 61]. It is unclear whether this represents a physiologically relevant phenomenon or a manifestation of modelling deficiency. Given the widespread application of the minimal model estimation technique and in view of the difficulties identified with nonlinear regression (NLR) approach, we examined the performance of the minimal model analysis of glucose kinetics data from T2D employing the Bayesian analysis.

The Bayesian inference treats unknown parameters as random variables. Hierarchical models exploit the flexibility this approach offers. The estimation of parameters of the hierarchical model using the Bayesian inference is termed Bayesian hierarchical analysis and such a procedure provides a natural setting for the analysis of data from IVGTT. Hence, we employed the Bayesian hierarchical framework for minimal modelling of glucose kinetics.

The methodology presented in this study has five key features; a physiological model, experimental data, a population model, prior information of the population parameters, and the Bayesian inference. Each of these features cannot work in isolation. The combination of these features facilitates the simultaneous estimation of the model parameters with the motives of pooling strengths to improve the precision of the estimates of each parameter, and of allowing for certainty in such estimates.

The Bayesian hierarchical analysis adopts two sets of assumptions. The first assumption is related to the underlying distribution of the parameters of interest. Secondly, prior distributions have to be specified. The log-normal distributions were adopted for the parameters of interest following an independent analysis, data not shown, and satisfying physiological constraints. The log-normal distribution provides positive estimates of the parameters and also positive credible intervals.

The identification of forms of the underlying distributions for sources of variability in a nonlinear hierarchical model is a difficult statistical task. We assumed a normal distribution with a zero mean and unknown variance for the measurement errors. Another approach for modelling measurement errors is to use a heavy-tailed distribution. Some researchers have shown how the t-distribution, instead of the normal distribution can easily be adopted for modelling the measurement errors [46]. The t-distribution is wider and flatter than the normal distribution. In the WinBUGS program, a t-distribution on ν degrees of freedom can be specified directly as a sample distribution, with priors being specified for its scale and location. The problem with a t-distribution is what

value of degrees of freedom should be used. Also, the use of multivariate t-distribution tends to increase the estimation accuracy of the inter-individual variances by guarding against the outlying individuals [132, 130, 48]. A multivariate normal distribution is assumed for this study, WinBUGS does not generate a multivariate t-distribution at the time of this study.

The Bayesian hierarchical model is more expensive in terms of computation time. The model estimation took about 12 hours on a desk-top PC. This compares less favourably with non-linear regression analysis with several seconds needed to run a single subject. However, when compared to the overall time-scale of population studies which normally take months to complete, the increase in computational time is negligible as it provides an information rich picture about individual and population parameters and improves precision of the estimates.

One advantage of the Bayesian hierarchical analysis is that it provides realistic credible intervals. The population posterior median estimate and credible interval for S_I (median(CI) = 1.07(0.82 - 1.36) x 10^{-5} ; min^{-1} per $pmol \cdot L^{-1}$) and S_G (median(CI) = 1.53 (1.41 - 1.64) x 10^{-2} ; min^{-1}) and other parameters from the Bayesian hierarchical analysis were realistic with plausible credible intervals.

The Bayesian hierarchical model provides useful quantitative assessment of uncertainty in the population, i.e. it measures the population characteristics rather than a single set of parameters representing the "average person".

Pillonetto et al [103] proposed the use of the Bayesian approach to reanalyse large databases of epidemiological studies [94, 107, 124], because the method offers a comprehensive and robust approach to model estimation. It will however also be useful to reanalyse these databases employing the Bayesian hierarchical framework we presented in this study, as it gives a better understanding of the spread of the inter-individual variability in the population.

3.8 Conclusion

We showed that the Bayesian hierarchical analysis is an appealing and feasible method to estimate population and individual insulin sensitivity and glucose effectiveness with the minimal model of the insulin-modified IVGTT.

Chapter 4

Comparison of Bayesian Hierarchical and Standard Two-Stage Approaches

4.1 Introduction

In the previous chapter we developed and evaluated a population-based approach to facilitate simultaneous estimation of individual and population insulin sensitivity and glucose effectiveness with the minimal model of insulin-modified IVGTT. We demonstrated that the Bayesian hierarchical analysis is an appealing and feasible approach.

In this study, our interest is to compare the parameter estimation capabilities of the minimal model of glucose kinetics using the Bayesian hierarchical analysis (BAY) and the standard two-stage approach using the non-linear regression (NLR) analysis, which tends to overestimate the population covariance matrix.

The concept of the Bayesian population analysis exploits the insight that nonlinear mixed-effects models are equivalent to the "nonlinear hierarchical models" modelling structure. The hierarchy has three stages and the details of the model and implementation methods were provided in Section 3.3.2 and 3.4, Chapter 3. The approach has enjoyed much interest recently, especially as a result of methodological advances and increased computational speed.

4.2 Subjects and Experimental Design

We employed IVGTT data from 65 newly presenting T2D subjects as described in Section 3.2.

4.3 Data Analysis

4.3.1 Bayesian Hierarchical Approach

An illustrative outline of the Bayesian analysis is shown in Figure 4.1. The analysis includes the development of a hierarchical model with individual $\boldsymbol{\theta}_i$ and population $\hat{\boldsymbol{\mu}}_\theta$ and $\boldsymbol{\Sigma}_\theta$ parameters as explained in Section 3.3.2, Chapter 3.

In the first stage, the relationships between the glucose and insulin concentrations, and time are modelled for a particular individual. In the second stage, the glucose kinetic parameters $\boldsymbol{\theta}_i$ defining individual profiles are assigned some distributional form. A Bayesian model then requires a third stage in which prior distributions are specified.

As explained earlier, we adopted 'vague' (non-informative) prior distributions representing a lack of information about parameter values. Each stage of the hierarchy and the data analysis are described in detail in Section 3.3.2 and 3.4, Chapter 3.

4.3.2 Standard Two-Stage Approach

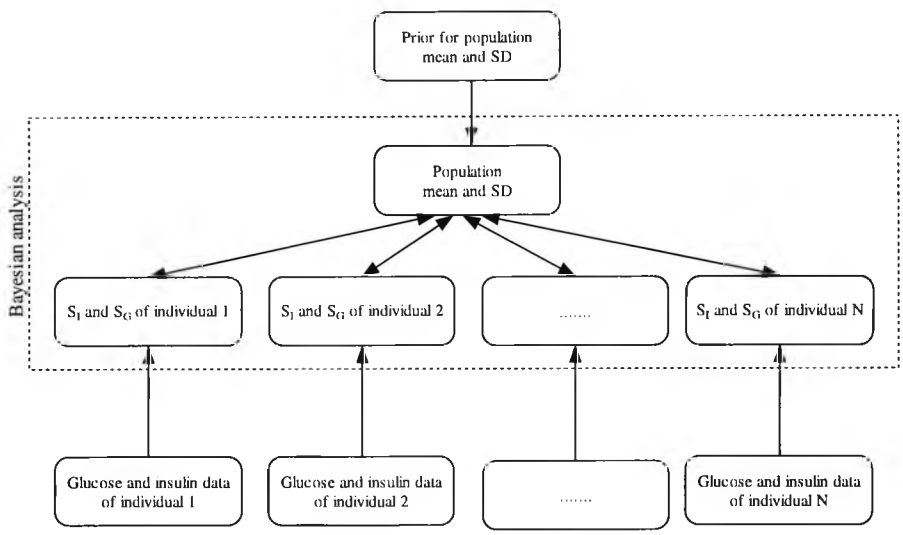
The principles of the standard two-stage approach are shown in Figure 4.2. In the first stage, a weighted non-linear regression analysis is employed to estimate the individual parameters $\boldsymbol{\theta}_i$ and σ_i.

Using least squares as the estimation procedure (or, equivalently, maximum likelihood under the assumption of normal distribution of measurement errors), we obtain estimates of $\boldsymbol{\theta}_i$ by minimising

$$\sum_{j=1}^{n_i} w_j [c_{ij} - f_{iv}(I_i, \boldsymbol{\theta}_i, D, t_{ij})]^2 \tag{4.1}$$

where w_j are the weights reflecting the relative uncertainty attached to individual measurements. The weight was defined as the reciprocal of the variance of the measurement error. The CV of the measurement error was assumed at the level of 1.5%.

Figure 4.1: Bayesian hierarchical analysis of the minimal model indices. Individual S_I and S_G values are estimated in parallel with the population characteristics (the mean and the standard deviation). All indices are treated as random variables and the estimation involves determining their probability density functions. Log-normal population distribution is assumed to reflect that S_I and S_G can attain only non-negative values. The Bayesian hierarchical analysis facilitates "information" flow between individuals (individual estimate depends on the measured glucose and insulin data and also on the population mean and standard deviation, which is in turn influenced by other individual values) and also results in estimates with higher precision contributing with a greater weight to the population characteristics.



We used the IS_CIBA package program (Insulin Sensitivity from Ciba, Ciba-Geigy Ltd, CH-4002 Basle Switzerland 1995 Author: Dr. G. H. Mehring / Medical Department / Biometrics Date of version: September 22nd, 1998), which employs non-linear regression analysis to carry out parameter estimation.

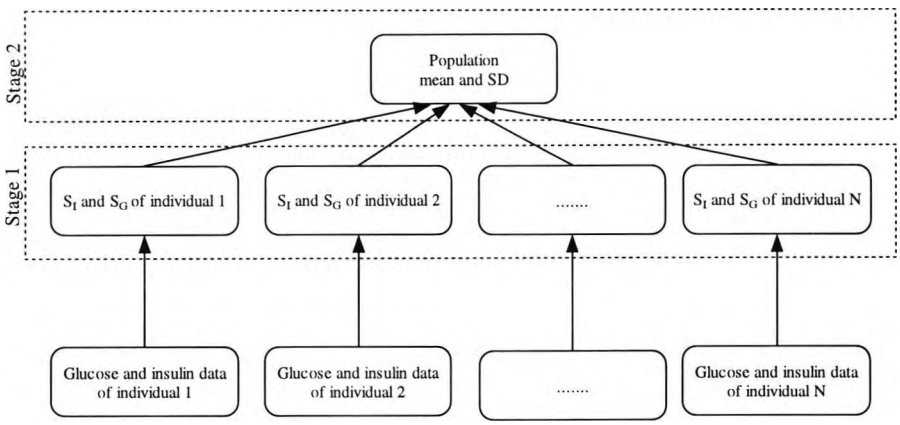
In the second stage, the resulting estimates are combined into an estimate of geometric mean $\hat{\boldsymbol{\theta}}$ and the geometric variance $S^2_{\hat{\boldsymbol{\theta}}}$ as

$$\boldsymbol{\theta} = \left(\prod_{i=1}^{N} \boldsymbol{\theta}_i\right)^{N^{-1}} \quad \text{(geometric mean)} \tag{4.2}$$

$$S^2_{\hat{\boldsymbol{\theta}}} = \left(2\prod_{i=1}^{N} \frac{\boldsymbol{\theta}_i}{\boldsymbol{\theta}}\right)^{N^{-1}} \quad \text{(geometric variance)} \tag{4.3}$$

from which the 95% confidence intervals of population S_I^{NLR} and S_G^{NLR} were calculated.

Figure 4.2: The standard two-stage analysis of minimal model derived insulin sensitivity S_I and glucose effectiveness S_G. In Stage 1, individual S_I and S_G values are estimated from experimental data with the use of a non-linear regression parameter estimator. In Stage 2, the individual values are used to calculate the population mean and the standard deviation (or another measure of variability) with individual values contributing equally irrespective whether they are estimated with high or low precision.



4.4 Statistical Analysis

Summary statistics of the results are given in terms of geometric means, medians, confidence intervals, credible intervals, and quartiles. The degree of relationships among variables was assessed by Spearman's correlation coefficient (r_s).

4.5 Results

4.5.1 Standard Two-Stage Analysis

The individual results are presented in Appendix D, Tables D.1-D.2. Estimates of glucose effectiveness (S_G^{NLG}) were obtained in all 65 subjects with acceptable precision (12%, 4% - 127%; median, range). Unlike S_G^{NLR}, the non-linear regression analysis successfully estimated insulin sensitivity (S_I^{NLR}) in all but four subjects, resulting in a 6% failure rate, see Appendix D, Table D.2. Estimation 'failure' is defined for any individual with a CV greater than 150%. The overall precision of the individual estimates of p_2^{NLR} was satisfactory (21%, 7% - 211%; median, range). The overall precision of the individual estimates of V^{NLR} was very good (2%, 1% - 10%; median, range). Population statistical summaries of S_I^{NLR}, S_G^{NLR}, p_2^{NLR} and V^{NLR} are given in Table 4.1.

Table 4.1: Population characteristics of the minimal model with the Bayesian hierarchical analysis (S_I^{BAY}, S_G^{BAY}, p_2^{BAY} and V^{BAY}) and the standard two-stage analysis (S_I^{NLR}, S_G^{NLR}, p_2^{NLR} and V^{NLR}) during the insulin-modified IVGTT in subjects with newly presenting T2D.

	Mean (95% CI of Mean)*	Interquartile range
S_I^{BAY} (10^{-5} x min^{-1} $pmol{\cdot}L^{-1}$)	1.07 (0.82 - 1.36)	1.43†
S_I^{NLR} (10^{-5} x min^{-1} $pmol{\cdot}L^{-1}$)	1.23 (0.97 - 1.56)	1.84
S_G^{BAY} (10^{-2} x min^{-1})	1.53 (1.41 - 1.64)	0.46†
S_G^{NLR} (10^{-2} x min^{-1})	1.45 (1.32 - 1.59)	0.53
p_2^{BAY} (10^{-2} x min^{-1})	5.35 (4.58 - 6.13)	3.43†
p_2^{NLR} (10^{-2} x min^{-1})	5.71 (4.87 -6.68)	3.82
V^{BAY} (10^{-2} x $ml{\cdot}kg^{-1}$)	13.8 (13.40 - 14.30)	3.30†
V^{NLR} (10^{-2} x $ml{\cdot}kg^{-1}$)	14.0 (13.60 - 14.50)	3.30

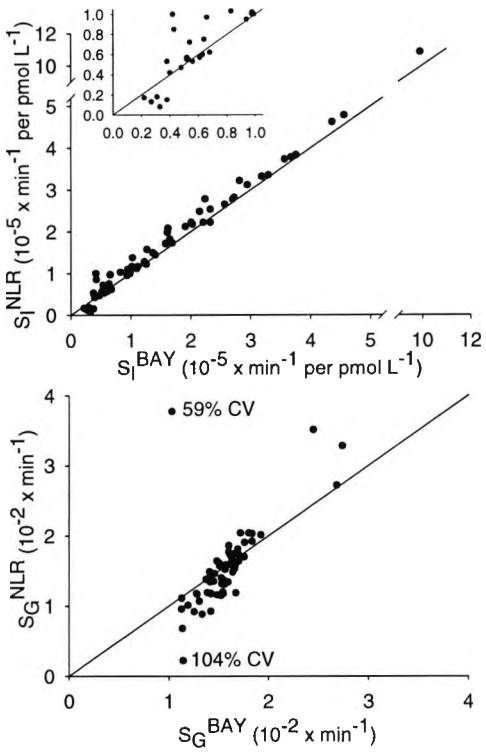
*CI stands for credible intervals for the Bayesian estimates; for two-stage estimates, geometrical mean and its confidence interval are reported.
†Calculated from the sample generated from the posterior population distribution.

4.5.2 Comparison of the Standard Two-Stage and Bayesian Hierarchal Analysis

The results are presented in Figures 4.3-4.6. The non-linear regression analysis failed in Subjects #5, #6, #31, and #44 to estimate insulin sensitivity with acceptable precision, whereas the Bayesian hierarchical analysis returned insulin sensitivity in these four subjects with acceptable precision (range of CV 46 - 59%). These four insulin sensitivity values were in the lower quartile and were 1st, 4th, 9th, and 14th lowest among the studied group at 0.21, 0.28, 0.39, and 0.50 x 10^{-5} min^{-1} per pmol L^{-1}. Their posterior density functions are shown in Figure 4.5.

Subjects #5, #6, and #44 had the lowest, and subject #31 had the 7th lowest precision as determined by the Bayesian hierarchical analysis (59%, 58%, 53%, 46%). Otherwise there was no apparent difference in the shape of the posteriors, which would provide a further insight into the failure of the non-linear regression analysis. This suggests that the non-linear regression analysis tends to fail in subjects with low and poorly defined insulin sensitivity.

Figure 4.3: Comparison of the minimal model indices determined by the Bayesian hierarchical analysis (S_I^{BAY} and S_G^{BAY}; median of the posterior density function) and the non-linear regression analysis (S_I^{NLR} and S_G^{NLR}) in subjects with newly presenting type 2 diabetes (N = 65). Top panel compares S_I estimates, bottom panel compares S_G estimates. Two S_G^{NLR} estimates with lowest precision (CV of the estimate given in bottom panel) differed most from their corresponding S_G^{BAY} values. Solid lines represent the unity slope.

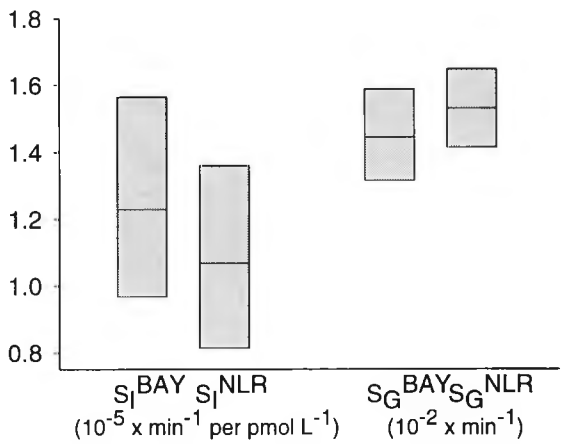


The posterior density functions of subjects across a range of insulin sensitivities are shown in Figure 4.6.

Individual insulin sensitivities estimated by the two approaches were highly correlated ($r_s = 0.98$, $P < 0.001$). However, the correlation in the lower 20% centile of the insulin sensitivity range was significantly lower than the correlation in the upper 80% centile ($r_s = 0.71$ vs $r_s = 0.99$; $P < 0.001$) further supporting the notion that the non-linear regression analysis has difficulties at low insulin sensitivities. The non-linear regression tended to provide slightly higher insulin sensitivity estimates, see Figure 4.3. The difference was not considered clinically significant. The inset in the top panel of Figure 4.3 highlights the comparison at lower values of insulin sensitivity.

The precisions of individual insulin sensitivity estimates provided by the two methods were highly correlated ($r_s = 0.82$, $P < 0.001$) and were similar in extent although the

Figure 4.4: Comparison of population characteristics of minimal model indices determined by the Bayesian hierarchical analysis (S_I^{BAY} and S_G^{BAY}; mean and 95% credible interval of a log-normal posterior distribution) and the non-linear regression analysis (S_I^{NLR} and S_G^{NLR}; geometric mean and 95% confidence interval of the mean) in subjects with newly presenting type 2 diabetes (N = 65).



Bayesian hierarchical analysis gave a tighter range. This is most likely due to the hierarchical nature of the analysis, i.e. borrowing strength across individuals.

A different picture emerged when considering glucose effectiveness. Estimates provided by the two methods were still highly correlated ($r_s = 0.77$, $P < 0.001$) but were not proportional (the unity line is different from a projected regression line, see Figure 4.3). The non-linear regression gave a wider range of glucose effectiveness. There were large differences in several subjects and these were generally in estimates with low precision as returned by the non-linear regression analysis, see Figure 4.3.

The precision of glucose effectiveness was not correlated between the two methods ($r_s = 0.12$, P = NS). The Bayesian hierarchical analysis returned estimates with identical precision as judged by the median but with a tighter dispersion (2 vs 16% SD of the precision) explained again by the ability of the Bayesian hierarchical analysis to borrow of strength across individuals.

The comparison of population characteristics is shown in Figure 4.4. In the case of Bayesian analysis, the mean and the credible interval were directly extracted from the posterior density of the population mean, and the interquartile range was obtained from a simulated posterior distribution of an individual parameter. In the case of non-linear regression analysis, the characteristics correspond to the log-normal distribution

Figure 4.5: Posterior density function of individual insulin sensitivity derived by Bayesian hierarchical analysis in subjects where non-linear regression analysis failed (#5, #6, #31, and #44).

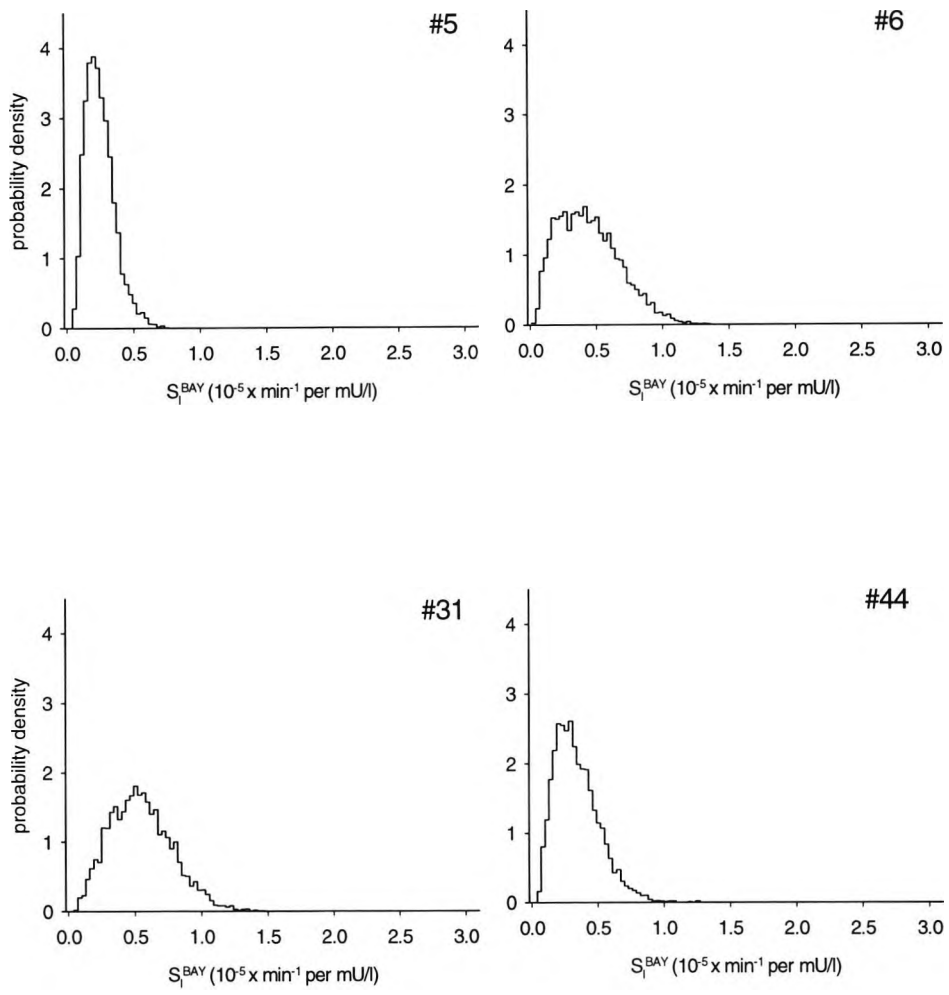
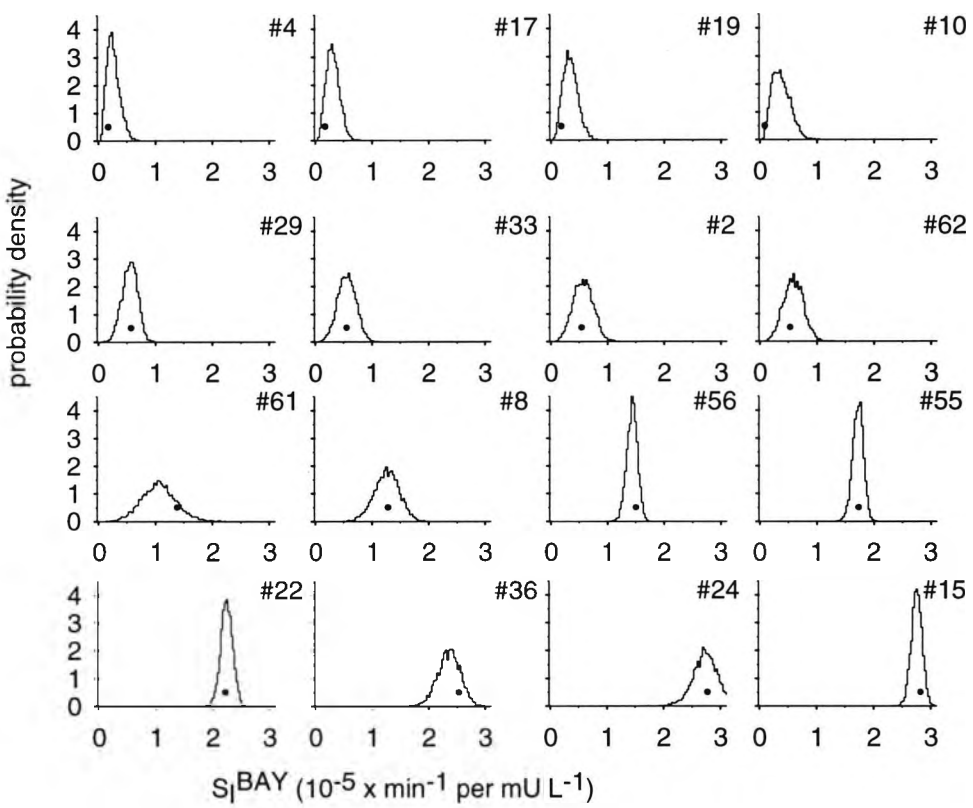


Figure 4.6: Posterior density function of insulin sensitivity derived by Bayesian hierarchical analysis (solid line) and point estimate obtained by the non-linear regression analysis (solid circle) in sample subjects representing a spectrum of insulin sensitivities. Each row includes subjects from successive quartiles starting with the lowest quartile.



of the parameter. There was no statistical difference between the two methods as demonstrated by overlapping confidence and credible intervals. However, the Bayesian hierarchical analysis gave tighter estimates as indicated by a smaller interquartile range for both insulin sensitivity and glucose effectiveness by 20% and 15%, respectively.

4.6 Discussion

The traditional method to analyse data with the minimal model is a standard two-stage approach (STS). The model parameters $\boldsymbol{\theta}_i$, Equation 3.6, are identified in each subject separately, and the mean $\boldsymbol{\mu}_\theta$ and the covariance $\boldsymbol{\Sigma}_\theta$ of the population are established as the sample mean and covariance. The main drawbacks of this approach are that equal weight is given to each individual's parameter estimate, though some estimates are more accurate than others and the method requires sufficient data for each individual to obtain the individual's parameter estimates. This approach has been shown to overestimate the population covariance [35]. These problems have led to attempts to develop more refined two stage approaches, the global two-stage and the iterative two-stage [118].

The first population kinetics alternative using a nonparametric approach was developed by Mallet [86]. An additional approach for population kinetics analysis, that lies between parametric and nonparametric, was proposed by Davidian and Gallant [34]. All the above approaches use the method of maximum likelihood as the basis for estimation. A Bayesian hierarchical approach was introduced to population kinetics analysis by Wakefield et al [132].

In this study, we investigated two methods to estimate both individual and population kinetic parameters of the minimal model, the Bayesian hierarchical approach (BAY) and the standard two-stage (STS) approach. The Bayesian hierarchical analysis has been proved effective in a number of applied and simulated studies [132, 131, 126, 2].

We found that both STS and BAY approaches gave similar results for the population mean and variance. However, the Bayesian hierarchical analysis has less variability in the individual precisions of S_I [S_I^{BAY} CV: 15% (2-67%) vs S_I^{NLR} CV 12% (4-127%); median(range)] and S_G [S_G^{BAY} CV: 13% (7-18%) vs S_G^{NLR} CV: 15% (5-104%); median(range)], thus giving more confidence in the estimates. The hierarchical Bayesian analysis allows the calculation of more precise individual estimates for all subjects including those where the STS parameter precisions are unacceptable.

The minimal model of IVGTT employing the traditional non-linear regression experienced a modest failure rate of 6% in subjects with low insulin sensitivity. The Bayesian

hierarchical analysis avoids the minimal model failures The results of this study demonstrate that the Bayesian hierarchical analysis is an appealing method to estimate insulin sensitivity and glucose effectiveness with the minimal model of IVGTT.

The advantage of the Bayesian hierarchical analysis over the standard two-stage approach is that it can improve on a drawback of the standard two-stage approach by offering a robust approach to model estimation [35]. It improves estimation in the data sets by borrowing strength across individuals. It provides a tighter credible interval of the population mean/median and also a tighter interquartile range, see Table 4.1. In the present study, a reduction in the interquartile range by 10-20% was observed despite including in the analysis subjects with very low insulin sensitivity values that were failed by the non-linear regression analysis. The Bayesian hierarchical analysis is able to accommodate differences in precision of the individual estimates; individual estimates with higher precision will influence to a greater extent the population characteristics such as the mean and the covariance matrix.

Unfortunately, different estimation approaches often converge to different sets of values or fail to converge at all. Bennett and Wakefield [13] made such a comparison for three population approaches implemented in commercially available software and explained how the problem of parameter estimation could confound the analyst's judgement of the correct model.

It would be beneficial to compare the performance of different population approaches (e.g. the iterative two-stage, NONMEN and the Bayesian hierarchical analysis) with the standard two-stage approach using the minimal model of IVGTT in both healthy and T2D subjects. The comparison could employ simulated data, in order to evaluate bias and the precision of these different estimation approaches. Such a comparison would provide further details about the merit of these approaches to estimate individual and population insulin sensitivity and glucose effectiveness with the minimal model.

4.7 Conclusions

The Bayesian hierarchical analysis was compared with the standard two-stage approach. Besides avoiding parameter estimation failure, the Bayesian hierarchical analysis provides reliable estimates of the population parameters, and allows estimation of individual parameters with very good precision.

Chapter 5

Evaluating Reduced Sampling Schemes with the Bayesian Hierarchical Modelling

5.1 Introduction

Steil et al [117] suggested that the number of blood samples that are required for estimating S_I and S_G in healthy subjects and subjects with diabetes can be reduced from the original 32 [full sampling schedule (FSS)] to 12 [reduced sampling schedule (RSS)] with a minor loss in precision, making the technique useful for population studies. The use of RSS has a considerable number of advantages in the experimental design It potentially reduces the experimental cost, labour, patient discomfort and can be carried over easily to paediatric populations. As a result, many reports have been published on reduced sampling protocols involving insulin-modified IVGTT in T2D [31, 117, 28]. Most of these approaches are limited to the standard two-stage technique for parameter estimation.

Recently, a population-based approach, an iterative two-stage technique, has been investigated [126, 128] and has been shown to improve precision compared to the standard two-stage approach. The strength of the population-based estimation technique is that the knowledge about the underlying population can be employed in the estimation process bringing about an improvement in the estimates of population and individual characteristics.

The present study employs the minimal model of glucose kinetics, in the Bayesian hierarchical framework as in the previous two chapters, to investigate the effect of reduced sampling schemes involving insulin-modified IVGTT in T2D patients.

5.2 Subjects and Experimental Design

We employed IVGTT data from 65 newly presenting T2D subjects as described in Section 3.2.

5.3 Sampling Schemes

The blood samples for the assessment of the changes in plasma glucose and insulin concentrations were drawn for three hours after the bolus administration according to a schedule comprising 30 samples (0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30,40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 minutes), see Section 3.2.2 for the experimental protocol. This frequent or full sampling schedule will be denoted as FSS and its parameter estimation results will be utilised as the reference. The parameter estimates of the full sample scheme are denoted by $S_{I(30)}$ and $S_{G(30)}$ for estimates of insulin sensitivity and glucose effectiveness, respectively. The investigation of sampling schemes with the Bayesian hierarchical will follow the work by Steil et al [117] and Coates et al [28]. The first reduced sampling scheme (RSS) included 12 samples drawn at 0, 2, 4, 19, 22, 30, 40, 50, 70, 90 and 180 minutes [117] and parameter estimates are denoted by $S_{I(12)}$ and $S_{G(12)}$. The second reduced sample scheme, 13 sample scheme, included an additional sample data at 25 minutes [28] and parameter estimates are denoted by $S_{I(13)}$ and $S_{G(13)}$.

5.4 Data Analysis

We employed the minimal model in combination with the Bayesian hierarchical analysis, as described in Section 3.4, to estimate values of model parameters using both full and reduced sampling schemes (30, 12, and 13 samples).

5.5 Statistical Analysis

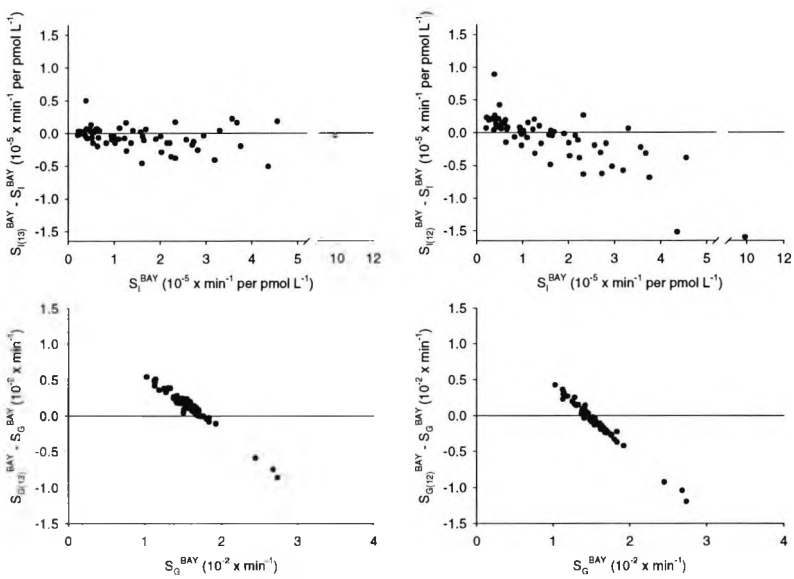
The reduced sampling schemes were assessed in terms of

- Comparability with the full sampling scheme using the Bland Altman plot [18];

- Bias of estimates (the presence of systematic errors) calculated as the percentage relative error [%REs = $100\%(\frac{\text{full - reduced}}{\text{full}})$];
- Precision of estimates expressed (CV) of parameter estimates.

The Wilcoxon's signed rank test was used to evaluate the bias of the reduced sampling schemes. Summary statistics for S_I and S_G estimates were obtained in terms of posterior median estimates, credible intervals, and interquartile ranges (IQRs).

Figure 5.1: Comparison of the full and reduced sampling schemes. The top left panel plots S_I determined by the full sampling scheme (regarded as the reference measurement) against the difference between $S_{I(13)}$ determined by the 13 sample scheme and S_I; the top right panel plots a similar relationship using $S_{I(12)}$ determined by the 12 sample scheme; the two bottom panels plot corresponding relationships for S_G.



5.6 Results

Estimated S_I and S_G are given in Appendix E, Table E.1 and E.2, together with corresponding CVs and %REs.

Table 5.1: Relative error of the 13 sample scheme and the 12 sample scheme during the insulin-modified IVGTT in subjects with newly presenting T2D (N = 65).

	Median %RE	95% CI*	P-value
$S_{I(13)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	4.66	(-0.47, 6.81)	0.899
$S_{I(12)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	-1.67	(-10.64, 3.00)	0.902
$S_{G(13)}$ (10^{-2} x min^{-1})	-9.30	(-13.13, -6.41)	0.009
$S_{G(12)}$ (10^{-2} x min^{-1})	5.95	(3.14, 8.47)	0.006

* Confidence interval of the median.

Table 5.2: Population characteristics of the minimal model with the Bayesian hierarchical analysis with the full-sampling scheme (30 samples), the 13-sample scheme, and the 12-sample scheme during the insulin-modified IVGTT in subjects with newly presenting T2D(N = 65).

	Median	(95% CI*)	Interquartile Range
$S_{I(30)}$ (10^{-5} x min^{-1} $pmol \cdot L^{-1}$)	1.06	(0.82 - 1.36)	(0.57 - 2.01)
$S_{I(13)}$ (10^{-5} x min^{-1} $pmol \cdot L^{-1}$)	1.03	(0.78 - 1.33)	(0.58 - 1.95)
$S_{I(12)}$ (10^{-5} x min^{-1} $pmol \cdot L^{-1}$)	1.16	(0.92 - 1.42)	(0.70 - 1.87)
$S_{G(30)}$ (10^{-2} x min^{-1})	1.53	(1.41 - 1.64)	(1.31 - 1.77)
$S_{G(13)}$ (10^{-2} x min^{-1})	1.69	(1.58 - 1.81)	(1.57 - 1.82)
$S_{G(12)}$ (10^{-2} x min^{-1})	1.47	(1.32 - 1.61)	(1.36 - 1.59)

* Credible interval.

Figure 5.2: Histogram of S_I relative errors (%RE) comparing the bias and accuracy of estimation of the 13 sample scheme (top panel) and the 12 sample scheme (bottom panel). Unbiased estimates have distribution centred around 0. Accuracy can be gauged from the spread of the distribution (the smaller the better) (N = 65).

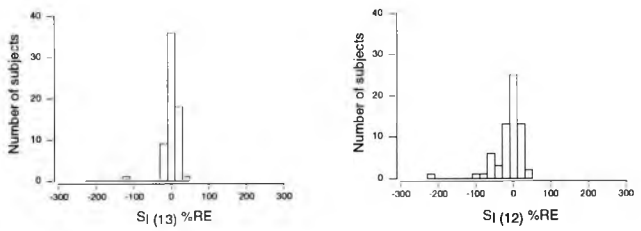
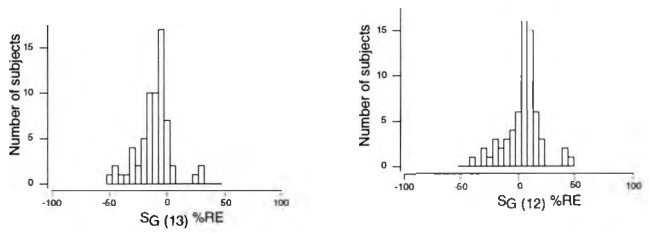


Figure 5.3: Histogram of S_G relative errors (%RE) comparing the bias and accuracy of estimation of the 13 sample scheme (top panel) and the 12 sample scheme (bottom panel). Unbiased estimates have distribution centred around 0. Accuracy can be gauged from the spread of the distribution (the smaller the better) (N = 65).

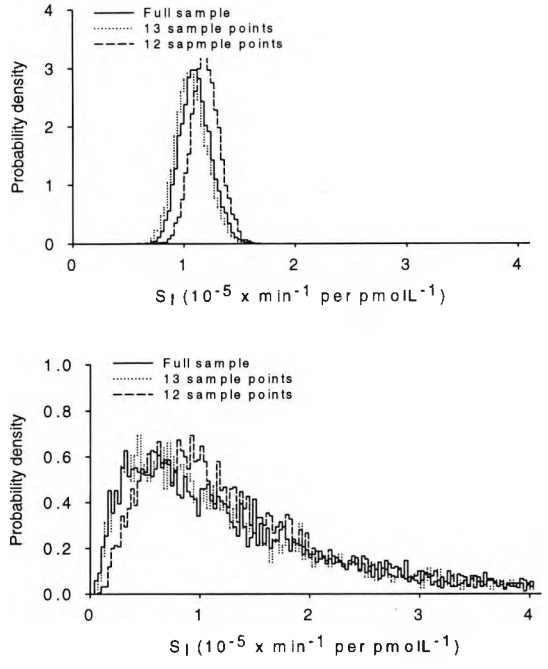


5.6.1 Bias of Estimates

Table 5.1 gives summarises of the bias introduced by the reduced sampling schemes. The results of S_I estimates imply statistical support for %RE of the median values equal to zero, indicting unbiased estimates by RSS. The estimated %RE of S_G showed biased estimates by RSS. The 13 sample scheme overestimated S_G [%RE median = -9.30%, CI = (-13.13, -6.41)], while the 12 sample scheme underestimated S_G [%RE median = 5.95%, CI = (3.14, 8.47)].

The visual comparison of individual estimates of insulin sensitivity and glucose effectiveness by the full, the 13 sample, and the 12 sample schemes is shown in Figure 5.1. From the clinical viewpoint, the 13 sample scheme provided identical individual estimates of S_I as the full sampling scheme (-0.1 $\pm$ 0.2$\times$ 10^{-5} min^{-1} per pmol L^{-1}; mean $\pm$

Figure 5.4: The posterior densities of the population mean (top panel) and the posterior densities of individual values (bottom panel) for insulin sensitivity estimated with the Bayesian hierarchical analysis using different sampling schemes.

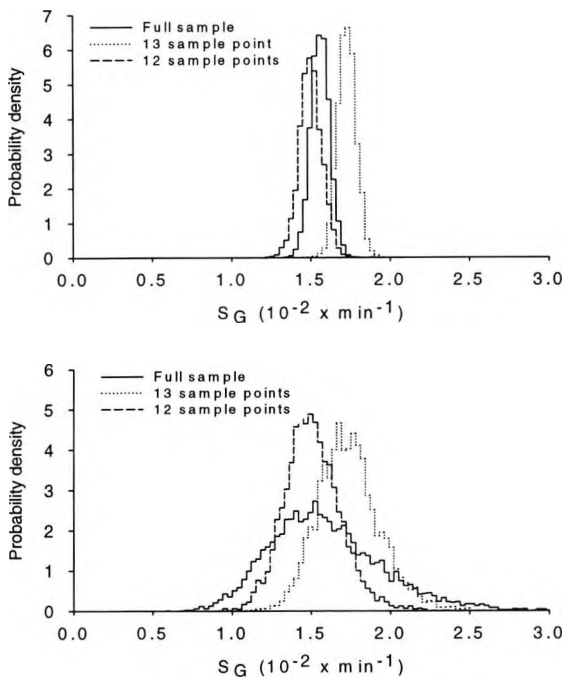


SD of the deviation between the 13 sample and the full sampling scheme). The 12 sample scheme gave a slightly biased estimate, i.e. overestimated low and underestimated high insulin sensitivity, and gave results on the borderline of clinical acceptance (-0.1 $\pm$ 0.4$\times$ 10^{-5} min^{-1} per pmol L^{-1}; mean $\pm$ SD of the deviation between the 12 sample and the full sampling scheme). The difference in the precision of the individual estimates between 13 and 12 sample schemes is illustrated by the histograms in Figures 5.2-5.3

Both the 13 sample and the 12 sample schemes gave unacceptable individual estimates of glucose effectiveness (S_G), see Figure 5.1. The two sampling schemes gave biased estimates indicating that these two sampling schemes do not contain sufficient data to estimate individual values of glucose effectiveness (0.1 $\pm$ 0.2$\times$ 10^{-2} min^{-1}; -0.1 $\pm$ 0.3$\times$ 10^{-2} min^{-1}).

The three sampling schemes gave similar population mean values of insulin sensitivity and glucose effectiveness, see Table 5.2, although 95% CI just failed to overlap for glucose effectiveness estimated by the full and the 13 sample scheme.

Figure 5.5: The posterior densities of the population means (top panel) and the posterior densities of individual values (bottom panel) for glucose effectiveness estimated with the Bayesian hierarchical analysis using different sampling schemes.



5.6.2 Precision of Estimates

The precision of S_I expressed as CV of the parameter estimate was almost identical among the three sampling schemes. It was 16% (CI: 12%, 23%), 18% (CI: 14%, 25%), and 15% (CI: 13%, 19%) for $S_{I(30)}$, $S_{I(13)}$, and $S_{I(12)}$, respectively. Counter intuitively, the precision of S_G was improved with the reduced sampling schemes. It was 14% (CI: 13%, 15%), 10% (CI: 9%, 10%), and 11% (CI: 10%, 11%) for $S_{G(30)}$, $S_{G(13)}$, and $S_{G(12)}$, respectively. The improvement is explained by a tighter population spread (reduced inter-individual variability with reduced sampling schemes), which propagates through the hierarchical model into tighter posterior density functions of individual estimates.

5.7 Discussion

The Bayesian hierarchical analysis offers the possibility to estimate parameters not only from dense data, but also from relatively sparse data (or from a combination of

dense and sparse data). The approach allows the analysis of data from a variety of unbalanced designs.

This study was motivated by the affinity of the hierarchical population analysis to analyse such data coupled with its potential to improve precision of parameter estimates when compared with nonlinear regression analysis.

The main aim was to investigate further the work of Steil et al [117] and Coates et al [28] on reduced sampling schemes with the Bayesian hierarchical framework. The advantage of analysing the complete population sample rather than the individual as a unit as compared to the previous work by Steil et al [117] and Coates et al [28] is that the population method of analysis estimates the population distribution of the parameters in addition to individual parameter estimates. A similar population approach with the minimal model of glucose kinetics in healthy subjects, the iterative two-stage approach (ITS) by Vicini and Cobelli [128], showed that the population estimation improves substantially the precision of parameter estimates with both full and reduced sampling schemes.

Our analysis assessed the bias, the precision and the accuracy of S_I and S_G from RSS during the insulin modified IVGTT in T2D subjects.

Unlike the results by Coates et al [28] in which a significant bias was introduced by the 12 sample scheme ($S_{I(12)}$) in the form of underestimation of the individual S_I, the percent relative error (%RE) demonstrated the absence of bias with 13 and 12 sample schemes for S_I. The results of achieving unbiased S_I from RSS in this study could be attributed to a better form of the parameter estimation approach. %RE of S_G demonstrated the presence of a bias with the RSS. S_G was overestimated with the 13 sample scheme and underestimated with the 12 sample scheme. These results agreed with those by Coates et al [28] and similar to their interpretation, they are judged not clinically significant.

The population characteristics, the population median, gave similar values of S_I for both the full and the reduced sampling schemes. This is demonstrated by overlapping CIs for the posterior density functions. Similar results were obtained for the population characteristics of S_G.

S_I from the reduced sampling schemes was estimated with acceptable precision, the distribution of %RE near 0 indicating a closer agreement. The introduction of the 25 min time-point resulted in an improvement in the precision of the estimation of the S_I from the 13 sample scheme when compared with the 12 sample scheme. The precision of S_G from the reduced sampling schemes was unacceptable and markedly reduced for

the 13 sample scheme. While the 13 sample scheme overestimated individual S_G, the 12 sample scheme underestimated the individual S_G.

The precision of estimates (CV) for S_I was similar for all sampling schemes. The results for S_G gave smaller CV for the reduced sampling schemes compared with the full sampling. The CV obtained for reduced sampling schemes for S_I appeared smaller than those reported by Coates et al [28]. The precision of estimates obtained for reduced sampling schemes for both S_I and S_G was within the acceptable range.

5.8 Conclusions

Our analysis of reduced sampling schemes with the Bayesian hierarchical analysis suggests that the adoption of the 13 sample scheme is preferable to that of the 12 sample scheme. This is in agreement with an observation made by Coates et al [28], who also recommended the 13 sample scheme. This scheme provides accurate estimates of individual and population insulin sensitivity, and population estimates of glucose effectiveness. However, it does not give accurate estimates of individual glucose effectiveness. The 12 sample scheme provides accurate estimates of population insulin sensitivity and glucose effectiveness but not individual estimates of these two parameters. It appears that the addition of a single sample after insulin modification substantially improves accuracy of the calculations.

Part II

Progression of Type 2 Diabetes

Chapter 6

Progression of Insulin Resistance and Insulin Secretion

6.1 Introduction

In the previous chapters, we developed and evaluated a stochastic model using data collected in newly presenting T2D subjects to estimate parameters with the minimal model of glucose kinetics. In the present chapter and Chapters 7 and 8, we will employ the population parameter estimation approach in combination with deterministic approaches to advance our knowledge of insulin sensitivity and insulin secretion in subjects with T2D followed over 2 years.

Three separate objectives relating to the progression of insulin sensitivity and insulin secretion will be investigated. In the present chapter, the primary objective is to study the progression of insulin sensitivity and insulin secretion, taking into consideration some covariates (BMI) in 54 newly presenting T2D subjects. Our primary objective in Chapter 7 is to investigate relationships between the progression of treatment efficacy as measured by HbA_{1C} and initial conditions of metabolic settings at diagnosis and change in metabolic settings over 2 years. We will investigate the inter-subject variability of clinical measures of glucose control during IVGTT and MTT in the same 54 subjects over 2 years in Chapter 8.

The analysis of the progression of the impaired insulin secretion and action is confounded by the need for the subjects to be treated and since the studied subjects were not randomly allocated to individual treatments, it is impossible to separate treatment effects from other variables. Thus, the results will reflect the natural progression of insulin secretion and insulin action, the effectiveness of the treatments, and subjects'

lifestyle. However, a separate analysis of diet treated subjects (16 out of 54 subjects over 2 years) will be carried out to contrast diet and oral pharmacological treatments.

T2D is a progressive disease characterised by deteriorating glycaemic control even after an adequate treatment intervention. Both the development and progression of T2D have been attributed to complex dynamic interactions between insulin tissue sensitivity and insulin secretion to maintain glucose homeostasis. Various longitudinal studies investigated measures of glucose control to evaluate relationships with the onset and progression of diabetes complications [96, 57, 54, 58].

The most recent study, the United Kingdom Prospective Diabetes Study (UKPDS), has provided a better insight into the progression of the indices of clinical measures of glucose control in T2D patients with various treatment approaches, using both pharmacological therapy and diet. In spite of this, the nature of the progression of indices that constitute both insulin sensitivity and insulin secretion is still not fully understood. Moreover, no study was able to show how glycaemic control is related to the progression of insulin resistance and insulin secretion.

This study investigated progression, over two years, of sets of parameters identified during IVGTT and MTT. The parameter set consists of clinical measures of glucose control (FPG, FPI and HbA_{1c}), postprandial glucose response ($AUC_{Glucose}$, $C_{Max,Glucose}$) and postprandial insulin response ($AUC_{Insulin}$, $C_{Max,Insulin}$) to MTT, measures of insulin sensitivity and glucose effectiveness (S_I and S_G), a composite measure of insulin sensitivity, disposition index (D_I), and measures of pancreatic responsiveness (M_I, M_O, and AIR_G).

Table 6.1: Characteristics of newly diagnosed subjects with T2D (N = 54; mean ± SE (range)).

Age (years)	56 ± 1 (36-74)
Gender	43/11 (m/f)
BMI ($kg \cdot m^{-2}$)	30.3 ± 0.6 (18.6-42.4
FPG ($mmol \cdot L^{-1}$)	11.0 ± 0.5) (6.3-18.4)

Table 6.2: IVGTT and MTT sample schemes over two years (N=54).

	IVGTT Sample Scheme			MTT Sample Scheme		
	FSS	RSS	Total	FSS	RSS	Total
Year 0	52	2	54	49	5	54
Year 1	49	5	54	54	0	54
Year 2	54	0	54	54	0	54

6.2 Subject and Experimental Protocol

6.2.1 Subjects

A total number of 278 subjects with newly presenting T2D according to WHO criteria participating in a longitudinal study over 2 years [year 0 (baseline), year 1, and year 2] were considered (Diabetes Research Unit, Llandough Hospital, Penarth, UK). From the set, 54 (19.4%) subjects were selected based on the data selection criteria given in Table 6.3.

The characteristics of the 54 subjects with newly presenting T2D selected for the longitudinal analysis from the database are shown in Table 6.1. The study was approved by the Bro Taf Local Research Ethics Committee, Cardiff, UK.

Table 6.3: Data selection criteria for longitudinal study of subjects with T2D (N=54).

Data Selection Criteria	MTT	IVGTT
Data profiles used	C-peptide, Insulin and Glucose	Insulin and Glucose
Pretest samples	Either of these (–30 or 0 minutes sample) must be available	Either of these (–30, -15 or 0 minutes sample) must be available
Accepted missing values for reduced sampling schemes	2 missing values accepted, at most 1 missing within to 0-180 minutes; Either of 210 and 240 minutes sample must be available	2 missing values accepted, at most 1 missing within 0-180 minutes.
Accepted missing values for full sampling schemes	3 missing values accepted, at most 2 consecutive values	4 missing values accepted, at most 3 consecutive values
End test samples	Either of these (210 or 240 minutes samples) must be available	
Treatments	Subjects must have treatment data	Subjects must have treatment data

Table 6.4: Treatment allocation for newly diagnosed subjects with T2D.

Treatment	Year 0	Year 1	Year 2
No Treatment	54	-	-
Diet	-	23	16
Sulphonylureas	-	6	7
Metformin	-	16	14
Metformin + Sulphonylureas	-	9	17
Total	54	54	54

Sulphonylureas: Gliclazide and Glibenclamide.

6.3 Experimental Protocol

Selected subjects were studied over 2 years, at year 0 (baseline), 1, and 2. The subjects were admitted on the study day to the Diabetes Research Unit, Llandough Hospital (Penarth, UK). The subjects were studied after an overnight fast for 12 hours. On one occasion, the subjects underwent the insulin-modified intravenous glucose tolerance test (IVGTT) consisting of a 300mg·kg^{-1} glucose bolus per body weight given at 0 minute over 2 minutes, followed by an insulin injection of 0.05U·kg^{-1} at 20 minutes. Blood samples were collected at (-30), (-15), (-1), (0), 1, (2), 3, (4), 5, 6, 7, (8), 10, 12, 14, 16, (19), (22), 23, 24, 25, 27, (30), (40), (50), 60, (70), 80, (90), 100, 120, 150 and (180) minutes for measurement of plasma glucose, insulin, and C-peptide. The values in the brackets indicate a reduced sampling scheme, which was adopted for a subset of subjects, see Table 6.2 for number of subjects with full and reduced IVGTT sampling schemes over two years.

On a separate occasion, on average of one month after the IVGTT, each subject underwent a meal tolerance test (MTT). The meal was mainly solid with a small amount of juice and milk. It consisted of 15g Weetabix, 10g skimmed milk, 250ml pineapple juice, 50g white chicken, 60g wholemeal bread, 10g of polyunsaturated margarine. Total energy was 482 cal; total carbohydrate was approximately 75g. The meal was eaten within 10 minutes. Blood samples were collected at (-30), (-1), (0), 1, 10, 20, (30), 40, 50, (60), 75, (90), (120), (150), (180), (210) and (240) min for measurement of plasma glucose, insulin, and C-peptide. The values in the brackets indicate a reduced sampling scheme, which was adopted for a subset of subjects, see Table 6.2 for the number of subjects with full and reduced MTT sampling schemes over two years.

6.3.1 Analytical Techniques

Plasma glucose concentration was assayed using glucose oxidase method (Yellow Spring Analyser, YSI 2300, USA; intra-assay CV $< 2\%$). Insulin and C-peptide were assayed using monoclonal antibodies method (intra-assay CV $< 5\%$) and CV $< 6\%$ respectively). Samples collected from the patients were sent to the laboratory immediately. Blood samples were then centrifuged (2000g, 5mins) in a refrigerated (4^0C) centrifuge and the plasma put into aliquot and frozen at -20^0C immediately. Samples remained frozen until assayed.

6.4 Data Analysis

6.4.1 Data Selection Criteria

Tables 6.3 presents the criteria employed for the data selection. A total of 54 out of 278 subjects satisfied the specified criteria. The treatments were both non-pharmacological and pharmacological. At year 1, the treatment was mainly related to dietary and exercise advice alone. Other pharmacological therapies, Metformin and sulphonylureas or their combination, were then followed as required, see Table 6.4 for treatment patterns.

6.4.2 Estimating Metabolic Indices

Estimating S_I and S_G

The Bayesian hierarchical analysis of the minimal model of IVGTT data gave estimates of metabolic indices (S_I and S_G). S_I and S_G measure insulin sensitivity and glucose effectiveness, respectively. See Section 3.4 for details of the Bayesian hierarchical analysis. The analysis was run for each year separately.

Estimating AIR_G and D_I

The first phase insulin response (AIR_G; a measure of pancreatic β-cell responsiveness) was calculated as the incremental area under the curve from 2-8 minutes during the IVGTT. The disposition index (D_I; a composite measure of insulin sensitivity and pancreatic β-cell responsiveness) was obtained as a product of AIR_G and S_I ($D_I = AIR_G \times S_I$).

Estimating M_O and M_I

The insulin secretion model, see Section 2.3.2, Figure 2.2, implemented in the Pancreatic Responsiveness package (CPR version 1.0, developed by Roman Hovorka and H. C. Subasinghe, MIM Centre, City University, UK, 1997) was used to estimate indices of pancreatic β-cell responsiveness M_O and M_I from MTT data. M_O represents fasting prehepatic insulin secretion divided by fasting plasma glucose and expresses the ability of fasting glucose to stimulate C-peptide secretion. M_I represents the increase in prehepatic insulin secretion given an increment in postprandial glucose and expresses the ability of postprandial glucose to stimulate C-peptide secretion.

Estimation of Clinical Measures of Glucose Control and Insulin Responses to MTT

Fasting plasma glucose (FPG) and fasting plasma insulin (FPI) were estimated as mean pretest values of IVGTT and MTT experiments. $C_{max.Glucose}$ and $C_{max.Insulin}$ were the maximum incremental plasma glucose and insulin concentrations during MTT. $AUC_{Glucose}$ and $AUC_{Insulin}$ were the incremental area under the curve of plasma glucose and insulin, respectively, during MTT from 0 to 180 minutes.

6.5 Statistical Analysis

All analyses were conducted using SPSS version 11.5 (SPSS Inc. Chicago, Illinois). Variables that were not normally distributed were log transformed before analysis and the results were expressed as means and confidence intervals (CI). For ease of visualisation, data are presented in the measured untransformed scale. Significant changes over time were assessed by two-way ANOVA allowing for both subject and time effects. Statistical significance was declared at $P < 0.05$.

6.6 Results

6.6.1 Clinical Measures of Glucose Control

FPG and HbA_{1C} decreased significantly ($P < 0.001$) from baseline (year 0) to year 1 and 2, see Figures 6.1 and Table 6.5. In year 0-1, FPG decreased by 20%, with no change in year 1-2, see Table 6.5. Over two years, HbA_{1C} changed significantly ($P < 0.001$), see Figure 6.1. The decrease for years 0-2 was 13%, with a decrease of about 22% in year 0-1 and a returne to 87% of its baseline value in year 2.

FPI significantly increased in years 0-2 ($P < 0.02$), with an 11% increase in year 0-1, see Figure 6.1, and a 6% increase in year 1-2. The differences over years 0-2 remained significant after adjusting for BMI ($P < 0.04$), see Table 6.5.

Similar analyses were performed for 16 subjects treated with diet over years 0-2. The results showed significant changes in year 0-1 in both FPG and HbA_{1C}, similar to the results obtained for all 54 subjects, see Figure 6.2. A significant decrease was also observed in FPG in years 0-2, but not in HbA_{1C}, see Figure 6.2. FPI remained unchanged over the two years.

Table 6.5: Progression of clinical measures of glucose control over two years after presentation of T2D (N=54).

Clinical measures of glucose control	Year	Mean*	95% CI**	P-value†
FPG ($mmol \cdot L^{-1}$)	0	10.6	(10.1, 11.0)	
FPG ($mmol \cdot L^{-1}$)	1	8.4	(8.0, 8.8)	0.000
FPG ($mmol \cdot L^{-1}$)	2	8.5	(8.1, 8.9)	
FPI ($pmol \cdot L^{-1}$)	0	57.6	(53.5, 62.1)	
FPI ($pmol \cdot L^{-1}$)	1	63.9	(59.3, 68.9)	0.042
FPI ($pmol \cdot L^{-1}$)	2	65.6	(60.9, 70.7)	
HbA_{1C} (%)	0	7.6	(7.3, 8.0)	
HbA_{1C} (%)	1	6.1	(5.8, 6.4)	0.000
HbA_{1C} (%)	2	6.6	(6.4, 7.1)	

* Geometric mean after adjustment for BMI.
** CI stands for confidence interval.
† ANOVA.

6.6.2 Plasma Glucose, Insulin, and C-peptide Profiles During IVGTT and MTT.

The profiles of plasma glucose, insulin, and C-peptide during IVGTT and MTT in years 0-2 are shown in Figure 6.3.

During IVGTT, the effect of exogenous insulin given at 20mins to lower plasma glucose was clearly visible. The glucose bolus failed to stimulate an immediate insulin response at the start of the experiment, resulting in a paradoxical temporary suppression of insulin secretion.

During MTT, the glucose and insulin levels remained elevated throughout the experiment and peaked within 60-100mins. The paradoxical suppression of endogenous insulin secretion was not present. Diet treated subjects had lower glucose profiles, see Figure 6.5. Plasma glucose, insulin, and C-peptide profiles for different treatment regimens in year 1-2 are given in Figures 6.6 and 6.7.

Figure 6.1: Geometrical mean and 95% confidence interval for clinical measures of glucose control in all subjects (N=54).

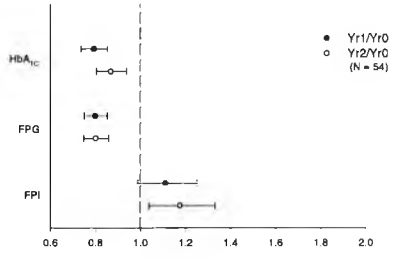
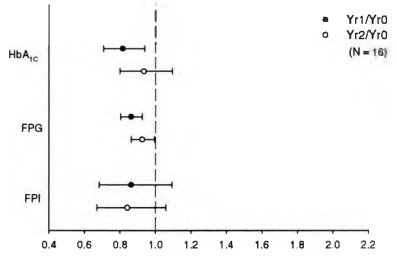


Figure 6.2: Geometrical means and 95% confidence interval for clinical measures of glucose control in diet treated subjects (N=16).



6.6.3 Indices of Postprandial Glucose and Insulin Responses to MTT

Plasma glucose and insulin responses to MTT showed significant changes over two years, see Table 6.6. Both $AUC_{Glucose}$ and $C_{max,Glucose}$ were reduced in years 0-2 ($P < 0.001$). The reductions were present in year 0-1 with an 18% drop observed for $AUC_{Glucose}$ and a 6% drop for $C_{max,Glucose}$, see Figure 6.8. The drops over years 0-2 for both variables were 18% and 12%, respectively. The significant differences for years 0-2 remained after adjusting for BMI.

$AUC_{Insulin}$ and $C_{max,Insulin}$ also changed significantly ($P < 0.001$) over years 0-2, see Table 6.6. $AUC_{Insulin}$ increased by 53% in year 0-1 and dropped by 5% in year 1-2. Unlike $AUC_{Insulin}$, the change is reversed in $C_{max,Insulin}$, which was reduced by 33% in year 1-2, with a drop of just 2% in year 0-1. Figures 6.8 and 6.9 show the proportional changes in mean values in year 0-1 and year 1-2 for glucose and insulin responses to MTT in subjects and all diet treated subjects, respectively, before adjusting for BMI.

Figure 6.3: Plasma glucose, insulin, and C-peptide mean profiles during IVGTT (left panels) and MTT (right panels) in year 0, 1, and 2 (N=54).

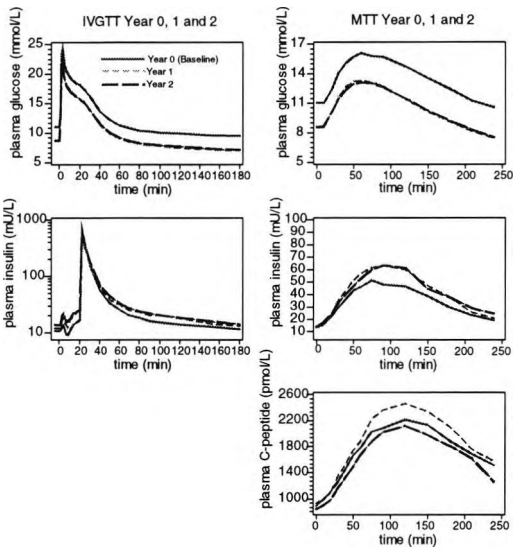


Figure 6.4: Plasma glucose, insulin, and C-peptide mean profiles during IVGTT (left panels) and MTT (right panels) in year 0, 1, and 2 (N=16).

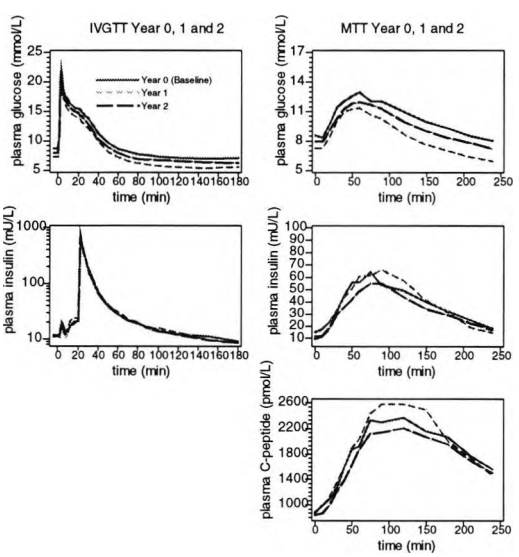


Figure 6.5:

Figure 6.6: Plasma glucose and insulin mean profiles for different treatment regimens during IVGTT in year 1 (left panels) and year 2 (right panels) (N=54).

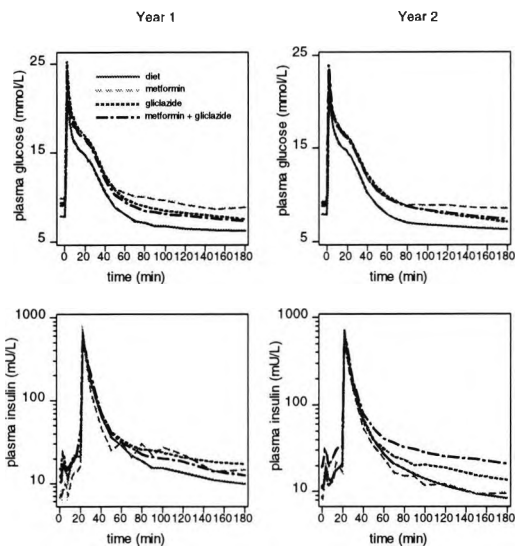


Figure 6.7: Plasma glucose and insulin mean profiles for different treatment regimens during MTT in year 1(left panels) and year 2 (right panels) (N=54).

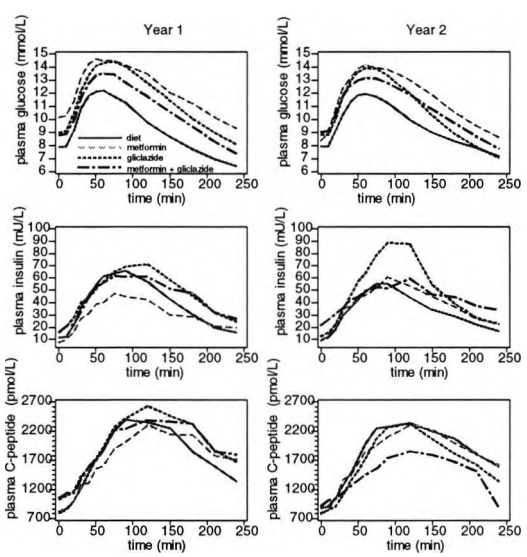


Table 6.6: Progression of post-prandial glucose and insulin responses to MTT over two years after presentation of T2D (N=54).

Postprandial responses to MTT	Year	Mean*	95%CI**	P-value†
$AUC_{Glucose}$ ($mmol \cdot L^{-1}$per 80min)	0	589	(551, 626)	
$AUC_{Glucose}$ ($mmol \cdot L^{-1}$per 80min)	1	485	(448, 523)	0.000
$AUC_{Glucose}$ ($mmol \cdot L^{-1}$per 80min)	2	488	(450, 526)	
$AUC_{Insulin}$ ($mmol \cdot L^{-1}$per 80min)	0	20.78	(18.25, 23.63)	
$AUC_{Insulin}$ ($mmol \cdot L^{-1}$per 80min)	1	31.66	(27.83, 36.02)	0.000
$AUC_{Insulin}$ ($mmol \cdot L^{-1}$per 80min)	2	29.14	(25.53, 33.25	
$C_{max,Glucose}(mmol \cdot L^{-1})$	0	5.16	(4.90, 5.42)	
$C_{max,Glucose}(mmol \cdot L^{-1})$	1	4.86	(4.63, 5.12)	0.005
$C_{max,Glucose}(mmol \cdot L^{-1})$	2	4.57	(4.34, 4.81)	
$C_{max,Insulin}$ ($pmol \cdot L^{-1}$)	0	292	(259, 330)	
$C_{max,Insulin}$ ($pmol \cdot L^{-1}$)	1	288	(256, 325)	0.000
$C_{max,Insulin}$ ($pmol \cdot L^{-1}$)	2	196	(174, 221)	

* Geometric mean after adjustment for BMI.
** CI stands for confidence interval.
† ANOVA

All these changes persisted after adjusting for BMI.

6.6.4 Metabolic Indices

Table 6.7 shows progression of insulin sensitivity and insulin secretion over years 0-2. S_I and S_G did not change over years 0-2. The disposition index D_I increased significantly ($P < 0.001$) in year 0-1 by 46% with no significant change in year 1-2. The proportional change in year 1-2 was just 4%. The trend for years 0-2 in D_I persisted after adjusting for the BMI, see Table 6.7 and Figure 6.10.

S_I, S_G, and D_I in diet treated subjects showed no significant differences over the two years, see Figure 6.11.

Measures of insulin secretion, M_O and AIR_G, increased significantly in year 0-1. The proportional increases in both variables were 31% and 26%, respectively. M_I showed

Figure 6.8: Geometrical mean and 95% confidence interval for postprandial glucose and insulin responses to MTT (N=54).

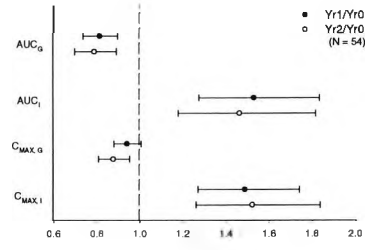
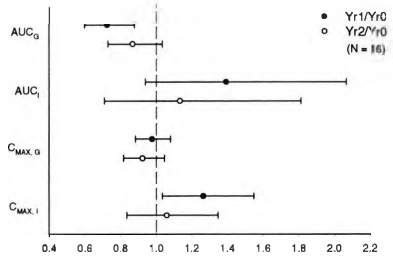


Figure 6.9: Geometical mean and 95% confidence interval for postprandial glucose and insulin responses to MTT in diet treated subjects (N=16).



no significant change over the years. Even after adjusting for BMI. The trend for M_O and AIR_G remained after adjusting for BMI. Figure 6.11, demonstrates no significant change in year 0-1 and years 0-2 in M_O, M_I, and AIR_G in diet treated subjects.

6.7 Discussion

Impaired insulin secretion and insulin resistance are major determinants of the dysregulation of glucose metabolism in T2D subjects. The resultant hyperglycaemia and hyperinsulinimia result in microvascular and macrovascular complications that comprise the main cause of morbidity and mortality in T2D.

The goal of any form of treatment is to reduce the risk of such complications by improving glycaemic control. Improved understanding of the pathophysiology and progression of the disease will support the development of rational therapeutic approaches based

Table 6.7: Progression of metabolic indices over two years after presentation of T2D (N=54).

Metabolic Index	Year	Mean*	95%CI**	P-value†
$S_I \times 10^{-5}(min^{-1}$ per $pmol \cdot L^{-1})$	0	1.12	(0.97, 1.29)	
$S_I \times 10^{-5}(min^{-1}$ per $pmol \cdot L^{-1})$	1	1.30	(1.13, 1.49)	0.300
$S_I \times 10^{-5}(min^{-1}$ per $pmol \cdot L^{-1})$	2	1.17	(1.02, 1.34)	
$S_G \times 10^{-2}(min^{-1})$	0	1.51	(1.47, 1.54)	
$S_G \times 10^{-2}(min^{-1})$	1	1.49	(1.45, 1.52)	0.070
$S_G \times 10^{-2}(min^{-1})$	2	1.54	(1.51, 1.58)	
AIR_G ($pmol \cdot L^{-1}$per 6min)	0	325	(296, 358)	
AIR_G ($pmol \cdot L^{-1}$per 6min)	1	409	(372, 449)	0.000
AIR_G ($pmol \cdot L^{-1}$per 6min)	2	471	(428, 518)	
$D_I \times 10^{-5}$ (min^{-1} per 6min)	0	364	(311, 425)	
$D_I \times 10^{-5}$ (min^{-1} per 6min)	1	532	(455, 621)	0.000
$D_I \times 10^{-5}$ (min^{-1} per 6min)	2	549	(470, 625)	
$M_I \times 10^{-9}$ (min^{-1})	0	16.40	(13.85, 19.43)	
$M_I \times 10^{-9}$ (min^{-1})	1	19.38	(16.36, 22.94)	0.206
$M_I \times 10^{-9}$ (min^{-1})	2	15.82	(13.33, 18.75)	
$M_O \times 10^{-9}$ (min^{-1})	0	4.81	(4.28, 5.42)	
$M_O \times 10^{-9}$ (min^{-1})	1	6.30	(5.58, 7.06)	0.020
$M_O \times 10^{-9}$ (min^{-1})	2	5.24	(4.65, 5.90)	

*Geometric Mean after adjustment for BMI.

** CI stands for confidence interval.

†ANOVA

Figure 6.10: Geometical mean and 95% confidence intervals for metabolic indices in all subjects (N=54).

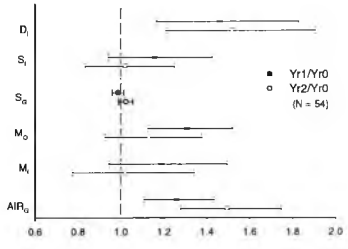
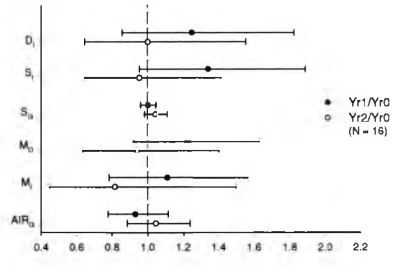


Figure 6.11: Geometical means and 95% confidence interval for metabolic indices in diet treated subjects (N=16).



on the relationships between impaired glucose-induced insulin secretion and insulin resistance and resultant effects.

To increase the understanding of the early progression of T2D, we investigated the progression of both insulin resistance and insulin secretion and associated clinical measures of glucose control over two years after diagnosis. Insulin resistance and insulin secretion have been well described in numerous studies [41, 36, 67, 109, 122], but only a few studies have examined their development over a of period years [97, 55, 57, 54, 83].

The study investigated 54 newly presenting T2D subjects over two years. Subjects were referred directly after diagnosis by their GPs. The subjects had no prior treatment or any dietary advice for diabetes before undergoing MTT and IVGTT. Subjects were put on intensive dietary therapy alone for the first 6 months of diagnosis. The subjects' elevated FPG levels were reviewed after 6 months and 12 monthly for two years. The outcomes of the reviews determined the type of therapies.

All 54 subjects were placed on intensive dietary therapy for 6 months after diagnosis. 23 subjects remained on the intensive dietary therapy at the end of the first year and the

remaining 31 subjects elevated to one or a combination of pharmacological therapies. Only 16 subjects remained with the dietary therapy at the end of the second year and 38 on pharmacotherapies.

The progression of glucose control and metabolic indices in this study reflects the combination of long-term degenerative development of insulin resistance and insulin secretion, the effectiveness of non-pharmacological and pharmacological therapies (sulphonylureas and Metformin) and the subjects' lifestyle. Sulphonylureas improve β-cell function but are associated with weight gain. Metformin improves insulin sensitivity [57, 54]. The long-term assessment of the progression of impaired insulin secretion and insulin action in this study is confounded by the need to treat the subjects and since the treatments were not randomly allocated, it is impossible to separate the treatment effects from the natural progression of insulin secretion and action over the two years. A robust statistical technique of separating treatment effect in this type of study design is the generalised linear mixed effect model technique, which was not employed here.

The presence of elevated fasting FPG at the baseline is an indication that the disruption of the normal relationship between β-cell function and insulin sensitivity is already well established in these subjects. The mean value of FPI at the baseline was within the range observed in normal subjects.

Fasting plasma glucose fell over years 0-2, which was associated with improved fasting β-cell responsiveness. Insulin sensitivity did not change.

The decrease in FPG over the two years demonstrated the need for intensive conventional pharmacological therapy. The failure of diet therapy alone was reported by Levy et al in their 10 years follow-up diet study in T2D subjects [83]. They showed that a progressive rise in FPG was associated with a progressive fall in an index of pancreatic β-cell function. They also reported no change in insulin sensitivity over the years.

The results of the present study showed that insulin sensitivity and glucose effectiveness were unchanged over 2 years, even with the conventional pharmacological therapies and progressive decrease in HbA_{1C}.

The disposition index D_I increased significantly in year 0-1 by 46% with no significant increase in year 1-2. This demonstrated that an improvement of β-cell function to compensate for insulin resistance is most effective in the first year of treatment and is maintained in the second year.

Both fasting β-cell responsiveness and first phase insulin secretion, M_O and AIR_G, improved significantly in years 0-2. The proportional increases in both variables are

31% and 26%, respectively. M_I showed no significant change over the years. The improvement of both M_O and AIR_G over the two years demonstrated that an adequate conventional treatment of T2D subjects early after diagnosis can have a profound improvement on the well being of the β-cell [57, 54].

6.8 Conclusions

We conclude that indices of insulin resistance and glucose effectiveness (S_I and S_G) do not improve over time in spite of the early therapeutic intervention in T2D subjects. Indices of insulin secretion (M_O and AIR_G) improved over years with main improvement in year 0-1.

The above analyses demonstrated that the progressive nature of T2D is rather due to the steady decline in the β-cell function, which can be ameliorated by adequate conventional treatment for at least two years after diagnosis of T2D.

Chapter 7

Progression of HbA_{1C}

7.1 Introduction

Monitoring blood glucose control is considered the cornerstone of diabetes care [51]. The Diabetes Control and Complications Trial (DCCT), a ten-year landmark study, demonstrated clearly that maintaining near-normal blood glucose levels significantly lowers a person's risk of developing complications related to type 1 diabetes [113]. Monitoring blood glucose control includes several different components: blood glucose testing, urine ketone testing, and glycated haemoglobin testing (haemoglobin A1c, glycosylated haemoglobin).

The UKPDS, the largest and the longest study of T2D, was the first to provide evidence of the benefit of tight glycaemic control in T2D. The UKPDS has demonstrated that a tight glycaemic control with oral agent and insulin combined with intensive monitoring could reduce complications and give a better quality of life for people with T2D. UKPDS data showed that a median decrease of 0.9% of HbA_{1C} level led to risk reduction in multiple areas, including diabetes related end-points and microvascular complications.

An assessment of long-term blood glucose control is best made by measuring HbA_{1C} every three or four months. HbA_{1C} is used because it correlates well with the average concentration and reflects improvements occurring gradually over time [19]. HbA_{1C} can be used to judge the treatment efficacy. An HbA_{1C} measurement refers to a series of stable haemoglobin components formed by the combination of glucose and haemoglobin [51]. Individuals with higher levels of blood glucose will have higher levels of glycated haemoglobin. Because the haemoglobin components are stable, the level provides an average indication of the overall blood glucose levels over two to three months period.

In the previous chapter, the early progression of T2D was evaluated. In the present chapter, the focus is on describing in detail the relationship between progression of

HbA_{1C} and initial conditions at the diagnosis of T2D with the aim to establish whether certain metabolic settings are more favourable for an early improvement of glucose control. The second objective is to map the temporal relationship between HbA_{1C} and other variables to establish the associations leading to rational treatment decisions.

7.2 Subjects and Experimental Protocol

We studied 54 subjects with newly presenting T2D as described in Chapter 6, Section 6.2, over 2 years.

7.3 Statistical Analysis

All analyses were conducted using SPSS version 11.5 (SPSS Inc. Chicago, Illinois). Variables that were not normally distributed were log transformed before analysis. Spearman's correlation analysis with a Boniferroni correction was used to examine the associations between the outcome variable quantifying treatment efficacy (a change in HbA_{1C}) and other variables (basal values of BMI, FPI, HbA_{1C}, and metabolic indices).

The stepwise linear analysis was used to relate changes in HbA_{1C} to basal values and changes in BMI, FPI, and metabolic indices.

7.4 Results

7.4.1 Relating Changes in HbA_{1C} (ΔHbA_{1C}) to Metabolic Settings at T2D Diagnosis

Table 7.1 shows the associations between changes in HbA_{1C} and the basal values of metabolic indices (BMI, FPI, AIR_G, M_I, M_O, S_G, S_I, and D_I).

Basal values of FPI, M_O, and M_I showed positive significant correlation with a change in HbA_{1C} in year 0-1, ($r_s = 0.45$, $P < 0.01$), ($r_s = 0.61$, $P < 0.001$), ($r_s = 0.54$, $P < 0.001$), respectively. The strongest correlation in year 0-1 was between a change in HbA_{1C} and its basal value. None of the basal values showed any significant association with the change in HbA_{1C} in year 1-2. The overall change in HbA_{1C} over year 0-2 was significantly correlated with FPI, M_O, and M_I, ($r_s = 0.63$, $P < 0.001$), ($r_s = 0.74$, $P <$

0.001), (r_s = 0.56, P < 0.001), respectively. AIR_G also showed a positive correlation with an increasing trend with the change in HbA_{1C} over the two years.

The graphical representation of ΔHbA_{1C} against S_I, M_I, and M_O is shown in Figures 7.1-7.3. Figure 7.1 a-c demonstrates a lack of relationship between changes in HbA_{1C} and the basal value of S_I. Figures 7.2-7.3 (a-c) show the relationships between changes in HbA_{1C} and the basal values of M_O and M_I. Subjects with low basal values of M_O and M_I had their HbA_{1C} values improved to the greatest extent, while subjects with high basal values of M_O and M_I did not experience any improvement in HbA_{1C}.

The results of the stepwise regression analysis are shown in Table 7.2. Out of the predictor indices (BMI, FPI, AIR_G, M_I, M_O, S_G, S_I, D_I), only M_O contributed significantly to the variation in ΔHbA_{1C} (year 0-1) (M_O = 0.58, P < 0.001, and R^2 = 33%). In year 1-2, only AIR_G and S_G contributed significantly to the variation in ΔHbA_{1C} (year 1-2) (AIR_G = 0.38, P < 0.01; S_G = 0.27, P < 0.05, and R^2 = 18%). For ΔHbA_{1C} in years 0-2, only basal M_O and AIR_G significantly contributed to its variation (M_O = 0.50, P < 0.01; AIR_G = 0.32, P < 0.05, and R^2 =53%). M_O and AIR_G accounted for about 53% of the total variation in change in HbA_{1C} over the two years.

Including basal HbA_{1C} among the predictor variables, the results of the stepwise regression analysis are shown in Table 7.3. Out of the predictor indices (BMI, FPI, AIR_G, M_I, M_O, S_G, S_I, D_I, HbA_{1C}), only basal HbA_{1C} contributed significantly to the variation in ΔHbA_{1C} in year 0-1 (HbA_{1C} = -0.82, P < 0.001, and R^2 = 67%). In year 1-2, only basal AIR_G and S_G contributed significantly to the variation in ΔHbA_{1C}. (AIR_G = 0.55, P < 0.01; HbA_{1C} = 0.37, P < 0.0,1 and R^2 = 27%). For ΔHbA_{1C}, in years 0-2, basal HbA_{1C}, M_O and AIR_G significantly contributed to its variation (HbA_{1C} = -0.47, P < 0.001; AIR_G = 0.26, P < 0.05, S_G = 0.24, P < 0.05, and R^2 =66%). HbA_{1C}, M_O and AIR_G accounted for about 66% of total variation in change in HbA_{1C} over the two years.

7.4.2 Relating Change in Glycated Haemoglobin to Change in Metabolic Indices, Change in Body Mass Index, and Change in Fasting Plasma Insulin

The correlation coefficients between changes in metabolic indices and changes in HbA_{1C} are given in Table 7.4.

Changes in M_O (r_s = -0.59, P < 0.001) and M_I (r_s = -0.40, P < 0.01) were negatively correlated with treatment efficacy (ΔHbA_{1C}) in the first year. The change in HbA_{1C} in year 1-2 showed no significant correlation with any of these parameters. The overall

Table 7.1: Spearman's coefficients correlation between a change (year 0-1, year 1-2, and year 0-2) in HbA_{1C} and basal values of BMI, FPG, HbA_{1C}, insulin sensitivity, and pancreatic responsiveness (AIR_G, M_I, M_O, S_G, S_I, D_I).

	Basal Values								
	BMI	FPI	AIR_G	M_I	M_O	S_G	S_I	D_I	HbA_{1C}
$\Delta_{1-0}HbA_{1C}$	030*	0.45**	0.34*	0.54***	0.61***	-0.25	-0.17	-0.23	-0.82***
$\Delta_{2-1}HbA_{1C}$	0.24	0.31*	0.39**	0.22	0.27*	0.33*	-0.22	0.13	0.13
$\Delta_{2-0}HbA_{1C}$	0.36**	0.63***	0.59***	0.56***	0.74***	-0.10	-0.21	0.42**	-0.75***

*P < 0.05; **P < 0.01; ***P < 0.001 (corrected for multiple comparisons using Boniferroni correction).

Table 7.2: Results of the stepwise linear regression analysis (z-scores). Dash (-) indicates independent variable (BMI, FPI, AIR_G, M_I, M_O, S_G, S_I, and D_I) not selected during the regression analysis to explain the change (year 0-1, year 1-2, and year 0-2) in the dependent variable (HbA_{1C}).

	Basal Values								
	BMI	$FPI^{\dagger}$	$AIR_G^{\dagger}$	$M_I^{\dagger}$	$M_O^{\dagger}$	$S_G^{\dagger}$	$S_I^{\dagger}$	$D_I^{\dagger}$	R^{2+}
$\Delta_{1-0}HbA_{1C}$	-	-	-	-	0.58****	-	-	-	0.33
$\Delta_{2-1}HbA_{1C}$	-	-	0.38**	-	-	0.27*	-	-	0.18
$\Delta_{2-0}HbA_{1C}$	-	-	0.32*	-	0.50***	-	-	-	0.53

*P < 0.05; **P < 0.01; ***P < 0.001 (corrected for multiple comparisons using Boniferroni correction); † data log transformed; + adjusted R^2.

Table 7.3: Results of the stepwise linear regression analysis (z-scores) including basal HbA_{1C} among the predictor variables. Dash (-) indicates independent variable (BMI, FPI, AIR_G, M_I, M_O, S_G, S_I, D_I, and HbA_{1C}) not selected during the regression analysis to explain the change (year 0-1, year 1-2, and year 0-2) in the dependent variable (HbA_{1C}).

	Basal Values									
	BMI	$FPI^{\dagger}$	$AIR_G^{\dagger}$	$M_I^{\dagger}$	$M_O^{\dagger}$	$S_G^{\dagger}$	$S_I^{\dagger}$	$D_I^{\dagger}$	$HbA_{1C}^{\dagger}$	R^{2+}
$\Delta_{1-0}HbA_{1C}$	-	-	-	-	-	-	-	-	-0.82****	0.67
$\Delta_{2-1}HbA_{1C}$	-	-	0.55**	-	-	-	-	-	0.37*	0.27
$\Delta_{2-0}HbA_{1C}$	-	-	0.26*	-	0.24*	-	-	-	-0.47***	0.66

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (corrected for multiple comparisons using Boniferroni correction); † data log transformed; + adjusted R^2.

treatment efficacy (ΔHbA_{1C}) over two years showed similar, but reduced, significant negative correlations with M_O (r_s = -0.49, $P < 0.001$) and M_I (r_s = -0.36, $P < 0.01$).

A stepwise linear regression analysis was performed to investigate which changes in metabolic settings (ΔS_I, ΔS_G, ΔM_I, ΔM_O, and ΔD_I), and ΔBMI could best describe changes in HbA_{1C} (ΔHbA_{1C}), see Table 7.5. The combination of changes in M_O, M_I, and S_G in year 0-1 explained 43% of variation in treatment efficacy in year 0-1. Only M_O was selected in the stepwise regression analysis in year 1-2 and it explained 6% variation in change of HbA_{1C} in year 1-2. Only 29% of the overall variation in the treatment efficacy measured by a change in HbA_{1C} over two years was explained by M_O and S_G.

An examination of the relationship between M_O and M_I over the two years showed a positive correlation with a decreasing trend (M_O vs M_I at year 0: r_s= 0.73, $P < 0.001$; at year 1: r_s= 0.52, $P < 0.001$; at year 2: r_s= 0.45, $P < 0.001$). The analysis of the relationship between a change in M_O to a change in M_I also indicated a positive significant association (r_s= 0.48, $P < 0.01$).

Table 7.4: Correlations between a change in HbA_{1C} and a change in BMI, FPG, and metabolic settings over year 1 (N = 54), year 2 (N = 52), and both years combined (N = 106).

	Change (Year 1 - Year 0)						
	ΔBMI	ΔAIR_G	ΔM_I	ΔM_O	ΔS_G	ΔS_I	ΔD_I
ΔHbA_{1C}	-0.34	-0.00	-0.40*	-0.59***	0.27	-0.17	-0.23
	Change (Year 2 - Year 1)						
	ΔBMI	ΔAIR_G	ΔM_I	ΔM_O	ΔS_G	ΔS_I	ΔD_I
ΔHbA_{1C}	0.13	-0.13	-0.19	-0.20	-0.12	-0.10	-0.13
	Change (Year 1 - Year 0 and Year 2 - Year 1)						
	ΔBMI	ΔAIR_G	ΔM_I	ΔM_O	ΔS_G	ΔS_I	ΔD_I
ΔHbA_{1C}	0.01	-0.02	-0.36*	-0.52***	0.21	-0.21	-0.17

Spearman's correlation coefficients are reported.
*P < 0.05; **P < 0.01; ***P < 0.001 (corrected for multiple comparison using Boniferroni correction).

7.5 Discussion

The relationship between HbA_{1C} and metabolic settings (insulin sensitivity and insulin secretion) over time is not fully understood. We have evaluated the relationship between changes in HbA_{1C} and basal values of insulin secretion, insulin sensitivity, and changes in metabolic settings over time.

The analysis indicated significant relationships between a change in HbA_{1C} and basal values of M_I and M_O. The analysis showed that subjects with low basal values of M_I and M_O experienced the largest improvement in HbA_{1C}.

Subjects with low M_O and M_I have severely reduced β-cell responsiveness. It appears that it is possible to bring about the largest improvement in glucose control in such subjects. This is also confirmed by the relationship between basal HbA_{1C} and the change in HbA_{1C}, which indicates that subjects with the poorest glucose control experience greatest improvement in the first two years after diagnosis of T2D.

The improvement in HbA_{1C} is accompanied by improvement in fasting (M_O) and also postprandial (M_I) β-cell responsiveness. This is consistent with the notion that β-cell

Table 7.5: A stepwise linear regression analysis between a change in HbA_{1C} and a change in BMI and metabolic settings over year 1 (N = 54), year 2 (N = 52) and both years combined (N = 106).

	Change (Year 1 - Year 0)							
	ΔBMI	ΔAIR_G	ΔM_I	ΔM_O	ΔS_G	ΔS_I	ΔD_I	R^{2+}
ΔHbA_{1C}	-0.32**	-	-	-0.47***	0.26*	-	-	0.43
	Change (Year 2 - Year 1)							
	ΔBMI	ΔAIR_G	ΔM_I	ΔM_O	ΔS_G	ΔS_I	ΔD_I	R^{2+}
ΔHbA_{1C}	-	-	-	-0.28*	-	-	-	0.06
	Change (Year 1 - Year 0 and Year 2 - Year 1)							
	ΔBMI	ΔAIR_G	ΔM_I	ΔM_O	ΔS_G	ΔS_I	ΔD_I	R^{2+}
ΔHbA_{1C}	-	-	-	-0.51***	0.19*	-	-	0.29

*P < 0.05; **P < 0.01; ***P < 0.001.
Standardised scores (z-scores) of the regression coefficients are reported.
Dash (-) indicates independent variables not selected during regression analysis.
+ adjusted R^2

responsiveness is the major determinant of glucose control at the presentation of T2D. Reversal of β-cell failure is accompanied by improved HbA_{1C}.

Improvement in HbA_{1C} showed no relationship with change in insulin sensitivity and glucose effectiveness. The highest amount of variation in the improvement of HbA_{1C} was explained in year 0-1 using BMI, M_O, and S_G indices.

7.6 Conclusions

T2D subjects with low basal values of M_O and M_I improved HbA_{1C} over two years to a greater extent than their counterparts with high basal values of M_O and M_I.

This suggests that naive treatment in the early years of T2D improves HbA_{1C} by improving M_O and M_I. The treatment efficacy improved over two years but the best results were achieved in the first year after the diagnosis. About 43% variations in HbA_{1C} were explained by M_O, BMI, and S_G in the first year.

Figure 7.1: Relationships between a change in HbA_{1C} with insulin sensitivity (S_I) at time of diagnosis.

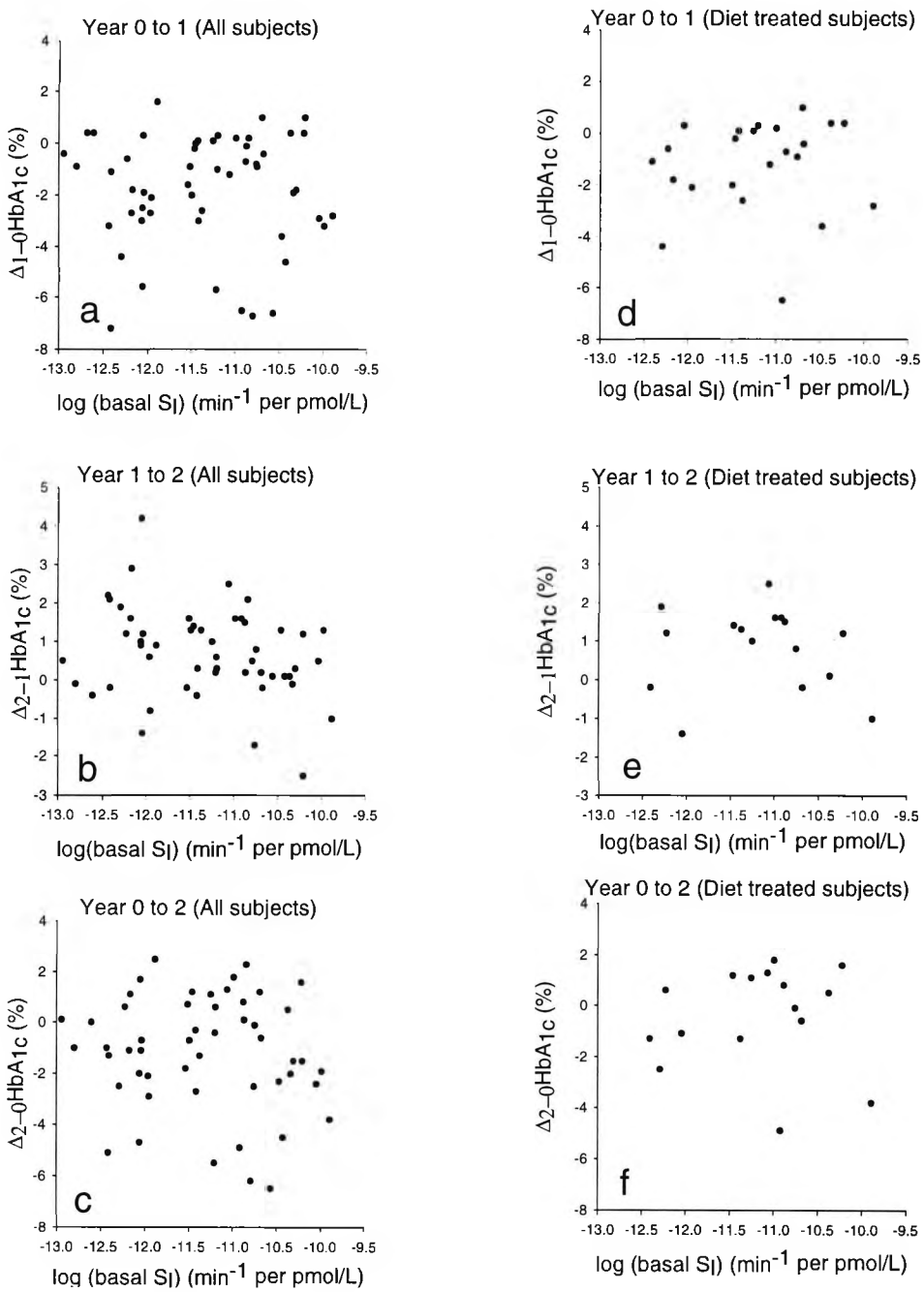


Figure 7.2: Relationships between a change in HbA_{1C} with post-prandial pancreatic β-cell responsiveness (M_I) at time of diagnosis.

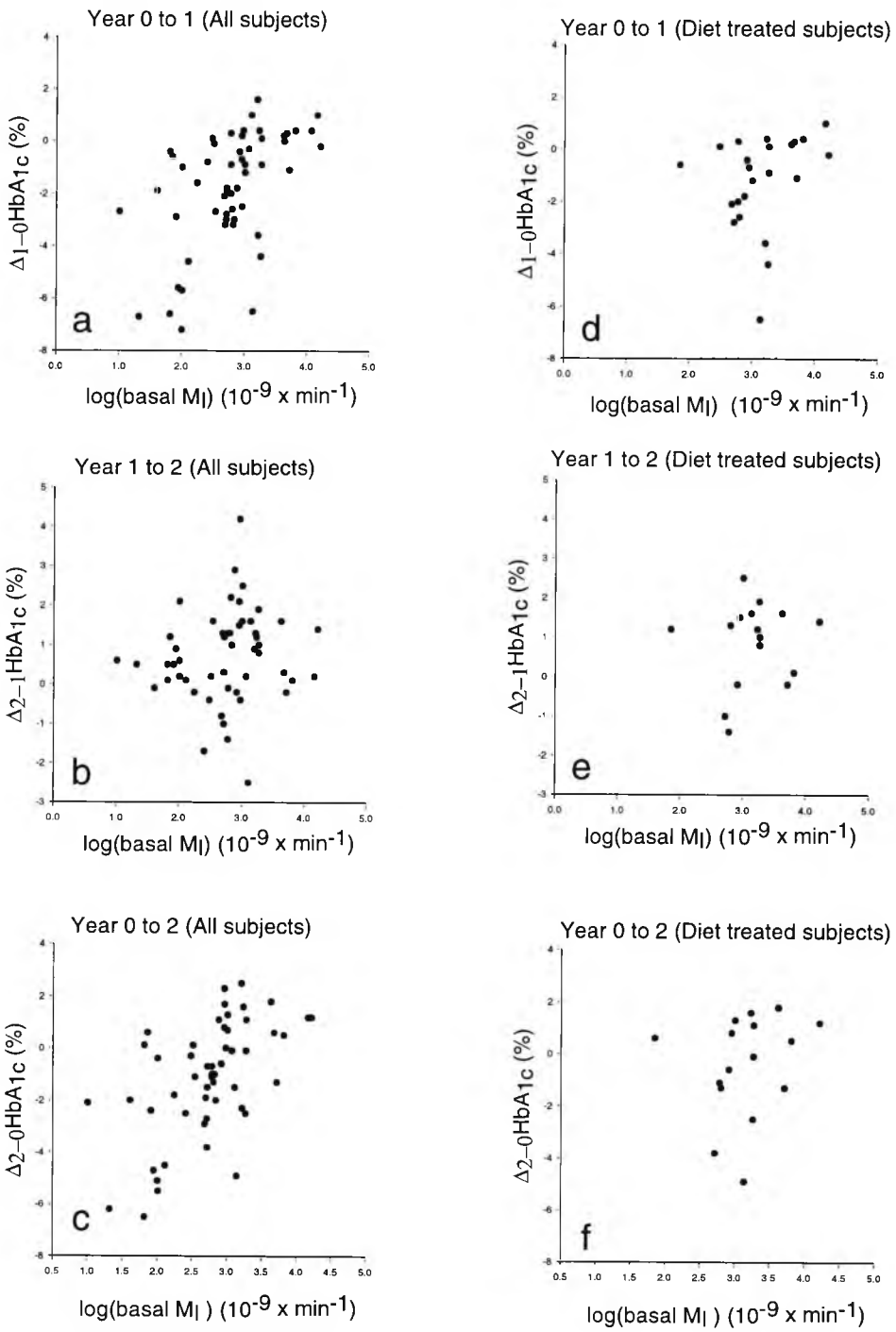


Figure 7.3: Relationships between a change in HbA_{1C} with basal pancreatic beta-cell responsiveness (M_O) at time of diagnosis.

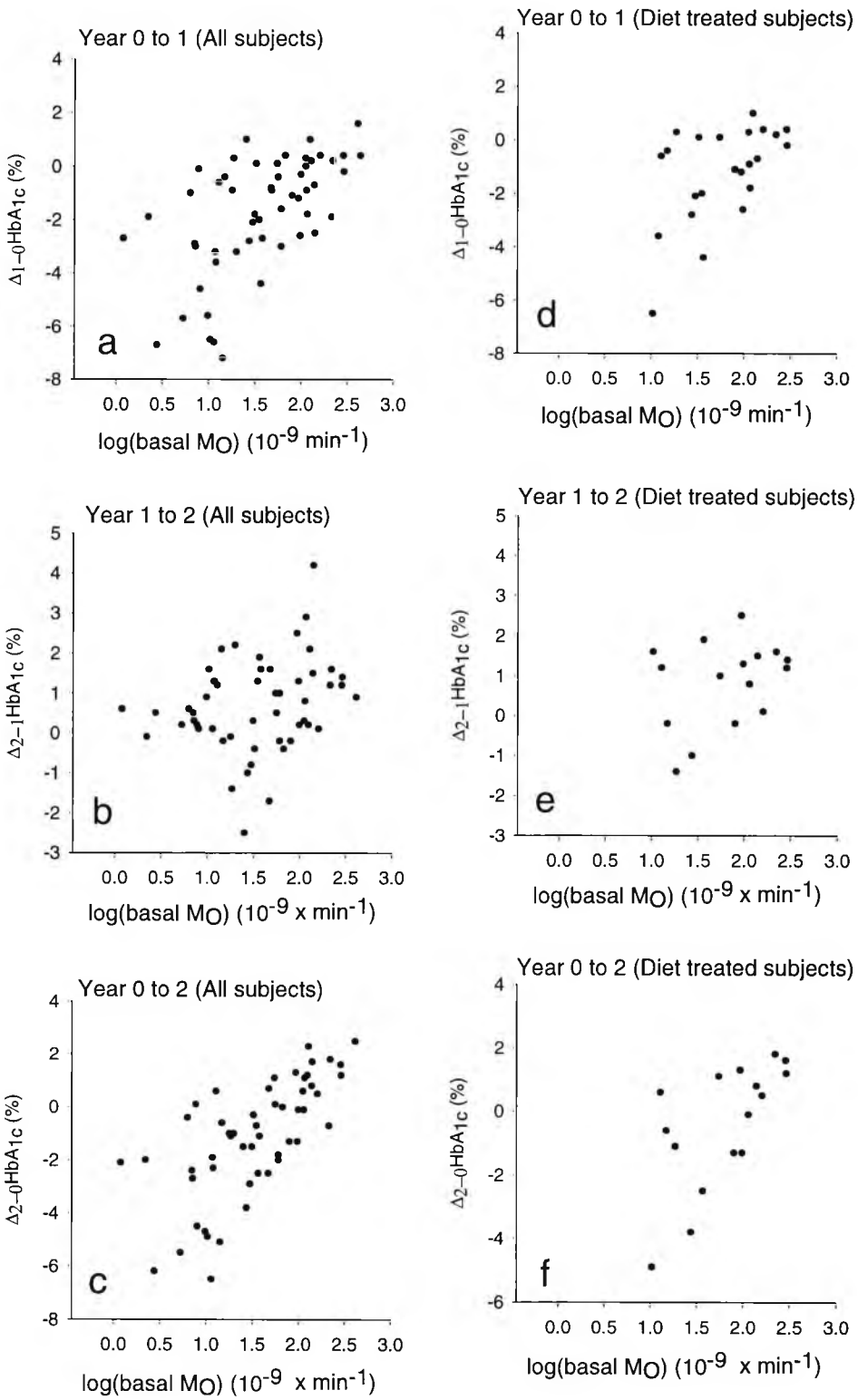
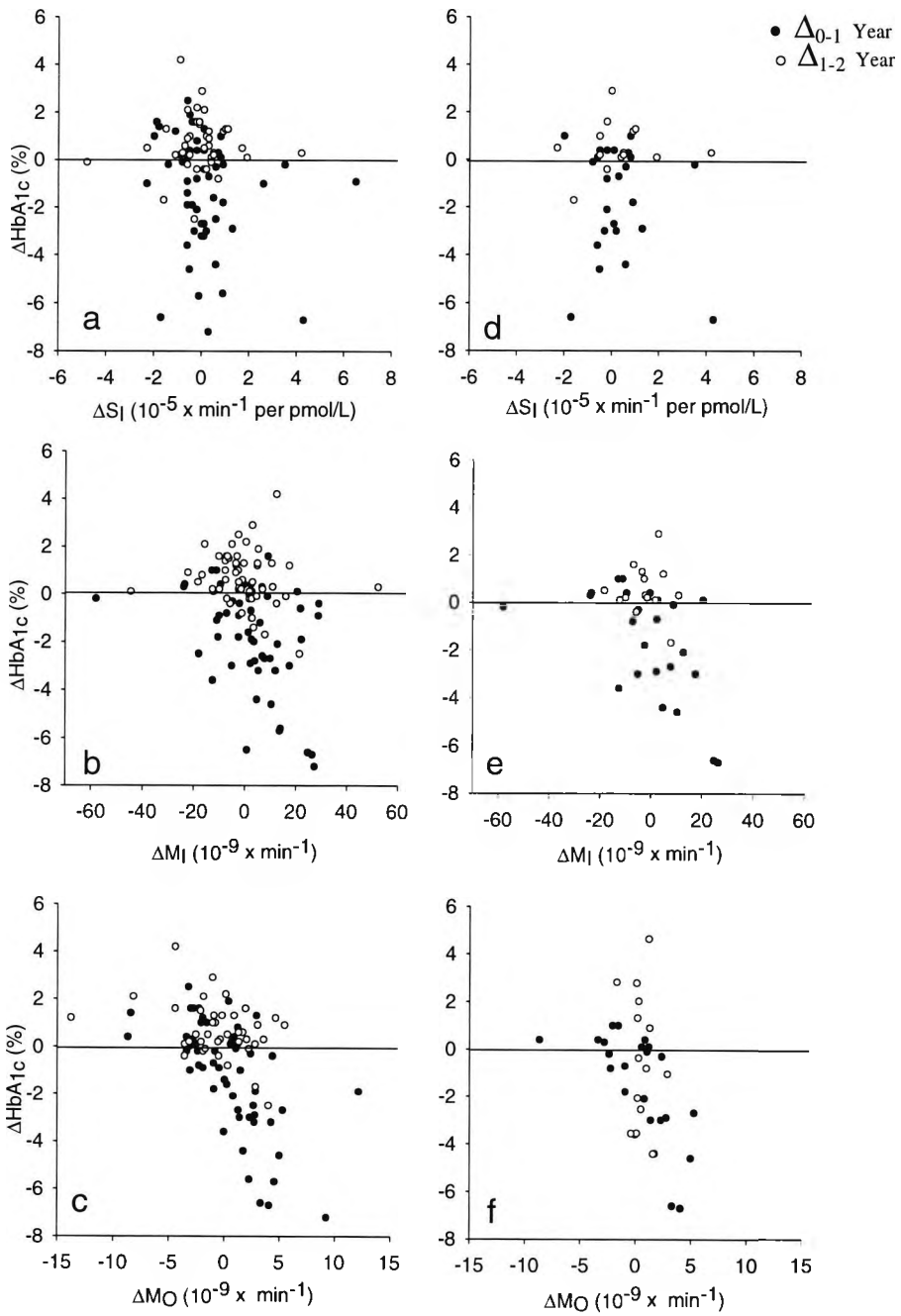


Figure 7.4: Scatter plots describing a change in HbA_{1C} (year 0-1 and year 1-2) aganist a change in metabolic indices S_I, M_I, and M_O.



Chapter 8

Explaining Inter-Subject Variability of Glucose Control

8.1 Introduction

In the last two chapters, we evaluated the early progression of T2D over 2 years and the relationship between the progression of HbA_{1C} and basal values of metabolic settings and other variables. In the present chapter, we will focus on whether indices of insulin sensitivity and insulin action are able to explain inter-individual variability of clinical measures of glucose control such as fasting plasma glucose and insulin, glycated haemoglobin, and the glucose and insulin responses to a meal over 2 years.

The pathogenesis of T2D is multifactorial involving both genetic and environmental factors. Genetic factors include defective pancreatic β-cell function and abnormal insulin action in target cells. Hyperglycaemia also impairs β-cell function and insulin action ("glucose toxicity") [42]. Environmental influences include obesity, age, lack of physical activity, and a high fat diet. Prenatal nutrition is also thought to determine predisposition to the disease. T2D can also occur secondary to other diseases and drugs [133, 134, 136, 81]. Various indices of both insulin resistance and insulin secretion have been investigated to understand the complexity of pathogenesis of T2D [7, 133].

In a recent cross-sectional study, it has been shown that M_I and M_O were the most informative variables to explain inter-individual variability of clinical measures of glucose control in newly presenting T2D. It was possible to explain 70-80% inter-individual variability of FPG, FPI, HbA_{1C} , and insulin responses to MTT, and 25-40% of glucose responses to MTT [5].

No longitudinal study has explained the characteristics and associations of the trend

among these factors in detail. The aim of this study was to assess whether the explained inter-individual variability at baseline as reported in [5] is maintained over the years. In this study, a total of 54 subjects with newly presenting T2D, as described in Section 6.2, were studied over two years.

8.2 Subjects and Experimental Protocol

The same 54 subjects with newly presenting T2D described in Chapter 6, Section 6.2, were studied over 2 years of therapeutic intervention.

8.3 Statistical Analysis

The statistical analyses were carried out using SPSS for Windows Version 11.5 (SPSS Inc., Chicago, IL, USA) and MINITAB Release 13.32 (MINITAB). Spearman's correlation analysis with a Boniferroni correction was used to assess the relationships between independent variables (clinical measures of glucose and postprandial glucose and insulin responses to MTT) and dependent variables (metabolic indices). Exploratory data analyses were performed to assess the distribution of both independent and dependent variables and appropriate transformations were carried out to ensure normality for regression analysis. A stepwise regression analysis was used to relate the measures of metabolic indices to the clinical measures of glucose control. The analysis of variance was used to calculate the extent of explained inter-subject variability.

8.4 Results

8.4.1 Correlation Analysis Over Year 0-2

Table 8.1 presents Spearman's correlations between the measures of glucose control, and indices of insulin sensitivity and pancreatic β-cell responsiveness over two years.

In year 0, FPG was negatively correlated with all measures of pancreatic responsiveness AIR_G, M_I, M_O, and D_I (AIR_G: r_s = -0.52; $P < 0.001$, M_I: r_s = -0.50; $P < 0.001$, M_O: r_s = -0.64; $P < 0.001$, D_I: r_s = -0.53; $P < 0.001$). In year 1, only AIR_G and D_I were significantly negatively correlated with FPG. While the same magnitude of the correlation coefficient was maintained for D_I (r_s = -0.54, $P < 0.001$) as in year 0, the

relationship with AIR_G (r_s = -0.35, $P < 0.05$) was reduced and borderline significant. The relationship between FPG and D_I continued into year 2 but weakened (D_I: r_s = -0.35, $P < 0.05$). In year 2, M_I showed a weak negative correlation with FPG ($P < 0.05$).

Table 8.3 shows similar correlation results for the 16 diet treated subjects. FPG was significantly correlated with M_O only in year 1 ($P < 0.001$). FPG showed no significant relationship with any of the indices of insulin sensitivity and pancreatic responsiveness in year 1. FPG only correlated with AIR_G in year 2.

Unlike FPG, FPI was positively correlated with all measures of pancreatic responsiveness (AIR_G, M_I, and M_O), (FPI vs AIR_G: $r_s = 0.83$, $P < 0.001$, FPI vs M_I: $r_s = 0.39$, $P < 0.05$, FPI vs M_O: $r_s = 0.65$, $P < 0.001$) and negatively correlated with S_I (FPI vs S_I: $r_s = 0.47$, $P < 0.001$). S_I, AIR_G, and M_O maintained these associations with FPI over the two years, and while the magnitude of the relationship is constant across the two years for AIR_G, it increased and decreased over time for S_I and M_O, respectively, see Table 8.1. Similar investigations on 16 diet treated subjects showed AIR_G to be significantly correlated with FPI over 2 years, Table 8.3.

At year 0, HbA_{1C} showed a negative significant association with all measures of pancreatic responsiveness and the disposition index (HbA_{1C} vs AIR_G: r_s = -0.43, $P < 0.01$, HbA_{1C} vs M_I: r_s = -0.62, $P < 0.001$, HbA_{1C} vs M_O: r_s = -0.64, $P < 0.001$, D_I: r_s = -0.44, $P < 0.01$). In the subsequent years, HbA_{1C} showed no significant association with any of the indices of insulin sensitivity, and was weakly negatively associated with D_I, M_I, and M_O in year 1. In the 16 treated diet subjects, the only variable with significant association with HbA_{1C} was M_I in year 0.

Postprandial insulin indices ($C_{max,Insulin}$ and $AUC_{Insulin}$) were positively correlated with all measures of insulin responsiveness (AIR_G, M_I, and M_O) at baseline year 0. This pattern was repeated in year 1 and with an additional significant negative correlation with insulin sensitivity. In year 2, both $C_{max,Insulin}$ and $AUC_{Insulin}$ showed a positive correlation with M_I and a negative correlation with S_I.

8.4.2 Explained Inter-individual Variability in Year 0, 1, and 2

The results of the stepwise regression analysis to identify explanatory variables (S_I, S_G, AIR_G, D_I, M_I, and M_O) for clinical measures of glucose control (FPG, FPI, HbA_{1C}, $C_{max,Glucose}$, $AUC_{Glucose}$, $C_{max,Insulin}$ and $AUC_{Insulin}$) are shown in Table 8.2 and Figure 8.1. M_O and D_I explained about 60% of variation in FPG in year 0, 36% variation with

an additional explanatory variable M_I in year 1, and only 14% variation was explained with one explanatory variable D_I in year 2. The total variation explained in year 0, 1, and 2 in FPI is 77% (S_I, AIR_G, and M_O), 75% (AIR_G, D_I, M_I, and M_O), and 68% (AIR_G and D_I) respectively. D_I and M_O explained 44% of the total variation in HbA_{1C} in year 0, 40% variation was explained by S_I, M_I and M_O in year 1, and only 10% in year 2 by S_I.

The variations in all postprandial glucose and insulin responses to MTT explained by one or more parameters of insulin sensitivity and pancreatic β-cell responsiveness range from 16% to 60%, see Table 8.2. In year 0, the variation of all postprandial glucose and insulin responses to MTT was explained by the indices of pancreatic responsiveness M_I and M_O. In year 1, a substantial extent of the variation was explained by M_I while the overall variation was explained by both indices of insulin sensitivity and pancreatic β-cell responsiveness, a large proportion was due to both M_I and M_O.

Table 8.1: Spearman's correlation coefficients between measures of glucose control (FPG, FPI, $AUC_{Glucose}$, $C_{max,Glucose}$, $AUC_{Insulin}$, and $C_{max,Insulin}$) and indices of insulin sensitivity and pancreatic β-cell responsiveness (S_I, S_G, AIR_G, D_I, M_O, and M_I) in year 0, 1, and 2 (N = 54).

	Year	S_I	S_G	AIR_G	D_I	M_I	M_O
FPG	0	-0.14	0.24	-0.52***	-0.53***	-0.50***	-0.64***
FPG	1	-0.28	-0.09	-0.35*	-0.54***	-0.31	-0.27
FPG	2	-0.18	-0.22	-0.25	-0.35*	-0.39	-0.26
FPI	0	-0.47***	0.05	0.83***	0.21	0.39*	0.65***
FPI	1	-0.53***	0.33**	0.82***	0.04	0.24	0.55***
FPI	2	-0.61***	-0.12	0.81***	0.04	-0.03	0.37*
HbA_{1C}†	0	-0.08	0.24	-0.43**	-0.44**	-0.62***	-0.64***
HbA_{1C}†	1	-0.34	0.14	-0.04	-0.37*	-0.30	-0.30*
HbA_{1C}†	2	-0.32	-0.01	0.30	-0.06	-0.22	0.12
$C_{max,Glucose}$	0	-0.12	0.15	-0.16	-0.26	-0.55***	-0.25
$C_{max,Glucose}$	1	-0.14	0.03	-0.04	-0.23	-0.32*	-0.01
$C_{max,Glucose}$	2	-0.12	0.10	-0.17	-0.28	-0.28	-0.12
$C_{max,Insulin}$	0	-0.28	-0.05	0.58***	0.21	0.68***	0.76***
$C_{max,Insulin}$	1	-0.50***	0.29	0.61***	-0.05	0.55***	0.44**
$C_{max,Insulin}$	2	-0.42**	-0.03	0.34	-0.14	0.51***	0.13
$AUC_{Glucose}$	0	-0.17	0.12	-0.23	-0.37	-0.67***	-0.39*
$AUC_{Glucose}$	1	-0.35	0.17	-0.08	-0.49***	-0.36*	-0.02
$AUC_{Glucose}$	2	0.40*	0.02	0.41**	-0.09	-0.49***	0.13
$AUC_{Insulin}$	0	-0.28	-0.03	0.54***	0.18	0.66***	0.71***
$AUC_{Insulin}$	1	-0.55***	0.35	0.58***	-0.14	0.56***	0.45**
$AUC_{Insulin}$	2	-0.42**	-0.11	0.31	-0.16	0.52***	0.10

*P < 0.05; **P < 0.01; ***P < 0.001 (corrected for multiple comparison using Boniferroni correction).

† N = 52

8.5 Discussion

T2D is characterised by defects in insulin secretion and insulin action [37, 75]. The development of T2D is a gradual process, and the relative role of impaired insulin secretion and resistance in the aetiology of the disease remain controversial. However, it is widely recognised that both defects are usually present in all patients with overt disease [37, 25].

In recent years, attempts have been made at understanding the complex relationships between insulin resistance and insulin secretion, and how these relationships affect glucose control. Several studies have quantified the associations between various clinical measures of glucose control, and measures of insulin resistance and insulin secretion to explain associations between the two sets of indices on presentation of T2D [5, 83].

Prospective studies that examined insulin resistance and impaired insulin secretion as risk factors for diabetes have demonstrated that the defects in insulin secretion and insulin action are independent predictors of the clinical measures of the disease [133, 22, 62, 61]. This study investigated insulin secretion and insulin resistance over two years after presentation of T2D and evaluated the relationship of these indices with measures of glucose control and the amount of variability explained by insulin secretion and resistance.

By analysing the data obtained from 54 newly presenting T2D subjects, following a naive treatment protocol, who had their insulin resistance and insulin secretion quantified with the use of the Bayesian hierarchical minimal model and the insulin secretion model, we investigated the relationships between these variables over two years.

The subjects in this study were between 36-74 year old. The subjects followed intensive dietary management for the first 6 months after diagnosis. The introduction of the dietary restriction was designed to improve insulin secretion capacity and not to improve insulin sensitivity [66]. Subjects were transferred to pharmacological therapies aimed at altering the metabolic processes after the failure of the intensive dietary treatment.

The above analyses showed that at the time of presentation of T2D, the fasting and postprandial pancreatic β-cell responsiveness (M_0 and M_1) play a major role in explaining the fasting glucose (FPG) level and postprandial glucose responses ($C_{max,Glucose}$ and $AUC_{Glucose}$) to MTT. These associations with fasting plasma glucose disappeared completely after a year. The postprandial glucose response to MTT ($AUC_{Glucose}$) maintained a weaker association with the postprandial β-cell responsiveness (M_1) over the two years.

Table 8.2: Results of the stepwise linear regression in the form of z-scores (regression coefficients when all variables are expressed in standardised form) (N = 54).

	Year	S_I	S_G	AIR_G	D_I	M_I	M_O	R^{2+}
FPG	0	-	-	-	-0.43***	-	-0.55***	0.59
FPG	1	-	-	-	-0.51***	-0.26**	-0.26**	0.36
FPG	2	-	-	-	-0.39*	-	-	0.14
FPI	0	-0.23*	-	0.68***	-	-	0.17*	0.77
FPI	1	-	-	0.83***	-0.28**	-0.16*	0.24*	0.75
FPI	2	-	-	0.90***	-0.20*	-	-	0.68
HbA_{1C}†	0	-	-	-	-0.25*	-	-0.57***	0.44
HbA_{1C}†	1	-0.59***	-	-	-	-0.28**	-0.46***	0.40
HbA_{1C}†	2	-0.35**	-	-	-	-	-	0.10
$C_{max,Glucose}$	0	-	-	-	-	-0.89***	0.40*	0.38
$C_{max,Glucose}$	1	-0.31**	-	-	-	0.37*	-	0.13
$C_{max,Glucose}$	2	-	-	-	-	-	-	-
$C_{max,Insulin}$	0	-	-	-	-	-	0.78***	0.59
$C_{max,Insulin}$	1	-0.35**	-	0.31*	-	0.34**	-	0.50
$C_{max,Insulin}$	2	-0.39**	-	0.25*	-	0.71***	-0.51***	0.44
$AUC_{Glucose}$	0	-	-	-	-	-0.68***	-	0.45
$AUC_{Glucose}$	1	-	-	-	-0.49***	-0.44***	-	0.33
$AUC_{Glucose}$	2	-	0.37**	-	-0.30*	-	-	0.16
$AUC_{Insulin}$	0	-	-	-	-	0.32*	0.46**	0.52
$AUC_{Insulin}$	1	-0.41**	-	0.29*	-	0.33*	-	0.55
$AUC_{Insulin}$	2	-0.70***	-	-	0.28*	0.77***	-0.57***	0.49

*P < 0.05; **P < 0.01; ***P < 0.001.

Dash(-) indicates that the independent variables (S_I, S_G, AIR_G, D_I, M_O and M_I) did not enter the the regression formula for the dependent variables (FPG, FPI, $C_{max,Glucose}$, $C_{max,Insulin}$, $AUC_{Glucose}$ and $AUC_{Insulin}$).

+ Adjusted R^2 for BMI; † N = 52.

Table 8.3: Spearman's correlation coefficients between measures of glucose control (FPG, FPI, $AUC_{Glucose}$, $C_{max,Glucose}$, $AUC_{Insulin}$, and $C_{max,Insulin}$) and indices of insulin sensitivity and pancreatic β-cell reponsiveness (S_I, S_G, AIR_G, D_I, M_O, and M_I) in diet treated subjects in year 0, 1, and 2 (N = 16).

	Year	S_I	S_G	AIR_G	D_I	M_I	M_O
FPG	0	-0.19	0.12	-0.33	-0.39	-0.41	-0.73**
FPG	1	0.04	-0.21	-0.59	-0.48	-0.01	-0.24
FPG	2	-0.16	-0.53	-0.70*	-0.60	0.11	-0.17
FPI	0	-0.53	0.31	0.76**	0.02	0.08	0.14
FPI	1	-0.40	0.17	0.78***	0.29	0.46	0.39
FPI	2	-0.21	-0.23	0.67*	0.32	0.33	0.59
HbA_{1C}	0	-0.07	0.47	-0.16	-0.22	-0.63*	-0.54
HbA_{1C}	1	-0.17	0.17	0.06	0.14	0.15	-0.19
HbA_{1C}	2	-0.00	-0.12	0.48	0.40	0.31	0.31
$C_{max,Glucose}$	0	-0.09	0.38	-0.26	-0.23	-0.71**	-0.36
$C_{max,Glucose}$	1	-0.08	-0.19	-0.17	-0.14	-0.31	-0.18
$C_{max,Glucose}$	2	-0.40	-0.12	-0.22	-0.34	0.15	0.07
$C_{max,Insulin}$	0	-0.25	0.20	0.38	0.12	0.65**	0.69**
$C_{max,Insulin}$	1	-0.20	0.02	0.79***	0.58	0.24	0.16
$C_{max,Insulin}$	2	-0.32	-0.16	0.61	0.28	0.73**	0.27
$AUC_{Glucose}$	0	-0.31	0.27	-0.32	-0.46	-0.68**	-0.62
$AUC_{Glucose}$	1	-0.57	0.32	-0.51	-0.90***	0.04	-0.39
$AUC_{Glucose}$	2	-0.69**	-0.24	-0.27	-0.61	0.28	-0.15
$AUC_{Insulin}$	0	-0.28	0.21	0.35	0.07	0.70**	0.58
$AUC_{Insulin}$	1	-0.29	0.21	0.80***	0.44	0.47	0.33
$AUC_{Insulin}$	2	-0.29	-0.30	0.58	0.27	0.77**	0.16

*P < 0.05; **P < 0.01; ***P < 0.001 (corrected for multiple comparisons using Boniferroni correction)

Table 8.4: Results of the step-wise linear regression in the form of z-scores (regression coefficients when all variables are expressed in standardised form) (N = 16).

	Year	S_I	S_G	AIR_G	D_I	M_I	M_O	R^{2+}
FPG	0	-	-	-	-	-	-0.67**	0.41
FPG	1	-	-	-0.57*	-	-	-	0.27
FPG	2	-	-0.48**	-0.71***	-	-	-	0.70
FPI	0	-0.28*	-	0.83***	-	-0.39**	-	0.82
FPI	1	-	-	0.80***	-	-	-	0.61
FPI	2	-	-	0.64**	-	-	0.45*	0.48
HbA_{1C}	0	-	-	-	-	-	-0.55*	0.25
HbA_{1C}	1	-	-	-	-	-	-	-
HbA_{1C}	2	-	-	-	-	-	-	-
$C_{max,Glucose}$	0	-	-	-	-	-0.62**	-	0.35
$C_{max,Glucose}$	1	-	-	-	-	-	-	-
$C_{max,Glucose}$	2	-	-	-	-	-	-	-
$C_{max,Insulin}$	0	-0.32*	-	-	-	-	0.90***	0.73
$C_{max,Insulin}$	1	-	-	0.79***	-	-	-	0.59
$C_{max,Insulin}$	2	-	-	0.70***	-	0.58**	-	0.61
$AUC_{Glucose}$	0	-	-	-	-	-	-0.60*	0.31
$AUC_{Glucose}$	1	-	-	-	-0.81***	-	-	0.65
$AUC_{Glucose}$	2	-	-	-	-0.66**	-	-	0.39
$AUC_{Insulin}$	0	-	-	-	-	0.72**	-	0.48
$AUC_{Insulin}$	1	-	-	0.77***	-	-	-	0.58
$AUC_{Insulin}$	2	-	-	0.96***	-0.42**	0.96***	-0.58***	0.90

*P < 0.05; **P < 0.01; ***P < 0.001.

Dash(-) indicates that the independent variables (S_I, S_G, AIR_G, D_I, M_O and M_I) did not enter the regression formula for the dependent variables (FPG, FPI, $C_{max,Glucose}$, $C_{max,Insulin}$, $AUC_{Glucose}$and $AUC_{Insulin}$).

+ Stands for adjusted R^2 for BMI.

Postprandial glucose responses ($C_{max,Glucose}$ and $AUC_{Glucose}$) were negatively correlated with postprandial β-cell responsiveness (M_I), while insulin responses ($C_{max,Insulin}$ and $AUC_{Insulin}$) were positively correlated with all measures of pancreatic responsiveness (AIR_G, M_o and M_I).

Fasting plasma insulin and the acute insulin response to IVGTT (AIR_G), a measure of β-cell well being, were consistently highly positively correlated over the two years. FPG was negatively correlated with D_I; the association was stronger in year 0 and year 1 when compared with year 2. D_I has already been reported for its usefulness in characterising the overall state of glucose metabolism [74].

Clinical measures of glucose metabolism except fasting plasma insulin (FPI) failed to demonstrate associations with insulin sensitivity (S_I). The lack of significant association between FPG and S_I is consistent with the results reported by Reaven [110] and Levy et al [83]. Reaven failed to find a relation between FPG and S_I in nonobese individuals and T2D subjects. Likewise, Levy et al documented that a fall in β-cell function is closely followed by a rise in FPG in their 10-year prospective study of newly presenting T2D, without a significant change in S_I. However, Van Haeften et al [125] found an association between fasting plasma glucose and insulin sensitivity estimated by the hyperglycaemic clamp in normal and impaired glucose tolerance subjects.

It is recognised that BMI is another determinant of insulin secretion in addition to the ambient plasma glucose levels. Obese insulin-resistant individuals secrete more insulin than lean insulin sensitive individuals at comparable plasma glucose levels. From results of the relationship between FPG and S_I in this study, it is unclear why there was a lack of associations at the baseline. As recently pointed out by Reaven [111], because of the feedback between plasma glucose concentration and insulin secretion, it is virtually impossible to develop diabetes due to the severity of insulin resistance found in most T2D subjects unless the capacity to secrete additional amount of insulin to compensate for insulin resistance is impaired. Thus, hyperglycaemia may be considered prima facie evidence for impaired insulin secretion.

FPI and insulin sensitivity are negatively correlated with an increasing trend over the two years. This relationship supported the methodological validity of estimating insulin sensitivity with insulin modified IVGTT.

At the baseline the inter-individual variability of clinical measures of glucose control was explained by only M_O and M_I with the exception of that of FPI, which was explained mainly by AIR_G. In year 1, both S_I and D_I were selected in the regression analysis in addition to M_O and M_I. In year 2, M_O was not selected at all. About 70% of inter-individual variability was consistently explained in FPI mainly by AIR_G.

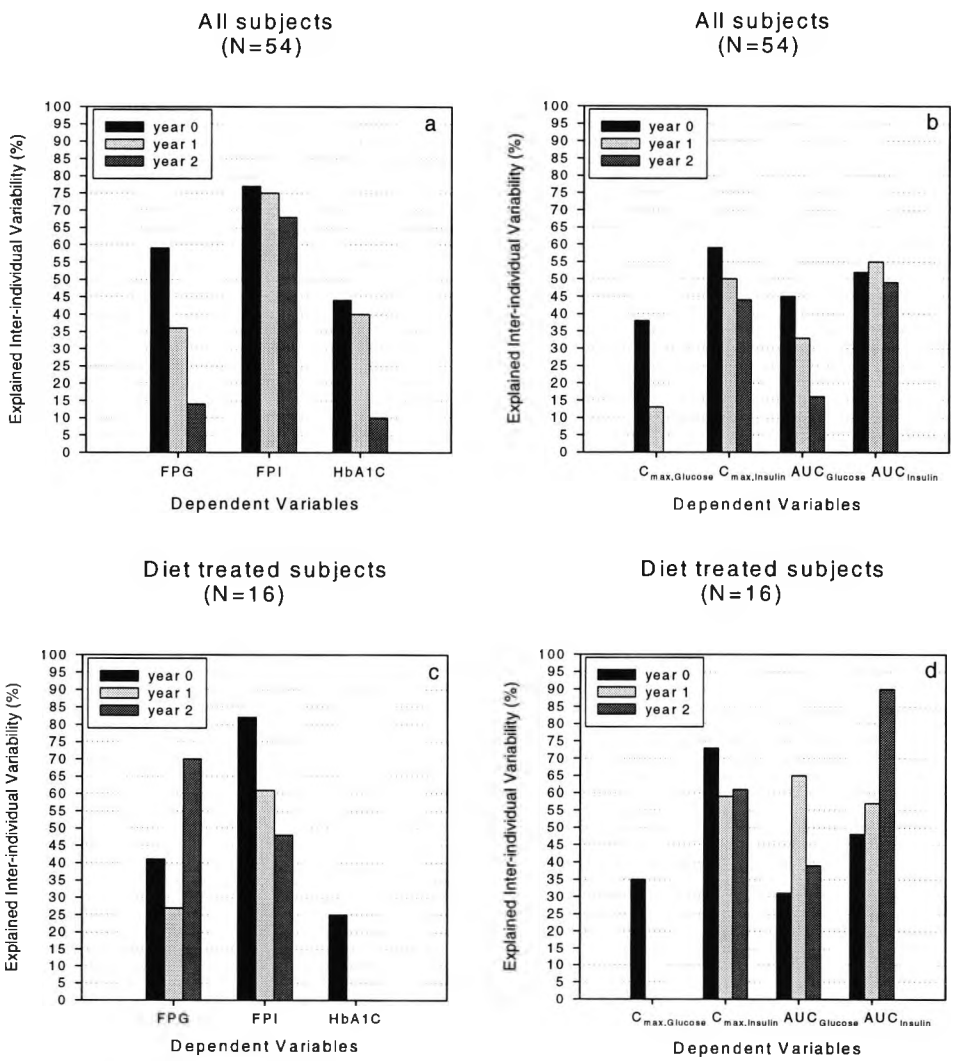
The results have also confirmed that both M_O and M_I were most informative in assessing the inter-individual variability of clinical measures of glucose at the time of presentation. The amount of explained variability ranges from 44% to 76% at the time of presentation of T2D and decreases over the years, ranging from 36% to 70% in year 1, and 10% to 68% in year 2. Various reasons could explain the reduced variability over time. The reasons leading to the reduced explained variability are unclear.

The results of the associations between all 54 subjects and the 16 diet treated subjects are comparable over the two years. Fewer significant associations in the diet treated group resulted from a smaller sample size. However, the trend of the associations over the two years between M_O and FPI was reversed in the diet treated subjects, and between AIR_G and FPG.

8.6 Conclusions

We conclude that the ability of indices of insulin sensitivity and pancreatic β-cell responsiveness to explain inter-individual variability of clinical measures of glucose control in newly presenting T2D decrease over time for all clinical measures of glucose control and measures of glucose and insulin responses to MTT with the exception of inter-individual variability in FPI and postprandial insulin response ($C_{max,Insulin}$ and $AUC_{Insulin}$), which remained the same over the two years.

Figure 8.1: Explained inter-individual variability of clinical measures of glucose control using indices of insulin resistance and pancreatic β-cell responsiveness.



Chapter 9

Final Discussion

The primary aim of this thesis was to employ the Bayesian hierarchical modelling approach to investigate methodological issues concerning parameter estimation of the minimal model of glucose kinetics using data collected during IVGTT, with the objective to avoid failures of the non-linear regression analysis.

The secondary aim was to use the Bayesian hierarchical modelling techniques in combination with other modelling techniques on data collected during IVGTT and MTT to improve our understanding of the pathophysiology of T2D on a longitudinal basis.

Type 2 diabetes results from the inability of the pancreas to augment insulin secretion in the presence of insulin resistance [37, 119]. Various methods have been developed to measure insulin secretion and insulin action - a methodological review has been given in Chapter 2.

The minimal model of glucose kinetics using IVGTT has become an invaluable method to estimate insulin sensitivity [5, 94, 107]. In spite of the wider application of the model, one reported problem with the minimal model studies is that, in a number of subjects, especially with T2D, insulin sensitivity (S_I) is calculated as $S_I = 0$ with the non-linear regression analysis [100]. The occurrence of S_I indistinguishable from zero is yet to be understood. This has been attributed to a possible manifestation of modelling deficiency. Solutions to the problem have generated a wider interest in finding more sophisticated parameter estimation techniques for more physiological values of S_I and at the same time improving the parameter estimates of the minimal model. Among these techniques is the Bayesian analysis with MCMC parameter estimation. In Chapter 3 we adopted the Bayesian analysis in a hierarchical framework to estimate S_I and S_G aiming to reduce the minimal model failures.

A population kinetic model combines individual and population parameters. The individual parameters quantify the kinetics of a particular individual to estimate the intra-

individual variability, while population kinetic parameters quantify population mean kinetics to estimate the inter-individual variability. The identification of these sources of variability and consequent parameter estimation in a non-linear hierarchical model, such as the minimal model of glucose kinetics, has its pitfalls. The traditional standard two-stage parameter estimates, obtained by fitting a kinetic model to individual data and then combining the individual parameter estimates to derive the population estimates, have been shown to overestimate the population covariance [35].

In Chapter 4, we compare the parameter estimation capabilities of the minimal model of glucose kinetics using the Bayesian hierarchical analysis and the standard two-stage approach, which employed the non-linear regression analysis for the individual parameters estimation. The Bayesian hierarchical analysis has proved effective in a number of applied and simulated studies and it is less difficult to implement in view of recent advances in statistical computation techniques and particularly in the MCMC techniques.

Our results from the Bayesian hierarchical analysis gave reliable estimates of the population parameters, with tighter credible intervals of the population mean/median parameters and also tighter interquartile ranges. This demonstrated the advantage of the Bayesian hierarchical analysis over the standard two-stage approach.

In Chapter 5, the Bayesian hierarchical analysis also demonstrated its ability to handle both dense data and relatively sparse data with the minimal model of glucose kinetics in T2D subjects. The approach can accommodate data from a variety of unbalanced designs.

The Bayesian hierarchical analysis gave reliable and unbiased estimates of S_I for 12 and 13 sample schemes, with acceptable precision of S_I estimates. The successful estimation of S_I without a loss in precision makes the technique useful for population studies by reducing cost, labour, and complexity of the studies.

In Chapter 6, we investigated the early progression of T2D subjects to increase our understanding of insulin resistance and insulin secretion and their associated clinical measures of glucose control over two years after diagnosis. The progressive nature of T2D with various treatments was demonstrated by the UKPDS [57]. In this chapter we investigated the trends in metabolic settings (insulin resistance and insulin secretion), clinical measures of glucose and insulin, clinical measures of glucose control, and postprandial glucose and insulin responses to MTT in 54 T2D subjects.

We showed that insulin sensitivity and glucose effectiveness do not change over time, irrespective of the early therapeutic intervention, although a progressive decrease in HbA_{1C} within the normal range reflected an association with a lower degree of insulin

resistance which could be attributed to treatment effects. Separating treatment effects was impossible since the subjects were not randomised with respect to the treatment allocation.

We also demonstrated that indices of insulin secretion (M_O and M_I) showed significant improvement only in year 0-1 and deteriorated after year 1 in spite of the treatment.

Our investigations demonstrated that the progressive nature of T2D is rather due to the steady decline in β-cell function, which can be ameliorated, at least for two years, by adequate conventional treatments starting after the diagnosis.

In Chapter 7, we demonstrated that subjects with lowest values of insulin secretion as measured by M_O and M_I can expect to achieve the highest improvement in HbA_{1C} if accompanied by improved M_O and M_I values. The results showed that the best improvement in HbA_{1C} is achieved in the first year after diagnosis.

The knowledge about the inter-individual variability in clinical measures of glucose control and postprandial glucose and insulin responses to MTT in T2D subjects was extended in Chapter 8 [5]. We related insulin resistance and secretion to clinical measures of glucose and insulin from IVGTT and MTT to increase our understanding of T2D pathology and the relationships among these sets of indices over two years.

The results of the analyses demonstrated changes and associations between model based indices of pancreatic β-cell responsiveness and indices describing the clinical measures of glucose and insulin responses. The analyses showed that at the time of presentation, fasting and postprandial pancreatic β-cell responsiveness plays major role in explaining fasting glucose and postprandial glucose responses to MTT. These associations with fasting plasma glucose disappeared completely with measures of pancreatic β-cell responsiveness (M_O and M_I) after year 1, except for the postprandial glucose response to MTT ($AUC_{Glucose}$), where the association was maintained but was weaker.

Postprandial glucose meal responses were negatively correlated with postprandial β-cell responsiveness (M_I), while postprandial insulin meal responses were positively correlated with all measures of pancreatic responsiveness (AIR_G, M_O, and M_I) suggesting that the elevated postprandial insulin responses to meals are reliable indices for assessing β-cell well-being. Fasting plasma insulin was shown to be a consistent and reliable index of insulin sensitivity over the two years.

Our results demonstrated that both M_O and M_I were most informative in assessing the inter-individual variability of clinical measures of glucose control at the time of presentation. The amount of explained variability ranged from 44% to 76% at the time

of presentation of T2D and decreased over the years ranging from 36% to 70% in year 1 and 10% to 68% in year 2. Similar results were obtained in a cross-sectional study by Albarrak et al [5] who found that the postprandial pancreatic responsiveness was the most powerful explanatory index of an impaired glucose control.

Chapter 10

Conclusions

The Bayesian hierarchical analysis with the minimal model of glucose kinetics of IVGTT is an appealing approach to estimate insulin sensitivity. The method avoids model failures and can conveniently accommodate both dense and relatively sparse data sets making the approach more acceptable for different target populations and experimental protocols.

The application of the Bayesian hierarchical analysis with the minimal model of glucose kinetic and of the insulin secretion model on a longitudinal basis has improved our understanding of T2D pathology at presentation and over a period of two years.

10.1 Achievement of Objectives

The achievements can be divided into two parts, namely, the methodological and pathophysiological.

The methodological achievements are outlined as follows:

- we have developed the minimal model of glucose kinetics within the Bayesian hierarchical framework. This approach facilitates simultaneous estimation of individual and population parameters;
- the Bayesian hierarchical analysis avoids parameter estimation failures and gives a smaller unbiased estimate of the population variance for both S_I and S_G;
- the parameter estimation capabilities of the minimal model of glucose kinetics using the Bayesian hierarchical analysis and the standard two-stage approach were successfully compared. The Bayesian hierarchical analysis provided reliable

estimates of the population parameters and allowed individual parameters to be estimated with good precision.

The successful implementation of the Bayesian hierarchical model to estimate insulin sensitivity and glucose effectiveness amounts to a significant contribution to the biomedical field.

Further achievements constitute new knowledge about pathophysiology of T2D:

- indices of insulin sensitivity and glucose effectiveness (S_I and S_G) did not improve over time in spite of the early therapeutic intervention;
- indices of insulin secretion (M_O and AIR_G) improved over years 0-2 with a significant improvement in year 0-1. M_I deteriorated over the two years in diet treated subjects;
- an improvement in HbA_{1C} was associated with an improvement in M_O and M_I. About 43% of improvement in HbA_{1C} was explained by BMI, M_O, S_G in year 1, while about 29% was explained in year 2;
- M_O and M_I were most informative in assessing the inter-individual variability of clinical measures of glucose at the time of presentation. The amount ranged from 44% to 76% at the time of presentation of T2D and decreased over the 2 years, ranging from 36% to 70% in year 1, and 10% to 68% in year 2.

The investigation of the progression of insulin sensitivity and insulin secretion over two years after diagnosis increases our understanding of the early progression of T2D. This is a contribution in the pathophysiological field.

10.2 Future Work

Several research questions were raised during the course of the study. Recommendations for future research in methodological and clinical aspects are as follows.

It is recommended to implement a t-distribution assumption for the measurement error with the minimal model of glucose kinetic using the Bayesian hierarchical analysis. The problem associated with using the t-distribution is deciding what value of ν (the degrees of freedom) should be used.

It is recommended to compare the performance of the parameter estimation capabilities of the minimal model of glucose kinetic using the Bayesian hierarchical analysis, the iterative two-stage analysis, and other population approaches in different pathophysiological states.

Understanding of the progression of T2D still relies solely on an understanding of complex dynamic interactions between insulin tissue sensitivity and insulin secretion. A statistical analysis of the progression of T2D using a generalised linear mixed effect model will be beneficial for adjusting for the treatments effects in the data employed in this study.

Part III

Appendices

Appendix A

Appendix A contains the WinBUGS specification of the Bayesian hierarchical model and the WinBUGS extensibility codes used for the implementation of the Bayesian hierarchical analysis with the minimal model, Section 3.4, Chapter 3.

WinBUGS Code

```
model
{
unmu.beta[1] <- exp(mu.beta[1]) # for monitoring purpose
unmu.beta[2] <- exp(mu.beta[2])
unmu.beta[3] <- exp(mu.beta[3])
unmu.beta[4] <- exp(mu.beta[4])

samp.tran[1:4]~dmnorm(mu.beta[] , R[ , ])
samp[1] <- exp(samp.tran[1] - samp.tran[4])
samp[2] <- exp(samp.tran[3])
samp[3] <- exp(samp.tran[3] + samp.tran[2])
samp[4] <- exp(samp.tran[4])
samp[5] <- exp(samp.tran[2])

for(i in 1:ind)                                    # loop over the subjects
{
beta[i ,1:4]~dmnorm(mu.beta[] , R[ , ])            # beta[i , j] represents pa-
rameter j in individual i.
betainf[i ,1:2]~dmnorm(muinf.beta[] , Rinf[ , ])   # beta[i , j] represents param-
eter j in individual i.
        theta[i, 1] <- exp(beta[i , 1] - beta[i , 4])   # ln(SG) = beta[1] /min
        theta[i, 2] <- exp(beta[i , 3])                 # ln(P2) = beta[3] /min
        theta[i, 3] <- exp(beta[i , 3]+beta[i , 2])     # ln(SI) =
beta[2] /min per mU/l
        theta[i, 6] <- exp(beta[i , 4])                 # ln(V)
= beta[4] l/kg
        theta[i, 4] <- 1.0                              # ln(K12) = betainf[1]
/min
        theta[i, 5] <- 0.0                              # ln(K21) = betainf[2]
/min

        sI[i] <-theta[i,3]/theta[i,2]/6.0 # insulin sensitivity min per pmol/l
        P1[i] <-theta[i,1] # glucose effectiveness per min


# generate measueremnts and predictions
    for(j in off.data.start1[i]:off.data.end1[i])
    {
    c1[i , j] ~dnorm(model1[i, j] , invvar1[i,j])      # c1[i,j] represents
concentration j in individal i
    model1[i, j] <- shola.model(theta[i,1:6], time1[j], 1.6653, i, 1, 1) # predicted glucose
concentration for IVGTT
    invvar1[i,j] <- tauC1/(model1[i,j]*model1[i,j])
    }
} # end of i loop
```

```
mu.beta[1:4] ~dmnorm(mean[], prec[ , ])
R[1:4 , 1:4] ~dwish(Omega[ , ], 4)
tauC1 ~dgamma(0.001, 0.001)
CV1<-1/sqrt(tauC1)
# calculate correlation matrix
    for (i in 1:4){
            for (j in 1:4){
                        S4[i,j] <- inverse( R[,],i,j)
                        }
            }
        sigma[1]<-sqrt(S4[1,1]) # SDs
        sigma[2]<-sqrt(S4[2,2])
        sigma[3]<-sqrt(S4[3,3])
        sigma[4]<-sqrt(S4[4,4])
        r[1,1] <- 1.0
        r[1,2] <- S4[1,2] / (sqrt(S4[1,1]) *sqrt(S4[2,2])); # correlation
        r[1,3] <- S4[1,3] / (sqrt(S4[1,1]) *sqrt(S4[3,3]));
        r[1,4] <- S4[1,4] / (sqrt(S4[1,1]) *sqrt(S4[4,4]));
        r[2,1] <- r[1,2]
        r[3,1] <- r[1,3]
        r[4,1] <- r[1,4]
        r[2,2] <- 1.0
        r[2,3] <- S4[2,3] / (sqrt(S4[2,2]) *sqrt(S4[3,3]));
        r[2,4] <- S4[2,4] / (sqrt(S4[2,2]) *sqrt(S4[4,4]));
        r[3,2] <- r[2,3]
        r[4,2] <- r[2,4]
    r[3,3] <- 1.0
        r[3,4] <- S4[3,4] / (sqrt(S4[3,3]) *sqrt(S4[4,4]));
        r[4,3] <- r[3,4]
    r[4,4] <- 1.0
} end of winbugs program


list(

ind = 65,

time1 = c(
0.00,1.00,2.00,3.00,4.00,5.00,6.00,7.00,8.00,10.00,12.00,14.00,
16.00,20.00,22.00,23.00,24.00,25.00,27.00,30.00,40.00,50.00,60
.00,70.00,80.00,90.00,100.00,120.00,150.00,180.00),

off.data.start1 = c(
9,  9,  9,  9,  9,  9,  9,  9,  9,  9,
9,  9,  9,  9,  9,  9,  9,  9,  9,  9,
9,  9,  9,  9,  9,  9,  9,  9,  9,  9,
9,  9,  9,  9,  9,  9,  9,  9,  9,  9,
9,  9,  9,  9,  9,  9,  9,  9,  9,  9,
```

```
9, 9, 9, 9, 9, 9, 9, 9, 9, 9,
9, 9, 9, 9, 9
),

off.data.end1 = c(
30, 30, 30, 30, 30, 30, 30, 30, 30, 30,
30, 30, 30, 30, 30, 30, 30, 30, 30, 30,
30, 30, 30, 30, 30, 30, 30, 30, 30, 30,
30, 30, 30, 30, 30, 30, 30, 30, 30, 30,
30, 30, 30, 30, 30, 30, 30, 30, 30, 30,
30, 30, 30, 30, 30, 30, 30, 30, 30, 30,
30, 30, 30, 30, 30
),


c1 = structure(
.Data = c(10.40 , 12.70 , 18.70 , 22.50 , 20.80 , 19.80 , 18.90 , 18.50 , 17.80 ,
17.20 , 16.80 , 16.40 , 15.90 , 15.90 , 15.70 , 15.60 , 15.60 , 15.60 , 15.40 ,
15.00 , 13.50 , 12.20 , 11.70 , 11.20 , 10.40 , 10.10 , 9.60 , 9.00 , 8.40 ,
7.90 ,
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. . . .
7.70 , 20.80 , 25.10 , 24.00 , 22.10 , 20.60 , 20.10 , 19.20 , 18.50 , 17.80 ,
17.30 , 16.70 , 16.20 , 15.80 , 14.90 , 15.10 , 14.90 , 14.60 , 14.30 , 13.50 ,
11.30 , 9.70 , 8.50 , 7.50 , 7.30 , 6.80 , 6.60 , 6.20 , 5.90 , 5.50
), .Dim = c(65,30)),

Omega = structure(
.Data = c(
0.02, 0, 0, 0,
0, 0.02, 0, 0,
0, 0, 0.02, 0,
0, 0, 0, 0.02),
.Dim = c(4,4)),

Rinf = structure(
.Data = c(
77.86, -70.07,
-70.07, 77.86
),
.Dim = c(2,2)),

mean = c(0,0,0,0),
muinf.beta = c(-2.66,-2.99),

prec = structure(
.Data = c(
0.000001,0,0,0,
0,0.000001,0,0,
0,0,0.000001,0,
0,0,0,0.000001
), .Dim = c(4,4))
)
```

```
list(
mu.beta = c(0,0,0,0),
tauC1 = 1,

beta = structure(
.Data = c(
-3.730, -9.309, -3.35, -2.049,
.    .    .    .    .    .
.    .    .    .    .    .
.    .    .    .    .    .
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.    .    .    .    .    .
-3.730, -9.309, -3.35, -2.049
), .Dim = c(65,4)),

samp.tran = c( -3.730, -9.309, -3.35, -2.049),

betainf = structure(
.Data = c(
-2.425, -2.847,
.    .    .    .
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.    .    .    .
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.    .    .    .
-2.425, -2.847
), .Dim = c(65,2)),

R = structure(.Data = c(1,0,0,0,
                        0,1,0,0,
                        0,0,1,0,
                        0,0,0,1
                              ),
                        Dim = c(4,4))
```

WinBUGS Extensibility Codes Used for Numerical Integration

```
MODULE MathDESolve;

IMPORT
GraphNodes,
TextMappers, TextModels, TextViews,
Converters, Files, Math, Views;

(*nInd = number of individuals;
nTimeI = number of observations for IVGTT;
nTimeM = number of observations for MTT;
nInits = number of initial values for the System of ODEs;
nMax = maximum number of obervation times.                *)

CONST
nInd = 65; nTimeI = 30; nTimeM = 14; nInits = 2; nMax = MAX(nTimeI, nTimeM);

TYPE
Vector = POINTER TO ARRAY OF REAL;
VecPair = ARRAY 2 OF Vector;

VAR
insulin: ARRAY 2 OF ARRAY nInd OF ARRAY nMax OF REAL;
allTimes : ARRAY 2 OF ARRAY nMax OF REAL;
inits: ARRAY 2 OF ARRAY nInd OF ARRAY nInits OF REAL;

thetaReal: POINTER TO ARRAY OF REAL;
        solution, parameters: POINTER TO ARRAY OF ARRAY OF VecPair;
        sTemp, pTemp: Vector;


    PROCEDURE NumTimes (form: INTEGER): INTEGER;
    BEGIN
        CASE form OF
        |0: RETURN nTimeI
        |1: RETURN nTimeM
        END
    END NumTimes;

PROCEDURE SolutionReady (ind, form, nPar: INTEGER): BOOLEAN;
    CONST
        eps = 1.0E-20;
    VAR
        solutionReady: BOOLEAN;
        i: INTEGER;
    BEGIN
```

```
        solutionReady := TRUE;
        i := 0;
        WHILE (i < nPar) & solutionReady DO
            IF ABS(thetaReal[i] - parameters[ind, form][1][i]) > eps THEN
            solutionReady := FALSE END;
            INC(i)
        END;
        IF solutionReady THEN RETURN TRUE END;
        solutionReady := TRUE;
        i := 0;
        WHILE (i < nPar) & solutionReady DO
            IF ABS(thetaReal[i] - parameters[ind, form][0][i]) > eps THEN
            solutionReady := FALSE END;
            INC(i)
        END;
        IF solutionReady THEN
            pTemp := parameters[ind, form][0]; sTemp := solution[ind, form][0];
            parameters[ind, form][0] := parameters[ind, form][1]; solution[ind, form][0]
:=
            solution[ind, form][1];
            parameters[ind, form][1] := pTemp; solution[ind, form][1] := sTemp;
            RETURN TRUE
        ELSE RETURN FALSE
        END
    END SolutionReady;


    PROCEDURE TimeIndex (time: REAL; form: INTEGER): INTEGER;
    CONST
        eps = 1.0E-20;
    VAR
        nTimes, i: INTEGER; found: BOOLEAN;
    BEGIN
        found := FALSE;
        nTimes := NumTimes(form);
        i := 0;
        WHILE (i < nTimes) & ~found DO
            IF ABS(time - allTimes[form, i]) < eps THEN found := TRUE END;
            INC(i)
        END;
        ASSERT(found, 56);
        RETURN i - 1
    END TimeIndex;


    PROCEDURE RK (dose: REAL; ind, form, model, nPar: INTEGER);
    CONST
```

```
        T = 10;      (* number of steps*)
        (*dt = 0.05;*)                    (* time step size *) (* deb *)
        eps = 1.0E-10;
    VAR
        i, m, p: INTEGER;
        KStep1, KStep2, KStep3, KStep4, LStep1, LStep2, LStep3, LStep4,
        TT, ins1, ins2, ins3, ins4, previous, current, insulinb, qBasal, q1, q2, ddt, S,
X0: REAL;
        ga1, ga2, ga4, Aga, Bga, Cga, F1 : REAL; (* this is for gut absorption *)
        P1, P2, P3, K12, K21, V, F, K13, K34, K45: REAL;
        partRemIns, partRemIns1 : REAL;
        dt: REAL;       (* time steps*)

    temp : REAL; (* debug*)
    BEGIN
(*          P1 := 0.024;
        P2 := 0.0351;
        P3 := 0.00000318;
        K12 := 0.0885;
        K21 := 0.0580;
        V := 0.129;
*)
        P1 := thetaReal[0];
        P2 := thetaReal[1];
        IF (P2 >= 1.0E30) THEN
            P2 := 1.0E30
        END;
        IF (P2 <= 1.0E-30) THEN
            P2 := 1.0E-30
        END;
        P3 := thetaReal[2];
        K12 := thetaReal[3];
        K21 := thetaReal[4];
        V := thetaReal[5];
        IF (model >= 2) & (model <=6) THEN
            F := thetaReal[6];
            K13 := thetaReal[7];
        END;
        IF (model = 3) OR (model = 4)THEN
            K34 := K13 + thetaReal[8];
        END;
        IF (model = 4) THEN
            K45 := K34 + thetaReal[9];
        END;
        insulinb := inits[form, ind, 0];
        qBasal := inits[form, ind, 1];                    (* basal mass in q1 *)
        q1 := qBasal*V;
```

```
IF (model = 1) THEN
    q1 := q1+dose;                    (* initial value for IVGTT for q1 *)
END;
q2 := K21*(qBasal*V)/K12;             (* initial value for IVGTT for q2 *)
KStep1 := 0.0; KStep2 := 0.0; KStep3 := 0.0; KStep4 := 0.0;
LStep1 := 0.0; LStep2 := 0.0; LStep3 := 0.0; LStep4 := 0.0;
ins1 := 0.0; ins2 := 0.0; ins3 := 0.0; ins4 := 0.0; X0 := 0;
previous := 0.0; TT := 0.0;

IF ( model = 4) THEN
    F1 := F*dose*K45*K34/(K34-K45);
    Aga := F1*K13/(K13-K45); (* auxilaries for gut absoprtion *)
    Bga := F1*K13/(K13-K34);
    Cga := -F1*K13/(K13-K34) + F1*K13/(K13-K45);
END;

p := 1;
solution[ind, form][0][0] := qBasal;
WHILE p < NumTimes(form) DO          (* loop through time-points *)
    current := allTimes[form, p];
    ASSERT(ABS(TT - previous) < eps, 33);
    ddt := current - previous;
S := (insulin[form, ind, p] - insulin[form, ind, p - 1]) / ddt;
    dt := ddt/T;
    (*T := SHORT(ENTIER(Math.Round(ddt / dt) + eps));*)
    m := 0;
    partRemIns := P3/P2*(insulin[form, ind, p-1]-S*previous-insulinb)
     -P3/(P2*P2)*S;
    partRemIns1:= X0-P3 /P2*(insulin[form, ind, p - 1] - insulinb)+P3/
    (P2*P2)*S;
    WHILE m < T DO
        ins1 := partRemIns+P3/P2*S*(TT) + partRemIns1*
        Math.Exp(-P2 *( TT-previous));

        CASE model OF (* gut absorption contribution *)
            |1: ga1:= 0;
            |2: ga1:= F*dose*Math.Exp(-K13*TT)*K13;
            |3: ga1:= F*dose*K34/(K13-K34)*
                (Math.Exp(-K34*TT)-Math.Exp(-K13*TT))*K13;
            |4:ga1:= Aga*Math.Exp(-K45*TT)-Bga*
                Math.Exp(-K34*TT)-Cga*Math.Exp(-K13*TT);
            |5:ga1:= F*dose*K13*K13*TT*Math.Exp(-K13*TT);
        END;

        KStep1 := dt*(-(P1+K21+ins1)*q1+K12*q2+ga1+P1*qBasal*V);
        LStep1 := dt*(K21*q1-K12*q2);
```

```
ins2 := partRemIns+P3/P2*S*(TT+dt/2) + partRemIns1*
Math.Exp(-P2 * (TT+dt/2-previous));
CASE model OF (* gut absorption contribution *)
     |1: ga2:=0;
     |2: ga2:= F*dose*Math.Exp(-K13*(TT+dt/2))*K13;
     |3: ga2:= F*dose*K34/(K13-K34)*
          (Math.Exp(-K34*(TT+dt/2))-Math.Exp(-K13*
          (TT+dt/2)))*K13;
     |4: ga2:=Aga*Math.Exp(-K45*(TT+dt/2))-Bga*
          Math.Exp(-K34*(TT+dt/2))-Cga*
          Math.Exp(-K13*(TT+dt/2));
     |5: ga2:= F*dose*K13*K13*(TT+dt/2)*
          Math.Exp(-K13*(TT+dt/2));
END;
KStep2 := dt*(-(P1+K21+ins2)*(q1+KStep1/2)+
K12*(q2+LStep1/2)+ga2+P1*qBasal*V);
LStep2 := dt*(K21*(q1+KStep1/2)-K12*(q2+LStep1/2));

ins3 := ins2;
KStep3:= dt*(-(P1+K21+ins3)*(q1+KStep2/2)+
K12*(q2+LStep2/2)+ga2+P1*qBasal*V);
LStep3 := dt*(K21*(q1+KStep2/2)-K12*(q2+LStep2/2));

ins4 := partRemIns+P3/P2*S*(TT+dt) + partRemIns1*
Math.Exp(-P2 * (TT+dt-previous));
CASE model OF (* gut absorption contribution *)
     |1: ga4:=0;
     |2: ga4:= F*dose*Math.Exp(-K13*(TT+dt))*K13;
     |3: ga4:= F*dose*K34/(K13-K34)*
          (Math.Exp(-K34*(TT+dt))-Math.Exp(-K13*(TT+dt)))*
          K13;
     |4: ga4:= Aga*Math.Exp(-K45*(TT+dt))-Bga*
          Math.Exp(-K34*(TT+dt))-Cga*Math.Exp(-K13*(TT+dt));
     |5: ga4:= F*dose*K13*K13*(TT+dt)*Math.Exp(-K13*
          (TT+dt));
END;
KStep4 := dt*(-(P1+K21+ins4)*(q1+KStep3)+K12*
(q2+LStep3)+ga4+P1*qBasal*V);
LStep4 := dt*(K21*(q1+KStep3)-K12*(q2+LStep3));

q1 := q1 + (1 / 6) * (KStep1 + (2 * KStep2) +
(2 * KStep3) + KStep4);
q2 := q2 + (1 / 6) * (LStep1 + (2 * LStep2) +
(2 * LStep3) + LStep4);
IF (q1 < 0.0) THEN          (* check for nonnegativity *)
    q1 := 1.0E-10
END;
```

```
                IF (q1 > 1.0E5) THEN
                    q1 := 1.0E5
                END;
                IF (q2 < 0.0) THEN
                    q2 := 1.0E-10
                END;
                IF (q2 > 1.0E5) THEN
                    q2 := 1.0E5
                END;
                TT := TT + dt;                    (* time increment *)
                INC(m)
            END;          (* end of m-loop *)

            X0 := partRemIns+P3/P2*S*(TT) + partRemIns1*Math.Exp(-P2 *
             (TT-previous));

            solution[ind, form][0][p] := q1 / V;
            previous := current;
            INC(p);

        temp := q1/V; IF ((p = 31) & (model = 4)) THEN
        ASSERT(insulinb = 0, 111);          (* debug *) END;
        END;                    (* end of p-loop *)
        (* once we have evaluated the solution we need to store the parameters that
        correspond to it *)
        i := 0; WHILE i < nPar DO parameters[ind, form][0][i] := thetaReal[i]; INC(i)
END;
    END RK;


    PROCEDURE InitSolution;
    VAR
        i, j, k: INTEGER;
    BEGIN
        NEW(solution, nInd, 2);
        i := 0;
        WHILE i < nInd DO
            j := 0;
            WHILE j < 2 DO
                k := 0; WHILE k < 2 DO NEW(solution[i, j][k], nMax); INC(k)
END;
                INC(j)
            END;
            INC(i)
        END;
        NEW(sTemp, nMax)
    END InitSolution;
```

```
    PROCEDURE InitParameters (nPar: INTEGER);
    VAR
        i, j, k, l: INTEGER;
    BEGIN
        NEW(thetaReal, nPar);
        NEW(parameters, nInd, 2);
        i := 0;
        WHILE i < nInd DO
            j := 0;
            WHILE j < 2 DO
                k := 0;
                WHILE k < 2 DO
                    NEW(parameters[i, j][k], nPar);
                    l := 0; WHILE l < nPar DO parameters[i, j][k][l] := MIN(INTEGER);
INC(l) END;
                    INC(k)
                END;
                INC(j)
            END;
            INC(i)
        END;
        NEW(pTemp, nPar)
    END InitParameters;

    PROCEDURE SolveShola* (theta: ARRAY OF GraphNodes.Node; time, dose:
GraphNodes.Node;
                                                    ind, form, model: INTE-
GER): REAL;
    VAR
        nPar, i: INTEGER; value: REAL;
    BEGIN
        nPar := LEN(theta);
        IF parameters = NIL THEN InitParameters(nPar) END;
        i := 0; WHILE i < nPar DO thetaReal[i] := theta[i].Value(); INC(i) END;
        IF SolutionReady(ind, form, nPar) THEN
            value := solution[ind, form][1][TimeIndex(time.Value(), form)]
        ELSE
        RK(dose.Value(), ind, form, model, nPar);
            value := solution[ind, form][0][TimeIndex(time.Value(), form)]
        END;
        RETURN value
    END SolveShola;


    PROCEDURE GetData;
    CONST
        path = "data\rsrc";
```

```
VAR
    loc: Files.Locator; v: Views.View; ask: BOOLEAN;
    conv: Converters.Converter; fileName: Files.Name; t: TextModels.Model;
    s: TextMappers.Scanner; i, j, k, nTime: INTEGER;
BEGIN
    loc := Files.dir.This(path);
    ask := (loc = NIL) OR (loc.res # 0);
    conv := NIL;
    fileName := "te-i-mod65_data";
    v := Views.Old(ask, loc, fileName, conv);
    ASSERT(v # NIL, 57);
    WITH v: TextViews.View DO
        t := v.ThisModel(); s.ConnectTo(t); s.SetPos(0);
        i := 0;                    (* define scanning loops for insulin *)
        WHILE i < 2 DO
            nTime := NumTimes(i);
            j := 0;
            WHILE j < nInd DO
                k := 0;
                WHILE k < nTime DO
                    s.Scan;
                    ASSERT((s.type = TextMappers.int) OR
                            (s.type = TextMappers.real), 58);
                    IF s.type = TextMappers.real THEN insulin[i, j, k] :=
                        s.real ELSE insulin[i, j, k] := s.int END;
                    INC(k)
                END;
                INC(j)
            END;
            INC(i)
        END;
        i := 0;                    (* define scanning loops for time *)
        WHILE i < 2 DO
            nTime := NumTimes(i);
            j := 0;
            WHILE j < nTime DO
                s.Scan; ASSERT((s.type = TextMappers.int) OR
                    (s.type = TextMappers.real), 59);
                IF s.type = TextMappers.real THEN allTimes[i, j] :=
                    s.real ELSE allTimes[i, j] := s.int END;
                INC(j)
            END;
            INC(i)
        END;
        i := 0;                    (* define scanning loops for inits *)
        WHILE i < 2 DO
            nTime := NumTimes(i);
```

```
                j := 0;
                WHILE j < nInd DO
                    k := 0;
                    WHILE k < nInits DO
                        s.Scan; ASSERT((s.type = TextMappers.int) OR
                            (s.type = TextMappers.real), 60);
                        IF s.type = TextMappers.real THEN inits[i, j, k] :=
                            s.real ELSE inits[i, j, k] := s.int END;
                        INC(k)
                    END;
                    INC(j)
                END;
                INC(i)
            END
        END
    END GetData;


    PROCEDURE Init;
    BEGIN
        thetaReal := NIL;
        solution := NIL; parameters := NIL;
        sTemp := NIL; pTemp := NIL;
        InitSolution;
        GetData;
    END Init;
BEGIN
    Init

END MathPKShola.
```

```
MODULE MathPKShola;
(*     form = IVGTT (1) or MTT (2)
    model =      1: IVGTT model only
        2: IVGTT + MTT (One Compantment)
        3: IVGTT + MTT (Two Compantment)
        4: IVGTT + MTT (Three Compantment)
    ind = individual number (1, 2, 3, ...)                *)
    IMPORT
        PKLinkSingleDose,
        GraphLogical, GraphNodes,
        MathDESolve;
    TYPE
        Node = POINTER TO RECORD (PKLinkSingleDose.Node)
            ind, form, model: INTEGER
        END;

        Factory = POINTER TO RECORD (GraphNodes.Factory) END;
    VAR
        fact-: GraphNodes.Factory;

    PROCEDURE (node: Node) Set (IN args: GraphNodes.Args; OUT res: INTE-
GER);
    CONST
        eps = 1.0E-10;
    VAR
        len, i, off, size: INTEGER;
    BEGIN
        res := 0;
        WITH args: GraphLogical.Args DO
            ASSERT(args.vectors[0].base # NIL, 21); len := LEN(args.vectors[0].base);
            ASSERT(args.vectors[0].start >= 0, 21);
            ASSERT(args.vectors[0].size > 0, 21);
            ASSERT(args.vectors[0].step > 0, 21);
            size := args.vectors[0].size; NEW(node.theta, size);
            i := 0;
            WHILE i < size DO
                off := args.vectors[0].start + i * args.vectors[0].step;
                    ASSERT(len > off, 21);
                node.theta[i] := args.vectors[0].base[off];
                INC(i)
            END;
            ASSERT(args.scalars[0] # NIL, 21); node.time := args.scalars[0];
            ASSERT(args.scalars[1] # NIL, 21); node.dose := args.scalars[1];
            ASSERT(args.scalars[2] # NIL, 21); node.ind :=
                SHORT(ENTIER(args.scalars[2].Value() - 1 + eps));
            ASSERT(args.scalars[3] # NIL, 21); node.form :=
                SHORT(ENTIER(args.scalars[3].Value() - 1 + eps));
```

```
            ASSERT(args.scalars[4] # NIL, 21); node.model :=
                SHORT(ENTIER(args.scalars[4].Value() + eps))
        END
    END Set;
    PROCEDURE (node: Node) Value (): REAL;
    BEGIN
        RETURN MathDESolve.SolveShola(node.theta, node.time, node.dose,
                                      node.ind, node.form, node.model);
    END Value;

    PROCEDURE (f: Factory) New (option: INTEGER): GraphNodes.Node;
    VAR node: Node;
    BEGIN
        NEW(node); node.Init; RETURN node
    END New;
    PROCEDURE Install*;
    BEGIN
        GraphNodes.SetFactory(fact)
    END Install;
    PROCEDURE Init;
    VAR f: Factory;
    BEGIN
        NEW(f); fact := f
    END Init;
BEGIN
    Init
END MathPKShola.
```

Appendix B

Appendix B includes three tables. Each table gives the results of CODA's diagnostic criteria (Geweke, Raftry and Lewis, and Heldelberger and Welch) used to assess the convergence of MCMC chains in Section 3.4, Chapter 3. The appendix also contains the plot of the iteration history of model parameters.

Table B.1: Geweke convergence diagnostic (Z-score).

Iterations used = 6000:10999
Thinning interval = 1
Sample size per chain = 5000

Fraction in 1st window = 0.1
Fraction in 2nd window = 0.5

Variable	unmubeta
unmu.beta[1]	-0.397
unmu.beta[2]	0.533
unmu.beta[3]	2.640
unmu.beta[4]	-0.419

Table B.2: Raftery and Lewis convergence diagnostics.

Iterations used = 6000:10999
Thinning interval = 1
Sample size per chain = 5000

Quantile = 0.025
Accuracy = +/- 0.005
Probability = 0.95

Variable	Thin (k)	Burn-in (M)	Total (N)	Lower bound (Nmin)	Dependence factor (I)
unmu.beta[1]	1	3	4293	3746	1.15
unmu.beta[2]	1	2	3995	3746	1.07
unmu.beta[3]	1	4	4955	3746	1.32
unmu.beta[4]	1	3	4470	3746	1.19

Table B.3: Heidelberger and Welch stationarity and interval halfwidth tests.

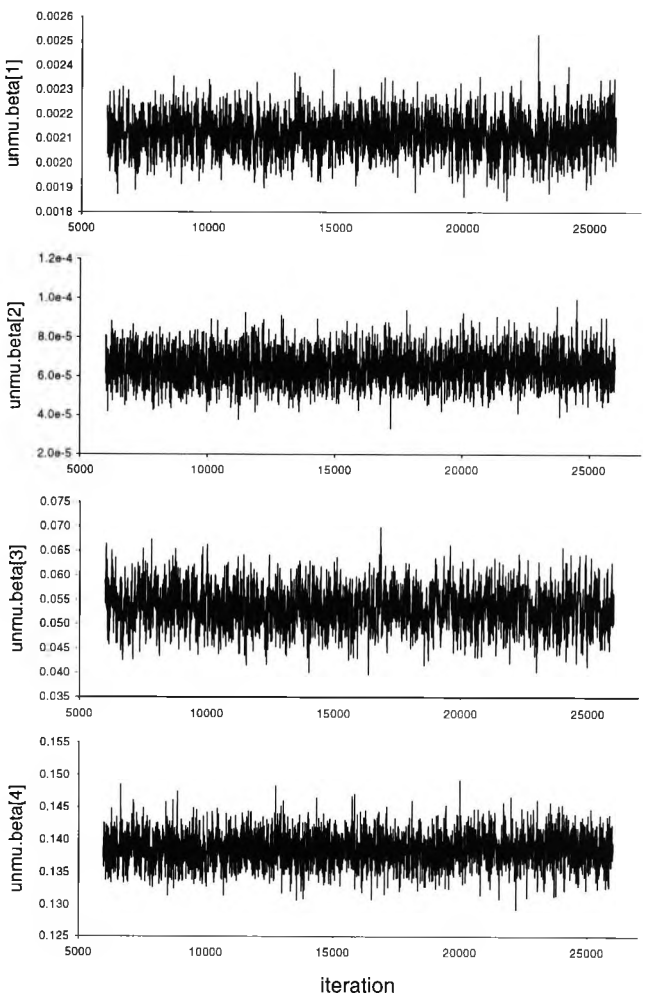
Iterations used = 6000:10999
Thinning interval = 1
Sample size per chain = 5000

Precision of halfwidth test = 0.1

Variable	Stationary test	# of iters. to keep	# 0f iters to discard	C-vonM stat
unmu.beta[1]	passed	5000	0	0.349
unmu.beta[2]	passed	5000	0	0.116
unmu.beta[3]	passed	4500	500	0.196
unmu.beta[4]	passed	4000	1000	0.441

	Halfwidth test	Mean	Halfwidth
unmu.beta[1]	passed	0.0021200	3.69e-006
unmu.beta[1]	passed	0.0000645	3.15e-007
unmu.beta[1]	passed	0.0534000	2.12e-004
unmu.beta[1]	passed	0.1390000	9.20e-005

Figure B.1: Plots of iteration history for the model parameters.



Appendix C

Appendix C has two tables for results in Chapter 3. The first table shows individual parameter estimates of the minimal model with BAY in 65 newly presenting T2D subjects. The second table shows individual estimates of glucose effectiveness and insulin sensitivity using the minimal model with BAY in the same 65 subjects.

Table C.1: Individual parameter estimates of the minimal model with the Bayesian hierarchical analysis of the insulin-modified IVGTT in subjects with newly presenting T2D.

Subject	p_1 ($10^{-2} \times$ min^{-1})	p_2 ($10^{-2} \times$ min^{-1})	p_3 ($10^{-5} \times$ min^{-2}pmol·L^{-1})	V (ml·kg^{-1})
1	1.53*	3.81*	0.97*	151.7*
2	1.42	4.47	1.42	156.6
3	1.42	9.58	16.93	115.7
4	1.51	4.49	0.58	122.5
5	1.54	3.50	0.44	151.3
6	2.74	6.30	1.45	107.9
7	2.45	5.94	8.23	127.0
8	1.56	5.28	3.92	138.6
9	1.80	4.49	1.28	139.3
10	1.60	4.36	0.86	139.7
11	1.14	7.46	7.21	149.8
12	1.25	4.37	3.31	134.6
13	1.38	5.02	6.45	143.7
14	1.39	6.48	6.13	120.8
15	1.37	5.36	8.77	145.7
16	1.14	5.75	7.68	144.6
17	1.60	4.58	0.74	134.0
18	2.68	8.39	49.96	146.9
19	1.61	5.15	0.95	116.0
20	1.63	4.93	2.80	127.5
21	1.63	5.33	3.16	141.2
22	1.70	5.16	2.99	157.2
23	1.31	5.35	6.50	154.7
24	1.59	5.85	9.42	174.1
25	1.13	3.43	6.56	151.1
26	1.48	5.10	4.99	168.3
27	1.70	5.25	1.92	141.9
28	1.55	7.44	14.74	155.6
29	1.53	5.10	1.59	154.9
30	1.33	6.66	7.63	147.8
31	1.84	4.59	1.37	146.6
32	1.13	4.95	4.79	139.9
33	1.76	4.34	1.37	135.3
34	1.56	4.20	2.08	168.7
35	1.42	4.28	1.09	135.6

* Median of posterior density function.

Table C.1 continues

Subject	p_1 ($10^{-2}\times$ min^{-1})	p_2 ($10^{-2}\times$ min^{-1})	p_3 ($10^{-5}\times$ min$^{-2}\mu$U·ml^{-1})	V (ml·kg^{-1})
36	1.19*	4.76*	6.63*	111.1*
37	1.55	4.55	1.78	154.9
38	1.41	4.23	6.51	157.2
39	1.84	7.17	18.77	123.5
40	1.62	3.85	0.87	146.3
41	1.52	7.40	8.90	145.7
42	1.42	7.27	16.04	121.0
43	1.68	4.49	1.38	160.4
44	1.62	3.60	0.62	175.9
45	1.28	5.03	1.94	134.7
46	1.46	5.42	3.57	112.7
47	1.53	5.68	3.23	153.0
48	1.64	7.10	4.76	139.4
49	1.03	6.31	10.68	135.5
50	1.92	7.14	5.36	143.6
51	1.66	6.35	3.69	140.9
52	1.67	7.07	5.93	161.8
53	1.28	8.13	18.31	108.8
54	1.48	4.23	5.62	138.6
55	1.42	6.10	6.19	132.8
56	1.45	5.18	4.24	132.9
57	1.70	6.58	2.67	115.5
58	1.55	7.38	15.81	134.5
59	1.50	5.22	14.29	123.1
60	1.76	5.71	1.35	129.0
61	1.66	4.71	2.87	143.5
62	1.57	5.21	1.75	127.4
63	1.57	4.08	2.49	135.1
64	1.72	4.90	1.14	126.1
65	1.67	5.13	1.95	115.5

* Median of posterior density function.

Table C.2: Individual estimates of glucose effectiveness S_G and insulin sensitivity S_I using the minimal model with the Bayesian hierarchical analysis of the insulin-modified IVGTT in subjects with newly presenting T2D.

Subject	S_G ($10^{-2}\times$ min^{-1})	CV (%)	S_I ($10^{-5}\times$ min^{-1} pmol·L^{-1})	CV (%)
1	1.53*	12**	0.42*	48**
2	1.42	14	0.54	32
3	1.42	17	2.95	5
4	1.51	7	0.22	48
5	1.54	13	0.21	49
6	2.74	13	0.39	56
7	2.45	16	2.33	14
8	1.56	15	1.23	18
9	1.80	14	0.48	37
10	1.60	12	0.33	46
11	1.14	18	1.62	7
12	1.25	15	1.27	15
13	1.38	13	2.15	6
14	1.39	16	1.58	12
15	1.37	13	2.73	3
16	1.14	16	2.24	11
17	1.60	10	0.27	42
18	2.68	7	9.91	4
19	1.61	8	0.31	41
20	1.63	14	0.94	21
21	1.63	15	0.99	15
22	1.70	16	0.98	22
23	1.31	14	2.03	7
24	1.59	17	2.70	8
25	1.13	14	3.19	5
26	1.48	17	1.65	12
27	1.70	16	0.61	29
28	1.55	15	3.30	5
29	1.53	15	0.52	26
30	1.33	16	1.91	7
31	1.84	12	0.50	45
32	1.13	13	1.61	14
33	1.76	13	0.53	30
34	1.56	17	0.83	24
35	1.42	13	0.43	41

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

Table C.2 continues

Subject	S_G (10^{-2}x min^{-1})	CV (%)	S_I (10^{-2} x min^{-1} $pmol \cdot L^{-1}$)	CV (%)
36	1.19*	16**	2.33*	9**
37	1.55	16	0.66	34
38	1.41	13	2.57	4
39	1.84	8	4.36	3
40	1.62	12	0.38	36
41	1.52	16	2.01	8
42	1.42	13	3.68	3
43	1.68	16	0.52	35
44	1.62	12	0.28	53
45	1.28	12	0.64	23
46	1.46	12	1.10	16
47	1.53	15	0.95	23
48	1.64	15	1.12	10
49	1.03	16	2.82	5
50	1.92	17	1.26	14
51	1.66	17	0.98	16
52	1.67	17	1.41	7
53	1.28	13	3.76	3
54	1.48	13	2.21	5
55	1.42	13	1.69	6
56	1.45	14	1.37	16
57	1.70	13	0.68	17
58	1.55	13	3.57	4
59	1.50	9	4.56	3
60	1.76	13	0.40	37
61	1.66	13	1.02	30
62	1.57	12	0.56	31
63	1.57	14	1.02	25
64	1.72	13	0.38	41
65	1.67	13	0.63	20

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

Appendix D

Appendix D has two tables. The first table shows the results of individual glucose effectiveness of the minimal model with the Bayesian hierarchical analysis and with the nonlinear regression analysis. The second table shows the results of individual insulin sensitivity of the minimal model with the Bayesian hierarchical analysis and with the nonlinear regression analysis.

Table D.1: Glucose effectiveness of the minimal model with the Bayesian hierarchical analysis (S_G^{BAY}) and with the nonlinear regression analysis (S_G^{NLR}) estimated during the insulin-modified IVGTT in subjects with newly presenting T2D.

Subject	S_G^{BAY} (10^{-2} x min^{-1})	CV (%)	S_G^{NLR} (10^{-2} x min^{-1})	CV (%)	%RE (%)
1	1.53*	12**	1.40*	11**	8***
2	1.42	14	1.18	17	17
3	1.42	17	0.92	36	35
4	1.51	7	1.57	17	-4
5	1.54	13	1.20	44	22
6	2.74	13	3.28	12	-20
7	2.45	16	3.51	8	-43
8	1.56	15	1.52	15	3
9	1.80	14	2.04	9	-13
10	1.60	12	1.86	5	-16
11	1.14	18	0.22	104	81
12	1.25	15	0.92	16	27
13	1.38	13	1.38	19	0
14	1.39	16	1.19	20	14
15	1.37	13	1.38	38	-0
16	1.14	16	0.68	29	40
17	1.60	10	1.77	7	-11
18	2.68	7	2.72	38	-1
19	1.61	8	1.73	7	-7
20	1.63	14	1.67	10	-2
21	1.63	15	1.60	11	2
22	1.70	16	1.64	11	4
23	1.31	14	1.07	15	18
24	1.59	17	1.34	16	16
25	1.13	14	1.11	33	2
26	1.48	17	1.16	13	22
27	1.70	16	1.73	8	-2
28	1.55	15	1.37	31	12
29	1.53	15	1.38	12	10
30	1.33	16	0.88	19	34
31	1.84	12	2.03	13	-11
32	1.13	13	0.95	33	15
33	1.76	13	1.90	8	-8
34	1.56	17	1.31	13	16
35	1.42	13	1.17	14	18

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error calculated as $100\%(\frac{S_G^{BAY} - S_G^{NLR}}{S_G^{BAY}})$.

Table D.1 continues

Subject	S_G^{BAY} (10^{-2} x min^{-1})	CV (%)	S_G^{NLR} (10^{-2} x min^{-1})	CV (%)	%RE (%)
36	1.19*	16**	1.01*	20**	15***
37	1.55	16	1.18	11	24
38	1.41	13	1.49	26	-6
39	1.84	8	1.92	35	-5
40	1.62	12	1.58	6	2
41	1.52	16	1.15	31	25
42	1.42	13	1.42	38	-0
43	1.68	16	1.62	13	4
44	1.62	12	1.61	15	1
45	1.28	12	1.18	19	8
46	1.46	12	1.46	10	-0
47	1.53	15	1.32	15	14
48	1.64	15	1.48	18	10
49	1.03	16	3.77	59	-266
50	1.92	17	2.01	21	-5
51	1.66	17	1.57	17	6
52	1.67	17	1.19	43	29
53	1.28	13	1.17	28	9
54	1.48	13	1.64	18	-11
55	1.42	13	1.34	26	5
56	1.45	14	1.35	12	7
57	1.70	13	1.81	8	-7
58	1.55	13	1.56	44	-1
59	1.50	9	1.61	38	-7
60	1.76	13	1.70	7	3
61	1.66	13	1.53	11	8
62	1.57	12	1.59	11	-1
63	1.57	14	1.52	8	3
64	1.72	13	2.04	5	-19
65	1.67	13	1.75	8	-5

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error calculated as $100\%(\frac{S_G^{BAY} - S_G^{NLR}}{S_G^{BAY}})$.

Table D.2: Insulin sensitivity of the minimal model with the Bayesian hierarchical analysis (S_I^{BAY}) and with the nonlinear regression analysis (S_I^{NLR}) during the insulin-modified IVGTT estimated in subjects with newly presenting T2D.

Subject	S_I^{BAY} (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	S_I^{NLR} (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	%RE (%)
1	0.42*	48**	1.00*	24**	-140***
2	0.54	32	0.72	23	-34
3	2.95	5	3.12	5	-6
4	0.22	48	0.17	127	21
5	0.21	49	NA	NA	NA
6	0.39	56	NA	NA	NA
7	2.33	14	2.22	14	5
8	1.23	18	1.28	16	-4
9	0.48	37	0.47	31	1
10	0.33	46	0.08	102	75
11	1.62	7	2.08	6	-29
12	1.27	15	1.58	8	-25
13	2.15	6	2.48	7	-16
14	1.58	12	1.72	10	-9
15	2.73	3	2.82	6	-3
16	2.24	11	2.78	9	-24
17	0.27	42	0.13	57	52
18	9.91	4	10.83	13	-9
19	0.31	41	0.18	58	41
20	0.94	21	0.95	15	-1
21	0.99	15	1.00	9	-1
22	0.98	22	1.02	12	-4
23	2.03	7	2.18	5	-7
24	2.70	8	2.77	4	-2
25	3.19	5	3.32	8	-4
26	1.65	12	1.82	5	-10
27	0.61	29	0.57	15	7
28	3.30	5	3.35	6	-1
29	0.52	26	0.57	16	-9
30	1.91	7	2.12	5	-11
31	0.50	45	NA	NA	NA
32	1.61	14	1.98	26	-23
33	0.53	30	0.55	19	-4
34	0.83	24	1.03	10	-24

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error was calculated as $100\% \left(\frac{S_I^{BAY} - S_I^{NLR}}{S_I^{BAY}}\right)$.

NA Stands for failed S_I estimate (CV $>$ 150%)

Table D.2 continues

Subject	S_I^{BAY} (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	S_I^{NLR} (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	%RE (%)
35	0.43*	41**	0.85*	22**	-99***
36	2.33	9	2.53	7	-8
37	0.66	34	0.97	12	-48
38	2.57	4	2.65	6	-3
39	4.36	3	4.62	10	-6
40	0.38	36	0.53	12	-41
41	2.01	8	2.22	10	-10
42	3.68	3	3.78	6	-3
43	0.52	35	0.55	26	-6
44	0.28	53	NA	NA	NA
45	0.64	23	0.75	28	-16
46	1.10	16	1.12	13	-2
47	0.95	23	1.10	16	-15
48	1.12	10	1.17	9	-4
49	2.82	5	3.22	5	-14
50	1.26	14	1.22	15	3
51	0.98	16	1.00	12	-2
52	1.41	7	1.45	6	-3
53	3.76	3	3.83	5	-2
54	2.21	5	2.22	6	-0
55	1.69	6	1.73	9	-2
56	1.37	16	1.50	12	-9
57	0.68	17	0.62	11	8
58	3.57	4	3.73	7	-5
59	4.56	3	4.78	9	-5
60	0.40	37	0.42	17	-5
61	1.02	30	1.38	18	-35
62	0.56	31	0.53	29	5
63	1.02	25	1.17	12	-14
64	0.38	41	0.15	47	61
65	0.63	20	0.60	14	5

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error was calculated as $100\%(\frac{S_I^{BAY} - S_I^{NLR}}{S_I^{BAY}})$.

NA stands for failed S_I estimate (CV > 150%).

Appendix E

Appendix E includes two tables. Each table shows individual results of the full sample and the reduced sample schemes using the minimal model with the Bayesian hierarchical analysis in subjects with newly presenting T2D.

Table E.1: Glucose effectiveness estimated with the full-sample scheme ($S_{G(30)}$), the 13 sample scheme ($S_{G(13)}$), and the 12 sample scheme ($S_{G(12)}$) and associated relative error (%RE) using the minimal model with the Bayesian hierarchical analysis during the insulin-modified IVGTT in subjects with newly presenting T2D.

Subject	Full-Sample		13-Sample			12-Sample		
	$S_{G(30)}$ (10^{-2} x min^{-1})	CV (%)	$S_{G(13)}$ (10^{-2} x min^{-1})	CV (%)	%RE (%)	$S_{G(12)}$ (10^{-2} x min^{-1})	CV (%)	%RE (%)
1	1.53*	12**	1.67*	8**	-9***	1.45*	11**	5***
2	1.42	14	1.68	9	-18	1.43	11	-1
3	1.42	17	1.70	11	-19	1.56	11	-10
4	1.51	7	1.59	7	-6	1.46	10	3
5	1.54	13	1.64	10	-7	1.41	12	9
6	2.74	13	1.88	12	31	1.54	11	44
7	2.45	16	1.86	11	24	1.52	11	38
8	1.56	15	1.71	9	-10	1.46	10	6
9	1.80	14	1.76	10	2	1.47	11	18
10	1.60	12	1.69	9	-5	1.46	11	9
11	1.14	18	1.65	9	-44	1.46	10	-28
12	1.25	15	1.63	10	-30	1.45	10	-16
13	1.38	13	1.65	9	-19	1.44	10	-4
14	1.39	16	1.64	10	-18	1.47	10	-6
15	1.37	13	1.62	9	-18	1.40	10	-2
16	1.14	16	1.60	9	-41	1.43	10	-26
17	1.60	10	1.70	9	-7	1.47	11	8
18	2.68	7	1.94	12	28	1.64	17	39
19	1.61	8	1.68	8	-4	1.50	10	7
20	1.63	14	1.69	10	-3	1.46	10	11
21	1.63	15	1.73	10	-6	1.47	10	10
22	1.70	16	1.78	11	-5	1.46	12	14
23	1.31	14	1.69	9	-29	1.44	10	-10
24	1.59	17	1.79	12	-12	1.46	13	8
25	1.13	14	1.54	11	-37	1.36	12	-20
26	1.48	17	1.73	11	-16	1.44	12	3
27	1.70	16	1.77	10	-4	1.48	11	13
28	1.55	15	1.78	11	-15	1.51	13	3
29	1.53	15	1.73	10	-13	1.46	12	5
30	1.33	16	1.71	9	-28	1.48	10	-11
31	1.84	12	1.76	10	4	1.47	11	20
32	1.13	13	1.62	9	-43	1.49	10	-32
33	1.76	13	1.75	10	1	1.48	11	16
34	1.56	17	1.78	11	-14	1.44	13	7
35	1.42	13	1.60	9	-12	1.43	11	-0

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error calculated as $100\%(\frac{\text{full - reduced}}{\text{full}})$.

Table E.1 continues

Subject	Full-Sample		13-Sample			12-Sample		
	$S_{G(30)}$ (10^{-2} x min^{-1})	CV %	$S_{G(13)}$ (10^{-2} x min^{-1})	CV %	%RE %	$S_{G(12)}$ (10^{-2} x min^{-1})	CV %	%RE %
36	1.19*	16**	1.55*	11**	-30***	1.46*	12**	-22***
37	1.55	16	1.72	10	-11	1.46	12	6
38	1.41	13	1.61	10	-15	1.37	11	3
39	1.84	8	1.80	8	2	1.61	10	12
40	1.62	12	1.69	9	-4	1.44	11	11
41	1.52	16	1.76	10	-15	1.50	10	2
42	1.42	13	1.64	9	-15	1.50	11	-6
43	1.68	16	1.78	11	-6	1.44	12	14
44	1.62	12	1.72	10	-6	1.43	13	12
45	1.28	12	1.60	9	-25	1.44	10	-13
46	1.46	12	1.63	10	-12	1.49	11	-2
47	1.53	15	1.73	10	-13	1.46	11	5
48	1.64	15	1.78	10	-9	1.50	10	9
49	1.03	16	1.57	9	-52	1.46	10	-41
50	1.92	17	1.82	11	6	1.50	11	22
51	1.66	17	1.78	10	-7	1.49	11	11
52	1.67	17	1.78	10	-6	1.47	12	12
53	1.28	13	1.62	10	-26	1.54	11	-20
54	1.48	13	1.65	9	-12	1.42	10	4
55	1.42	13	1.64	8	-16	1.45	9	-2
56	1.45	14	1.68	9	-16	1.49	10	-3
57	1.70	13	1.70	10	0	1.49	11	12
58	1.55	13	1.67	9	-8	1.49	10	4
59	1.50	9	1.55	9	-3	1.42	11	6
60	1.76	13	1.76	10	0	1.50	10	15
61	1.66	13	1.73	9	-4	1.48	11	11
62	1.57	12	1.68	9	-7	1.47	10	6
63	1.57	14	1.71	10	-9	1.47	10	6
64	1.72	13	1.71	10	0	1.49	11	14
65	1.67	13	1.69	10	-1	1.48	10	11

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error calculated as $100\%(\frac{\text{full - reduced}}{\text{full}})$.

Table E.2: Insulin sensitivity estimated with the full-sample scheme ($S_{I(30)}$), the 13 sample scheme ($S_{I(13)}$), and the 12 sample scheme ($S_{I(12)}$) and associated percentage relative error (%RE) using the minimal model with the Bayesian hierarchical analysis during the insulin-modified IVGTT in subjects with newly presenting T2D.

Subject	Full-Sample		13-Sample			12-Sample		
	$S_{I(30)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	$S_{I(13)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	%RE (%)	$S_{I(12)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	%RE (%)
1	0.42*	48**	0.34*	45**	19***	0.55*	32**	-33***
2	0.54	32	0.39	38	27	0.60	27	-12
3	2.95	5	2.91	7	1	2.43	9	18
4	0.22	48	0.25	47	-14	0.45	36	-109
5	0.21	49	0.18	46	11	0.28	33	-36
6	0.39	56	0.89	29	-129	1.27	18	-226
7	2.33	14	2.50	14	-7	2.59	12	-11
8	1.23	18	1.15	18	7	1.28	15	-4
9	0.48	37	0.53	31	-11	0.69	22	-46
10	0.33	46	0.33	44	-1	0.54	31	-67
11	1.62	7	1.57	9	3	1.65	10	-2
12	1.27	15	1.00	20	21	0.95	18	25
13	2.15	6	2.00	9	7	2.10	8	2
14	1.58	12	1.60	13	-1	1.54	13	3
15	2.73	3	2.60	6	5	2.10	7	23
16	2.24	11	1.88	14	16	1.85	14	17
17	0.27	42	0.30	40	-11	0.46	28	-70
18	9.91	4	9.87	4	0	8.31	5	16
19	0.31	41	0.33	43	-8	0.51	29	-67
20	0.94	21	0.90	20	4	1.02	16	-8
21	0.99	15	0.95	17	4	1.02	15	-3
22	0.98	22	0.92	22	6	0.95	17	3
23	2.03	7	1.74	9	14	1.67	9	17
24	2.70	8	2.52	12	7	2.39	12	12
25	3.19	5	2.78	9	13	2.61	10	18
26	1.65	12	1.54	15	6	1.60	15	3
27	0.61	29	0.67	27	-10	0.75	20	-23
28	3.30	5	3.34	7	-1	3.36	7	-2
29	0.52	26	0.53	26	-1	0.60	21	-15
30	1.91	7	1.82	9	5	1.89	9	1
31	0.50	45	0.63	35	-28	0.92	22	-85
32	1.61	14	1.15	22	29	1.12	20	31
33	0.53	30	0.54	26	-3	0.65	19	-23
34	0.83	24	0.68	27	18	0.76	21	8

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error calculated as $100\%(\frac{\text{full - reduced}}{\text{full}})$.

Table E.2 continues

Subject	Full-Sample		13-Sample			12-Sample		
	$S_{I(30)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	$S_{I(13)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	%RE (%)	$S_{I(12)}$ (10^{-5} x min^{-1} per $pmol \cdot L^{-1}$)	CV (%)	%RE (%)
35	0.43*	41**	0.35*	44**	19***	0.51*	35**	-18***
36	2.33	9	1.95	12	17	1.69	13	28
37	0.66	34	0.59	37	10	0.71	28	-8
38	2.57	4	2.47	7	4	2.37	7	8
39	4.36	3	3.85	4	12	2.84	4	35
40	0.38	36	0.33	34	13	0.42	26	-11
41	2.01	8	1.96	10	3	1.85	10	8
42	3.68	3	3.84	5	-4	3.36	6	9
43	0.52	35	0.45	35	13	0.59	24	-13
44	0.28	53	0.27	50	7	0.48	34	-69
45	0.64	23	0.44	33	31	0.49	26	24
46	1.10	16	1.01	19	8	1.02	17	7
47	0.95	23	0.85	25	11	0.96	20	-0
48	1.12	10	1.20	10	-8	1.27	9	-14
49	2.82	5	2.56	7	9	2.65	8	6
50	1.26	14	1.42	14	-12	1.46	13	-15
51	0.98	16	0.89	17	9	0.78	14	21
52	1.41	7	1.45	9	-3	1.24	10	12
53	3.76	3	3.56	5	5	3.07	6	18
54	2.21	5	2.06	7	7	2.09	7	6
55	1.69	6	1.75	7	-3	1.70	7	-1
56	1.37	16	1.22	18	11	1.47	16	-7
57	0.68	17	0.72	16	-7	0.76	14	-13
58	3.57	4	3.79	6	-6	3.34	6	6
59	4.56	3	4.74	5	-4	4.17	5	9
60	0.40	37	0.47	30	-18	0.66	21	-65
61	1.02	30	0.94	27	9	1.03	22	-1
62	0.56	31	0.57	29	-2	0.71	22	-27
63	1.02	25	0.87	25	14	1.05	18	-2
64	0.38	41	0.43	36	-13	0.58	26	-52
65	0.63	20	0.70	17	-11	0.82	14	-30

* Median of posterior density function.

** Precision of parameter estimate expressed as the CV of the posterior density function.

***Percentage relative error (%RE) calculated as $100\%(\frac{\text{full - reduced}}{\text{full}})$.

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Clinical Science (2003) **105**, 551–560 (Printed in Great Britain)

comment

Bayesian hierarchical approach to estimate insulin sensitivity by minimal model

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ABSTRACT

We adopted Bayesian analysis in combination with hierarchical (population) modelling to estimate simultaneously population and individual insulin sensitivity (S_I) and glucose effectiveness (S_G) with the minimal model of glucose kinetics using data collected during insulin-modified intravenous glucose tolerance test (IVGTT) and made comparison with the standard non-linear regression analysis. After fasting overnight, subjects with newly presenting Type II diabetes according to World Health Organization criteria ($n = 65$; 53 males, 12 females; age, 54 ± 9 years; body mass index, 30.4 ± 5.2 kg/m^2; means $\pm$ S.D.) underwent IVGTT consisting of a 0.3 g of glucose bolus/kg of body weight given at time zero for 2 min, followed by 0.05 unit of insulin/kg of body weight at 20 min. Bayesian inference was carried out using vague prior distributions and log-normal distributions to guarantee non-negativity and, thus, physiological plausibility of model parameters and associated credible intervals. Bayesian analysis gave estimates of S_I in all subjects. Non-linear regression analysis failed in four cases, where Bayesian analysis-derived S_I was located in the lower quartile and was estimated with lower precision. The population means of S_I and S_G provided by Bayesian analysis and non-linear regression were identical, but the interquartile range given by Bayesian analysis was tighter by approx. 20% for S_I and by approx. 15% for S_G. Individual insulin sensitivities estimated by the two methods were highly correlated ($r_S = 0.98$; $P < 0.001$). However, the correlation in the lower 20% centile of the insulin-sensitivity range was significantly lower than the correlation in the upper 80% centile ($r_S = 0.71$ compared with $r_S = 0.99$; $P < 0.001$). We conclude that the Bayesian hierarchical analysis is an appealing method to estimate S_I and S_G, as it avoids parameter estimation failures, and should be considered when investigating insulin-resistant subjects.

INTRODUCTION

The minimal model analysis of intravenous glucose tolerance test (IVGTT) data has become an invaluable method to estimate insulin sensitivity (S_I) and glucose effectiveness (S_G). The analysis has been used in both small- and large-scale studies, such as the Insulin Resistance Atherosclerosis study [1] and the FUSION (Finland–United States Investigation on NIDDM genetics) study [2].

The insulin modification of IVGTT addressed the early problems with the minimal model 'failures', i.e. the situation when the minimal model analysis returns S_I indistinguishable from 'zero' or the precision of S_I

Key words: Bayesian analysis, insulin sensitivity, minimal model, Type II diabetes.

Abbreviations: CI, confidence interval; CrI, credible interval; CV, coefficient of variation; IVGTT, intravenous glucose tolerance test; S_G, glucose effectiveness; S_G^{BAY}, Bayesian estimate of S_G; S_G^{NLR}, non-linear regression analysis estimate of S_G; S_I, insulin sensitivity; S_I^{BAY}, Bayesian estimate of S_I; S_I^{NLR}, non-linear regression analysis estimate of S_I; V, volume of distribution.

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is too low. Nevertheless, it has been documented [3] that the minimal model fails in up to 50 % of cases in highly insulin-resistant subjects, although, with insulin modification and careful data analysis, the failure rate is normally approx. 10 %. Godsland and Walton [4] reported even higher failure rates with a high-glucose dose (0.5 g/kg), but without modification, and showed the importance of basal glucose levels to improve the success rate.

The standard minimal model data analysis employs the non-linear regression technique to obtain estimates of S_I and S_G [5]. Alternative calculation techniques have become available recently, specifically the Bayesian analysis with the so-called Markov chain Monte Carlo computational strategies [6]. These have been adopted to estimate S_I and S_G by analysing data on an individual basis [7,8], with the aim of reducing minimal model failures and providing physiologically plausible confidence intervals [CIs; credible intervals (CrIs) within the context of Bayesian analysis] of the estimates.

Recently, a population-based approach [9], an iterative two-stage technique, has been investigated with reduced sampling and has been shown to improve precision compared with the standard non-linear regression approach. The strength of the population-based estimation techniques is that the knowledge about the underlying population distribution (usually normal or log-normal) can be employed in the estimation process, bringing about an improvement in the estimates of population and individual characteristics.

The present study extends these recent advances and reports on a Bayesian hierarchical analysis of the minimal model data. The combination of Bayesian methodology and hierarchical analysis (see Appendix for comments on Bayesian and hierarchical analyses) promises to be suitable to reduce/avoid minimal model failures and to extract correctly, and in full, all information, such as inter-subject variability, from the experimental data. The use of population analysis is also reported for the first time for subjects with Type II diabetes.

METHODS

Subjects and experimental protocol

Subjects with newly presenting Type II diabetes according to World Health Organization criteria participated in the study [$n = 65$; 53 males, 12 females; age 54 ± 9 (33–71) years; body mass index 30.4 ± 5.2 (20.9–43.4) kg/m^2; mean $\pm$ SD (range)]. The study was approved by Bro Taf Local Research Ethics Committee, Cardiff, U.K., and all subjects gave written informed consent.

The subjects were admitted to the Diabetes Research Unit, Llandough Hospital, Penarth, U.K. following an overnight 12 h fast and underwent IVGTT consisting of a 0.3 g of glucose bolus/kg of body weight given at time zero over 2 min, followed by 0.05 unit of insulin/kg of body weight (Actrapid; Novo Nordisk, Denmark) at 20 min. Blood was taken via an indwelling intravenous cannula, which was inserted into the antecubital fossa vein and connected via a three-way tap to a slow running saline infusion to maintain the patency of the vein. Samples were taken at -30, -15, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 min for measurement of plasma glucose and insulin.

Assays

Glucose was assayed using glucose oxidase method [YSI 2300, Yellow Springs Analyzer; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.; intra-assay coefficient of variation (CV) < 2 %]. Insulin was assayed using immunoassay utilizing monoclonal antibodies (Dako Diagnostics, Ely, Cambs., U.K.; intra-assay CV < 5 %).

Data analysis

Minimal model of glucose tolerance during IVGTT

The minimal model defines S_I (ability of insulin to enhance the net glucose disappearance from plasma) and individual S_G (ability of glucose to promote its own disposal) [10,11] and is described by two differential equations

$$dg_t/dt = -(p_1 + x_t) \cdot g_t + p_1 \cdot gb \qquad g_0 = D/V \quad (1)$$

$$dx_t/dt = -p_2 \cdot x_t + p_3 \cdot (i_t - i_b) \qquad x_0 = 0 \quad (2)$$

where g_t is the plasma concentration of glucose, i_t is the plasma insulin concentration, x_t is a variable associated with the remote insulin compartment, g_b is the (end-experimental) basal glucose concentration, i_b is the (end-experimental) basal insulin concentration, D is the amount of exogenous glucose injected at time 0, p_1, p_2 and p_3 are model parameters and V is the volume of the distribution. S_I is given as the ratio of p_3/p_2, and S_G is given by p_1. Glucose concentrations from 0–5 min were excluded from the parameter estimation with all parameter estimation approaches.

Bayesian hierarchical analysis

An illustrative outline of the Bayesian analysis is shown in Figure 1. The analysis included the development of a hierarchical model with individual and population parameters.

Within the context of the Bayesian analysis, prior distributions of parameters were specified. We adopted 'vague' (non-informative) prior distributions representing the lack of prior information about parameter values. The prior distributions were 'updated' from glucose and insulin measurements adopting the Bayes theorem giving the individual posterior distributions. These posterior distributions 'correspond' to point estimates derived

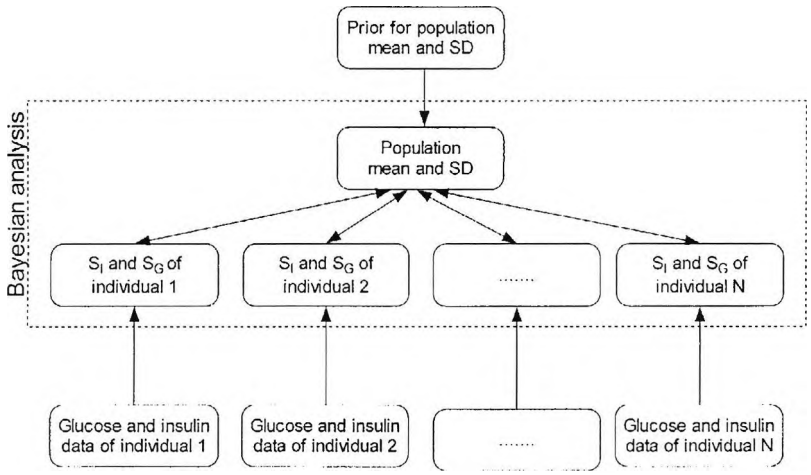

Figure 1 Bayesian hierarchical analysis of the minimal model indices

Individual S_I and S_G values were estimated in parallel with the population characteristics (the mean and S.D.). All indices were treated as random variables and the estimation involves determining their probability density functions. Log-normal population distribution is assumed to reflect that S_I and S_G can attain only non-negative values. The Bayesian hierarchical analysis facilitates 'information' flow between individuals (individual estimate depends on the measured glucose and insulin data and also on the population mean and S.D., which is, in turn, influenced by other individual values) and also results in estimates with higher precision contributing with a greater weight to the population characteristics.

by the non-linear regression analysis in the sense that they contain the result of the estimation procedure [12]. However, the posterior distributions are information-rich and can be used, for example, to determine directly the precision of the estimate using a measure of dispersion, such as the 95 % CrI or the CV of the posterior distribution.

The Bayesian analysis demands the form of underlying distributions to be specified. We assumed that individual parameters such as S_I and S_G are log-normally distributed, guaranteeing their non-negativity, and thus the physiological plausibility of associated 95 % CrI. We denote S_I^{BAY} and S_G^{BAY} as Bayesian estimates of S_I and S_G respectively.

Individual estimates of S_I^{BAY} and S_G^{BAY} were calculated as medians of the posterior distributions and the precision of the estimates as the CV of the posterior distributions. The median was chosen as it is a more robust measure than the mean (subsequent analysis indicated, however, that median and mean of individual estimates are nearly identical). Owing to the nature of the Bayesian analysis, population parameters (mean, CrI and interquartile range) are not obtained by statistical evaluations of individual results, but rather as an integral part of the Bayesian analysis. This reflects that individual parameters estimated with higher precision contribute with 'greater' weight to the population characteristics.

For the calculations, we employed the public domain WinBUGS program [13] extended by a purpose-made module implementing the numerical solution of eqns (1) and (2). The WinBUGS program adopted the Metropolis–Hastings algorithm [14] to calculate a single chain with 26 000 samples (with thinning of four), from which the first 6000 samples were discarded and the remaining 20 000 samples were used in a further analysis. Convergence criteria of the chain were tested using the Geweke method and the Raftery–Lewis method implemented in the CODA package [15]. The calculations were performed on a computer running the Microsoft Windows NT operating system with 512 MB RAM and a single 650 MHz Pentium processor. The generation of the chain with 26 000 samples took approx. 12 h.

Further details about the Bayesian hierarchical analysis are given in the Appendix.

Standard two-stage minimal model analysis

The two-stage minimal model analysis is the traditional method to determine population values. In the first stage, a weighted non-linear regression analysis was employed to estimate the model parameters and, specifically, S_I and S_G were denoted S_I^{NLR} and S_G^{NLR} respectively. The weight was defined as the reciprocal of the variance of the measurement error [16]. The CV of the measurement error of unlabelled glucose was assumed at the level of 1.5 %. The parameter estimation procedure provided the precision of a parameter estimate expressed as the CV of the parameter estimate from the Fisher information matrix [16].

In the second stage, the population (geometric) mean and 95 % CI of S_I^{NLR} and S_G^{NLR} was calculated.

Statistical analysis

Values are reported as means (95 % CI for two-stage analysis or CrI for the Bayesian hierarchical analysis).

The results of the Bayesian analysis are reported as medians of the posterior density functions, reflecting that medians are more robust than means. SPSS for Windows V9.0 (SPSS Inc., Chicago, IL, U.S.A.) was used to carry out statistical calculations associated with the two-stage analysis. S_I and S_G estimated by the two methods were correlated using Spearman correlation (r_s) to account for skewed distributions. The correlation coefficients for 20:80 % centiles of S_I were compared using Fisher z transformation [17].

RESULTS

Plasma glucose and plasma insulin

Plasma insulin and glucose data are shown in Figure 2.

Bayesian hierarchical analysis

The Bayesian analysis provided estimates of S_I^{BAY} and S_G^{BAY} in all subjects with good precision [16 % (3–59 %) and 14 % (7–18 %) respectively; median (range)]. Precision of individual estimates of p_2^{BAY} and V^{BAY} was also good [19 % (8 %–41 %) and 4 % (3 %–7 %)]. Population characteristics of S_I^{BAY}, S_G^{BAY}, p_2^{BAY} and V^{BAY} are given in Table 1.

The posterior density of the population mean of S_I^{BAY} and S_G^{BAY} is shown in Figure 3. The posterior density of individual values of S_I^{BAY} and S_G^{BAY} is also shown (obtained by sampling from the population posterior distribution, see Appendix for details).

The population mean densities in Figure 3 (thick lines) are narrow and nearly symmetrical. This means that the population mean for the two parameters is well defined and its CrIs are symmetrical around the mean. The individual density of S_I in Figure 3 (upper panel, thin line) is skewed to the right, specifying that individuals with newly presenting Type II diabetes have very low S_I, with a most likely value (the mode) of 1.2×10^{-5} min^{-1} · pmol^{-1} · l^{-1}, but that there is also a non-negligible number of individuals with 10 (and more)-fold higher S_I. Individual values of S_G are nearly symmetrically distributed around the most likely value of approx. 1.5×10^{-2} min^{-1},

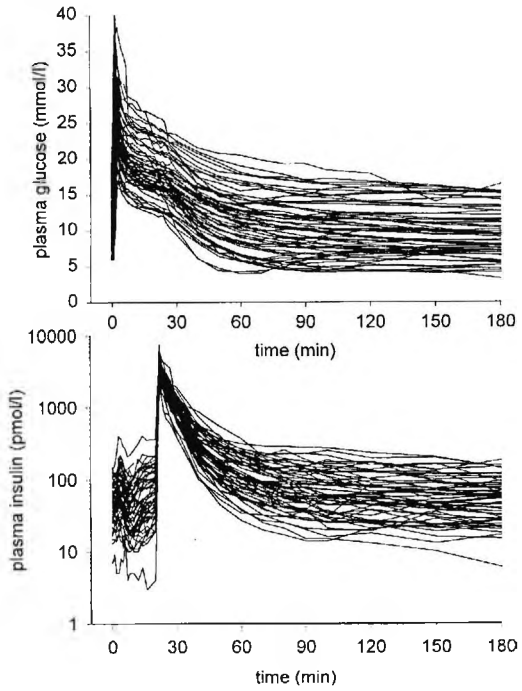

Figure 2 Plasma glucose (upper panel) and plasma insulin (lower panel) during insulin modified IVGTT in newly presenting Type II diabetes

Individual profiles are plotted ($n = 65$).

Table 1 Population characteristics of the minimal model with the Bayesian hierarchical analysis (S_I^{BAY}, S_G^{BAY}, p_2^{BAY} and V^{BAY}) and the standard two-stage analysis (S_I^{NLR}, S_G^{NLR}, p_2^{NLR} and V^{NLR}) during the insulin-modified IVGTT in subjects with Type II diabetes ($n = 65$)

*For the Bayesian hierarchical analysis characteristics (S_I^{BAY}, S_G^{BAY}, p_2^{BAY} and V^{BAY}), the interval is the CrI, and for the standard two-stage analysis (S_I^{NLR}, S_G^{NLR}, p_2^{NLR} and V^{NLR}) the interval is the CI. ‡Calculated using a sampling approach: the posterior distribution of the population parameters was used to generate a sample containing 20 000 individual S_I and S_G (see Appendix for details). V^{BAY} and V^{NLR}, Bayesian and non-linear regression analysis estimate of V respectively.

Population characteristic	Mean (95 % interval)*	Interquartile range
S_I^{BAY} ($\times 10^{-5}$ · min^{-1} · pmol^{-1} · l^{-1})	1.07 (0.82–1.36)	1.43‡
S_I^{NLR} ($\times 10^{-5}$ · min^{-1} · pmol^{-1} · l^{-1})	1.23 (0.97–1.56)	1.84
S_G^{BAY} ($\times 10^{-2}$ · min^{-1})	1.53 (1.41–1.64)	0.46‡
S_G^{NLR} ($\times 10^{-2}$ · min^{-1})	1.45 (1.32–1.59)	0.53
p_2^{BAY} ($\times 10^{-2}$ · min^{-1})	5.35 (4.58–6.13)	3.43‡
p_2^{NLR} ($\times 10^{-2}$ · min^{-1})	5.71 (4.87–6.68)	3.82
V^{BAY} ($\times 10^{-2}$ · l^{-1} · kg^{-1})	13.8 (13.4–14.3)	3.3‡
V^{NLR} ($\times 10^{-2}$ · l^{-1} · kg^{-1})	14.0 (13.6–14.5)	3.3

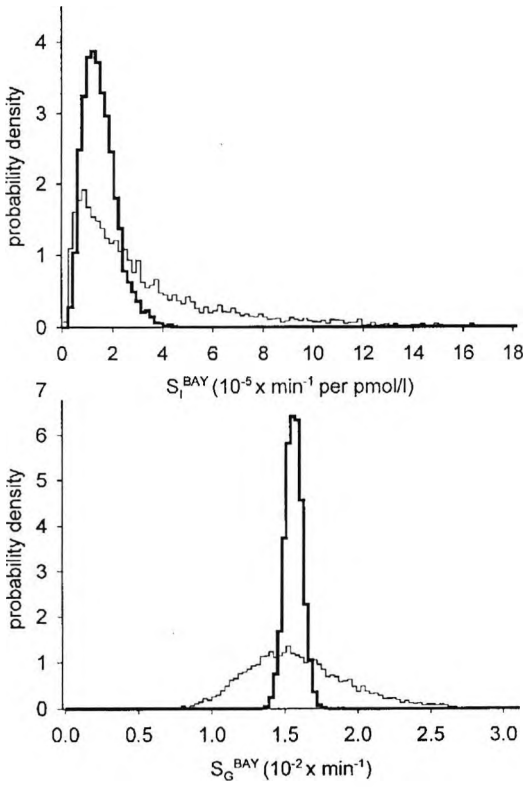

Figure 3 Posterior density of the population mean (thick line) and the posterior density of individual values (thin line) for S_I (upper panel) and S_G (lower panel) estimated with the Bayesian hierarchical analysis

See text for details.

with a small inter-subject variability (nearly no subjects below 0.7×10^{-2} min^{-1} or above 2.7×10^{-2} min^{-1}).

Standard two-stage analysis

The non-linear regression analysis successfully estimated S_I^{NLR} in all but four subjects (a 6 % failure rate) when the CV of the parameter estimate was $\gg 150$ %. The precision of the remaining S_I^{NLR} estimates was acceptable [12 % (4–127 %); median (range)]. Estimates of S_G^{NLR} were obtained in all subjects with an acceptable precision [15 % (5–104 %)]. Precision of individual estimates of p_2^{NLR} was satisfactory [21 % (7–211 %)]. Precision of individual estimates of V^{NLR} was very good [2 % (1–10 %)]. Population characteristics of S_I^{NLR}, S_G^{NLR}, p_2^{NLR}, and V^{NLR} are given in Table 1.

Comparison of the standard two-stage and Bayesian hierarchical analysis

The non-linear regression analysis failed to estimate S_I with precision in subjects #5, #6, #31 and #44, whereas the Bayesian hierarchical analysis returned S_I in these four subjects with acceptable precision (range of CV 46–59 %). These four S_I values were in the lower quartile and were 1st, 4th, 9th and 14th lowest among the studied group at 0.21, 0.28, 0.39, and 0.50×10^{-5} min^{-1} · pmol^{-1} · l^{-1}. Their posterior density function is shown in Figure 4. Subjects #5, #6 and #44 had the lowest, and subject #31 had the 7th lowest precision, as determined by the Bayesian hierarchical analysis (59 %, 58 %, 53 % and 46 % respectively). Otherwise, there was no apparent difference in the shape of the posterior density functions, which would provide a further insight into the failure of the non-linear regression analysis. This suggests that the non-linear regression analysis tends to fail in subjects with low and poorly defined S_I.

Individual S_I values estimated by the two methods were highly correlated ($r_S = 0.98$; $P < 0.001$). However, the correlation in the lower 20 % centile of the S_I range was significantly lower than the correlation in the upper 80 % centile ($r_S = 0.71$ compared with $r_S = 0.99$; $P < 0.001$), supporting further the notion that the non-linear regression analysis has difficulties at low-insulin sensitivities. The non-linear regression tended to provide slightly higher S_I estimates, as shown in Figure 5. The difference was not considered clinically significant. The inset in the upper panel of Figure 5 highlights the comparison at lower values of S_I.

The precision of individual S_I estimates provided by the two methods was highly correlated ($r_S = 0.82$; $P < 0.001$) and was similar in extent, although the Bayesian hierarchical analysis gave a tighter range. This is most probably due to the hierarchical nature of the analysis, i.e. borrowing of strength across individuals.

A different picture emerged when considering S_G. Estimates provided by the two methods were still highly correlated ($r_S = 0.77$; $P < 0.001$), but were not proportional (the unity line is different from a projected regression line; Figure 5). The non-linear regression gave a wider range of S_G. There were large differences in several subjects and these were generally in estimates with low precision, as returned by the non-linear regression analysis (Figure 5).

The precision of S_G was not correlated between the two methods ($r_S = 0.12$; $P =$ not significant). The Bayesian hierarchical analysis returned estimates with identical precision as judged by the median, but with a tighter dispersion (2 % compared with 16 % S.D. of the precision), explained again by the ability of the Bayesian hierarchical analysis to borrow of strength across individuals.

The comparison of population characteristics is shown in Table 1. In the case of the Bayesian analysis, the mean and CrI were directly extracted from the posterior density of the population mean and the interquartile range was obtained from a simulated posterior distribution of an individual parameter (Figure 3). In the case of the non-linear regression analysis, the characteristics correspond to the log-normal distribution of the parameter. There was no statistical difference between the two methods as demonstrated by overlapping CIs and CrIs. However,

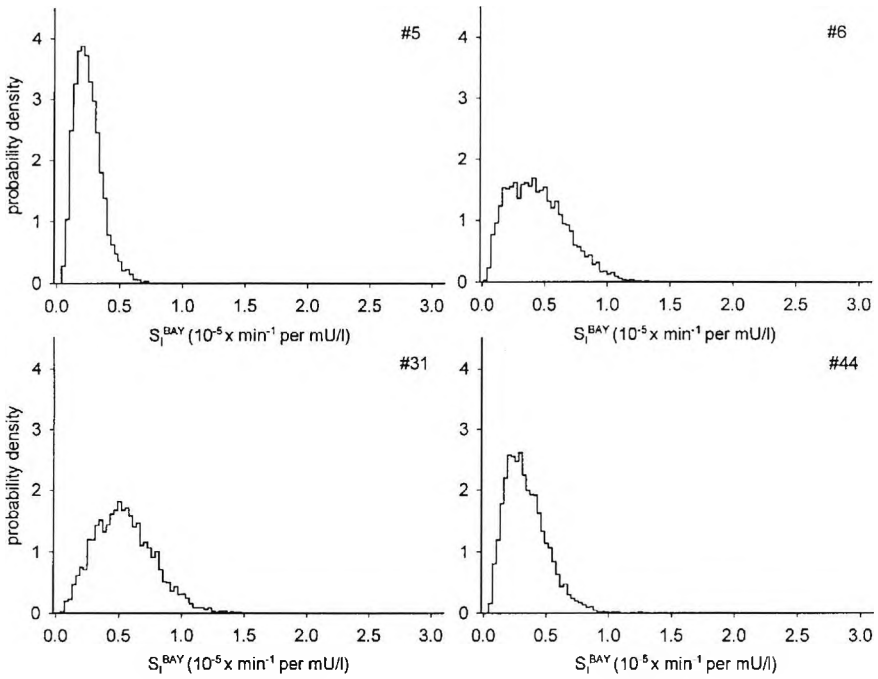

Figure 4 Posterior density function of individual S_I derived by Bayesian hierarchical analysis in subjects where non-linear regression analysis failed

Subjects #5, #6, #31 and #44 were used.

the Bayesian hierarchical analysis gave tighter estimates, as indicated by a smaller interquartile range for both S_I and S_G by 20 % and 15 % respectively.

DISCUSSION

The present study demonstrates that the Bayesian hierarchical analysis is an appealing method to estimate population and individual S_I and S_G with the minimal model analysis of IVGTT. The appeal is due to the reliability of the parameter estimation process (100 % success rate) underpinned by coherent theoretical foundations of the methodology.

The Bayesian hierarchical analysis avoids minimal model failures, which occur in subjects that combine low S_I and low precision of its estimate, as shown in the present study. We experienced a modest failure rate of 6 % with the use of insulin modification and the standard non-linear regression analysis. The failure rate is expected to increase with the use of protocols without insulin or tolbutamide modification, as the insulin stimulus is invariably smaller and inadequate to estimate S_I in the insulin-resistant state. In such studies, the Bayesian hierarchical analysis will have a greater impact and will avoid a potential bias toward a higher estimate of population S_I (this bias was not statistically significant in the present study).

An independent estimate of S_I is not available. Ideally, our present results should be confirmed by, for example, clamp studies. However, the results are consistent as the failures occur in subjects with low sensitivity. The reliability of the Bayesian hierarchical analysis is inferred from an excellent correlation with the non-linear regression analysis.

The Bayesian hierarchical approach is computationally demanding and cumbersome to deal with changes in the data set, i.e. adding a new subject or eliminating an outlier requires a completely new run. This should not be a problem in laboratory (non-epidemiological) studies due to shorter runs, but particular care needs to be exercised when dealing with data sets of our size or larger. In this situation, it becomes essential to prepare data well to avoid wasteful long runs.

The individual Bayesian method [8] is an alternative approach, which is more flexible (but overall the computation complexity is similar) than the Bayesian population approach and avoids 'failures' using a 'somewhat' informative prior distribution. The Bayesian individual analysis specifies that S_I above a certain fixed threshold is less probable. The Bayesian population analysis avoids this assumption. The distribution of S_I within the studied population co-determines S_I within a particular individual. We believe that the latter is more appropriate as it exploits information present in a particular subject group.

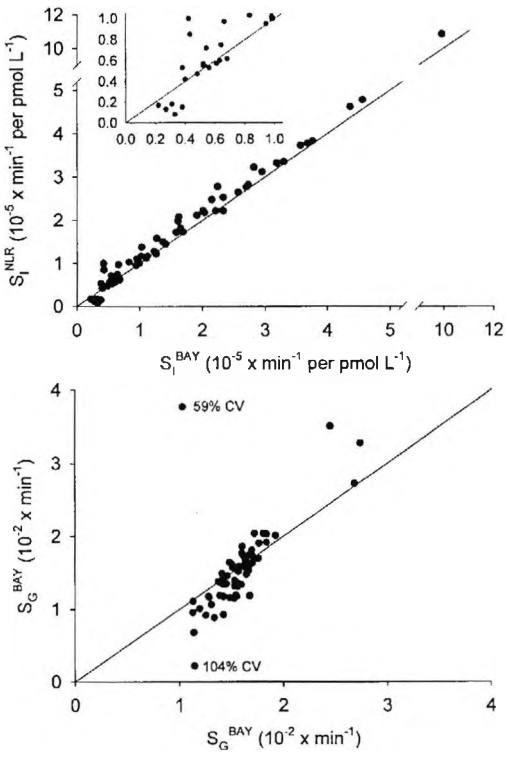

Figure 5 Comparison of the minimal model indices determined by the Bayesian hierarchical analysis (S_I^{BAY} and S_G^{BAY}) and the non-linear regression analysis (S_I^{NLR} and S_G^{NLR}) in subjects with newly presenting Type II diabetes

Upper panel, comparison of the S_I estimates. Lower panel, comparison of the S_G estimates. Two S_G^{NLR} estimates with lowest precision (CV of the estimate given in the lower panel) differed most from their corresponding S_G^{BAY} values. Solid lines represent the unity slope. The inset in the upper panel highlights the comparison at lower values of S_I. $n = 65$.

The Bayesian hierarchical analysis adopts two sets of assumptions. First, the type of the underlying distributions needs to be chosen. Secondly, prior distributions have to be specified. The type of underlying distributions for parameters, i.e. the log-normal distribution, adopted by the present study is both compatible with results of the standard two-stage analysis (results not shown) and physiologically feasible. It provides positive estimates of the parameters and also positive CrIs. We adopted a vague non-informative prior distribution, which was sufficient to obtain well-defined population and individual posteriors. This contrasts with the Bayesian 'individual' analysis [8], which required a somewhat informative prior distribution for S_I to avoid large CrIs associated with S_I, suggesting that additional information has to be provided for the individual Bayesian analysis to compensate for the lack of information about the underlying population characteristics.

The Bayesian hierarchical analysis is 'expensive' in terms of the computational time. The calculations took approx. 12 h on a mid-specification computer. This compares less favourably with the non-linear regression analysis with several seconds needed to run a single subject. However, compared with the overall time-scale of epidemiological studies, which normally take months to complete, the increased computational time is negligible. The appeal of the Bayesian approach originates from its ability to provide theoretically coherent plausible individual, population and precision estimates. However, care needs to be taken during data preparation to avoid wasteful runs.

An alternative, computationally faster, population-based approach is the iterative two-stage analysis, which has been shown to improve precision of minimal model parameters with reduced sampling using unmodified IVGTT in healthy subjects [9]. The method is computationally attractive and implements an empirical Bayesian hierarchical estimator. A full comparison using a common data set of the Bayesian hierarchical analysis and the iterative two-stage method is warranted to explore whether simplifications made by the latter method influence results at low S_I as observed with the standard two-stage analysis.

Our analysis of a mid-size group of newly presenting Type II diabetes subjects gave a modest 6 % failure rate of the non-linear regression analysis. This small failure rate could be attributed to frequent sampling, a relatively high dose of insulin (0.05 unit/kg of body weight) and a high accuracy of analytical techniques. The failures occurred in subjects with very low S_I. Any relaxation of the conditions given above is likely to give a higher failure rate, most likely in subjects with low S_I, distorting the population characteristics.

We have evaluated further the Bayesian hierarchical analysis with a small number of subjects when the population distribution is likely to be estimated with a lower confidence. We have carried out an analysis on a subset containing nine subjects, including two subjects failing S_I estimation, with the non-linear regression analysis. The results demonstrated that Bayesian hierarchical analysis is suitable for smaller subject groups too. All nine subjects provided well-defined S_G estimates (CV of parameter estimates < 25 %) and acceptable S_I estimates (CV < 90 %; higher CV values for lower S_I). Individual S_I estimates were highly correlated among the full set and the subset ($r = 0.99$; $P < 0.001$) without introducing bias ($P =$ not significant; paired Student's t test). A similarly high correlation ($r = 0.98$; $P < 0.001$) and a lack of bias ($P =$ not significant) were observed for S_G.

Population analysis with the minimal model of the tolbutamide-modified IVGTT has been reported using the non-linear mixed effect model implemented within the NONMEM package for healthy subjects [18]. The authors reported improved precision of population estimates compatible with our present results, but the population variability was very similar between the standard

two-stage analysis and the NONMEM results. The authors [18] did not report NONMEM-derived individual estimates of S_I and S_G, although these can be in principle obtained with the so-called 'post-hoc' option.

Population kinetics methods have become essential to study drug pharmacokinetics. The knowledge of population variability facilitates the design of efficacious and safe dosing schemes. The size of population studies normally prevents a complex experimental set-up and only limited number of samples per subject can be taken; however, this does not prevent the use of population approaches.

The physiological modelling field is following the trend. The main appeal of the present application is due to the avoidance of estimation failures. However, as the computational and conceptual complexity of Bayesian methods is considerable, the development of more user-friendly tools is needed to support wider deployment.

The simplest methods to assess S_I, such as HOMA [19] and QUICKI [20], rely on fasting glucose and insulin. These techniques estimate so-called basal S_I, whereas most techniques measure stimulated S_I [21]. Basal S_I primarily reflects sensitivity of endogenous glucose production, whereas stimulated sensitivity also contains the sensitivity of glucose disposal [22]. When stimulated S_I is of interest, the Bayesian hierarchical analysis is a method to be considered, especially in insulin-resistant subjects with or without Type II diabetes.

In conclusion, Bayesian hierarchical analysis is an appealing method to estimate population and individual S_I and S_G with the minimal model of the insulin-modified IVGTT. The method avoids parameter estimation failures and gives a smaller unbiased estimate of the population dispersion for both S_I and S_G.

APPENDIX

Comments on Bayesian analysis

The Bayesian analysis is underpinned by a formula derived from the Bayes theorem

$$p(P \mid c) \propto p(c \mid P)p(P) \tag{3}$$

which states that the probability $p(P \mid c)$ of parameters P given measurements c is proportional to the product of the so-called likelihood $p(c \mid P)$, and the prior probability of P, $p(P)$. In our case, c represents a vector of plasma glucose measurements, and P represents a vector of parameters. The vector P can be conveniently divided into three components $P = \{\xi, \mu, \Sigma\}$: ξ contains individual parameters (e.g. individual S_I and S_G), μ contains population means (e.g. mean S_I and mean S_G) and Σ contains population variance–covariance (e.g. the intersubject variability of S_I and S_G).

The probability $p(P \mid c)$ represents the posterior probability as it denotes probability of P after we observed c. Assuming, for the sake of simplicity, that P includes just one parameter, say S_I for one subject, $p(P \mid c)$ defines a probability density function, which assigns probability for all possible values of S_I.

The calculation of posterior probability is the objective of the Bayesian analysis. It should be stressed that the posterior probability is 'richer' than the estimate provided by the non-linear regression analysis. For example, the Bayesian analysis returns a value (probability) for each value of S_I, whereas non-linear regression analysis returns just one value, the point estimate of S_I. The Bayesian analysis enables the assessment of multimodal solutions (there might be two or more parameter values that provide a good fit to the data).

Another method, the maximum a posteriori Bayesian analysis works in a similar way to the non-linear regression analysis and returns a value at which the posterior probability attains its maximum value.

The Bayesian analysis requires the provision of the prior probability $p(P)$, i.e. probability of P prior to having any observation. Two conceptual types of prior probability can be considered, either informed or vague. The former specifies that certain values are more likely than others, the latter that all values occur with a similar probability. The property of the former is that we bring into the calculations our informed prior knowledge, but the drawback is that the prior knowledge might be too strong and influence the results, possibly too much. The latter avoids the use of informed knowledge (even if available) and makes the Bayesian analysis driven by the observations.

Comments on hierarchical analysis

As described above, the vector P includes individual parameters (ξ) and population parameters (μ and Σ). Mixing up individual and population parameters contributes to the difficulties with the specification and solution of Bayesian problems.

The hierarchical analysis separates levels and simplifies the specification of the problem and the calculation of the solution. The first level describes how observations are obtained from individual parameters, i.e. the likelihood $p(c \mid \xi)$ is defined from the minimal model equations. The second stage describes how individual parameters are obtained (drawn from) population parameters, i.e. the likelihood $p(\xi \mid \mu, \Sigma)$ is defined using the equation representing the chosen population probability distribution such as the log-normal distribution. The third and final stage describes how the population parameters are drawn from prior distributions.

Technical details of the Bayesian hierarchical analysis of the minimal model

A Bayesian framework for modelling the time-varying glucose profile during IVGTT and inter-individual variability requires a three-stage hierarchical model. At

the first stage, glucose values g_{ij} in subject i at time t_j were obtained as the solution to eqns 1 and 2

$$g_{ij} = g \cdot (t_j, p_{1i}, p_{2i}, S_{Ii}, V_i) \cdot (1 + \varepsilon_{ij}) \quad (4)$$

where p_{1i}, p_{2i} S_{Ii} and V_i are parameters of subject i, ε_{ij} is the random term representing the multiplicative measurement error and the model specification error and other unaccounted variability. The random term ε_{ij} is drawn from a normal distribution with a zero mean and an unknown variance σ^2. Our earlier investigations (results not shown) suggested that reparameterization with p_{1i}, p_{2i}, S_{Ii} and V_i is preferable (converges faster) to the original parameterization p_{1i}, p_{2i}, p_{3i} and V_i, where $S_{Ii} = p_{3i}/p_{2i}$.

The second stage is characterized by making assumptions about individual parameters. In particular, we assumed that the individual parameters are drawn from a multivariate log-normal distribution guaranteeing non-negativity of parameters

$$(p_{1i}, p_{2i}, S_{Ii}, g_{0i}) \sim \text{LNORMAL} \cdot (\boldsymbol{\mu}, \boldsymbol{\Sigma}) \quad (5)$$

where $\boldsymbol{\mu}$ is an unknown population mean vector, $\boldsymbol{\Sigma}$ is an unknown covariance matrix and LNORMAL is the log-normal distribution.

At the third stage, prior distributions for population parameters $\boldsymbol{\mu}$, $\boldsymbol{\Sigma}$, and σ^2 were specified. These prior distributions were vague representing 'lack' of prior knowledge

$$\begin{aligned} \boldsymbol{\mu} &\sim Normal\left[0, \begin{pmatrix} 10^6 & 0 & 0 & 0 \\ 0 & 10^6 & 0 & 0 \\ 0 & 0 & 10^6 & 0 \\ 0 & 0 & 0 & 10^6 \end{pmatrix}\right] \\ \boldsymbol{\Sigma} &\sim Wishart\left[4, \begin{pmatrix} 50 & 0 & 0 & 0 \\ 0 & 50 & 0 & 0 \\ 0 & 0 & 50 & 0 \\ 0 & 0 & 0 & 50 \end{pmatrix}\right] \quad (6) \\ \sigma^{-2} &\sim Gamma(0.001, 0.001) \end{aligned}$$

These prior distributions specify virtually 'flat' distributions, i.e. they indicate that all values occur with nearly the same probability. In principle, informative prior distributions could be used as there is a wealth of information about parameters of the minimal model in various populations. However, in the present study, we limited the use of such information in order to allow the experimental data to drive the estimation process, although information is contained in the form of chosen distribution, e.g. log-normal for individual parameters.

A general discussion about the form of vague prior distribution can be found in the literature, for example Gamerman [23].

Eqn (6) implements the common assumption that population parameters are not correlated, but allows the posterior estimates to demonstrate correlation.

The main purpose of the Bayesian inference [24] is to determine the posterior probability of unknown quantities such as individual parameters p_{1i}, p_{2i}, S_{Ii} and V_i and population parameters $\boldsymbol{\mu}$, $\boldsymbol{\Sigma}$ and σ^2. For our model, this cannot be achieved by direct (analytical) computation. Instead, sampling techniques such as the Markov chain Monte Carlo method [6] have to be used. These sampling techniques provide a large sample of the posterior distributions normally running into thousands to tens of thousands of samples.

The posterior distributions were summarized by the median, the mean and 95 % CrI respectively.

The posterior distribution of the parameters $\boldsymbol{\mu}$ and $\boldsymbol{\Sigma}$ was used to generate 20 000 samples of individual parameters, which determined the individual posterior distributions (thin line) in Figure 3.

ACKNOWLEDGMENTS

O. F. A. was supported by the Engineering and Physical Sciences Research Council (EPSRC) U.K. (CASE studentship).

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Received 27 March 2003/2 June 2003; accepted 6 June 2003
Published as Immediate Publication 6 June 2003, DOI 10.1042/CS20030117