MODELLING METHODOLOGIES TO ASSESS GLUCOSE METABOLISM IN TYPE 2 DIABETES

by

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TWO JOURNAL ARTICLES AT THE END OF THIS THESIS HAVE NOT BEEN DIGITISED BY REQUEST OF THE UNIVERSITY

Abstract

The aetiology and pathogenesis of type 2 diabetes (T2D) are yet to be fully understood. However, there is a degree of agreement that the most important pathological factors of T2D are β-cell dysfunction and insulin resistance. Moreover T2D is characterised by varying degrees of impaired pancreatic β-cell responsiveness and/or insulin resistance. The ability to easily quantify insulin sensitivity is useful for investigating the role of impaired insulin secretion and action in the pathophysiology of T2D.

The current work provided novel knowledge in both clinical and methodological areas. On the clinical side, it provided new information about pathology of T2D. On the methodological side, it assessed validity, performance, and/or reproducibility of two powerful models to assess insulin responsiveness and sensitivity.

The aim was to use modelling techniques employing data collected during tolerance tests to progress our understanding of pathology of type 2 diabetes. This research evaluated and/or validated two approaches namely, the insulin secretion model and the minimal model, respectively, to assess pancreatic β-cell responsiveness and insulin sensitivity. These methods were then applied to study the aetiology and pathology of T2D on its first clinical appearance (newly diagnosed subjects).

The insulin secretion model was assessed with two reduced sampling schemes. The model was validated during the oral glucose tolerance test (OGTT), and its performance was compared during OGTT and the meal tolerance test (MTT). The reproducibility of the model indices was also assessed during MTT and OGTT. The one and two compartmental minimal model performance was evaluated and compared to the clamp in subjects with T2D. The insulin secretion model and the one compartment minimal model were then applied to study newly presenting T2D in order to gain more understanding of the disease pathology. The output results showed the ability of these approaches to explain the inter-individual variability of important glucose clinical measures such as FPG and HbA_{1C}.

In conclusion the insulin secretion model and the one compartment minimal model demonstrated their validity and utility in assessing insulin sensitivity and β-cell responsiveness to provide better understanding of type 2 diabetes.

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Declaration

I hereby declare that as the author of this document, that all the analysis, results, etc., described throughout the document were performed by myself. Assistance by other persons has been acknowledged, and all previous published work has been identified and acknowledged

This work is original and has not been submitted for any other degree.

A. Albarrak

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Abbreviations

Following is a list of the most commonly used abbreviations, however other abbreviations have been used and are defined in the text where appropriate.

T2D	Type 2 diabetes
ISM	Insulin secretion model
MTT	Meal tolerance test
OGTT	Oral glucose tolerance test
BMI	Body mass index (kg/m^2)
IV	Intravenous
IVGTT	Intravenous glucose tolerance test
FPG	Fasting plasma glucose (mmol/L)
FPI	Fasting plasma insulin (pmol/L)
FPC	Fasting plasma C-peptide (nmol/l)
ICMM	One compartment minimal model
2CMM	Two compartment minimal model
HbA_{1C}	Glycated haemoglobin (%)
$C_{max,G}$	Maximum (above fasting) plasma glucose during MTT (mmol/L)
$C_{max,I}$	Maximum (above fasting) plasma insulin during MTT (pmol/L)
AUC	Incremental (above fasting) area under curve during MTT
S_I	Insulin sensitivity (1/min per pmol/L)
S_G	Glucose effectiveness (IVGTT-derived) (1/min)
M_1	Postprandial β-cell sensitivity (MTT-derived) (1/min)
M_0	Fasting β-cell sensitivity (MTT-derived) (1/min)
AIR_G	First phase insulin response during IVGTT (pmol/L per 6 min)
D_I	Disposition index ($D_I = S_I * AIR_G$) (IVGTT-derived) (1/min per 6 min)

1. Introduction

1.1. Overview

Diabetes mellitus is a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The persistent hyperglycaemia of diabetes is associated with long-term complications, and dysfunction of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The estimated global prevalence of diabetes among adults was 7.4% in 1995 and is expected to rise to 9% by 2025 (1). However, specific populations and subgroups have a much higher prevalence of the disease. These subgroups have certain attributes or risk factors that either directly cause diabetes or are associated with it.

In the United Kingdom the estimated number of diabetics in year 2000 was about 1.5 million (2--3%) (2). In Manchester, the people with prevalence of known and new type 2 diabetes, detected by oral glucose tolerance test, was 20% in Europeans, 22% in Afro-Caribbeans, and 33% in Pakistanis (3). These findings provide an idea about the potential size of the diabetes epidemic facing the United Kingdom. The epidemic will have major implications for the NHS, which will have to provide diabetic services for these patients, as a substantial proportion of the total health care budget is spent on the care of diabetic patients as well as to deal with the clinical and psychosocial complications resulting from diabetes.

Type 2 diabetes (T2D) is the most common metabolic disease in the world and is one of the most common chronic diseases. It accounts for about 85--95 % among all diabetes (2). It is associated with a number of complications, such as nephropathy, retinopathy, arteriosclerotic heart disease, and peripheral neuropathy, which most often result from the prolonged exposure to hyperglycaemia.

Type 2 diabetes mellitus is a heterogeneous syndrome resulting from a combination of insulin resistance and β-cell dysfunction. In addition, it is characterised by insulin resistance and impaired pancreatic responsiveness (4). Although insulin resistance may not be required for the development of T2D (2, 3), defects in insulin sensitivity and β-cell function have been demonstrated in most subjects with type 2 diabetes. In

addition, insulin resistance is associated with a number of other diseases, including obesity, hypertension, dyslipidaemias, and coronary artery disease (5; 6).

The assessment of insulin sensitivity and β-cell function is essential to investigate the pathophysiology and epidemiology of T2D and to follow the clinical course of patients on various therapeutic regimens. In addition, the ability to easily quantify insulin sensitivity in large numbers of subjects will be useful for investigating the role of impaired insulin secretion and action in the pathophysiology of these major public health problems.

1.2. Thesis hypothesis

The aetiology and pathophysiology of T2D are yet to be fully understood and have not been fully characterised (7). It has been generally accepted that both insulin resistance and deficient β-cell function are the primary cause for the development of T2D mellitus (8). However a considerable uncertainty exists about the sequence and the nature of the earliest biochemical changes and their relative contributions to the deterioration in glucose tolerance and development of T2D.

The generally accepted but as yet not confirmed hypothesis is that the glucose tolerance tests in a large group of newly diagnosed patients provide a unique insight into the dynamic of the glucose/insulin interaction system after meal and iv glucose stimuli. This detailed information helps to further examine the natural history of Type 2 diabetes and provides a greater understanding of the processes involved in the development and progression of diabetes. Furthermore facilitates the estimation of essential indices of the whole-body carbohydrate metabolism with aid of model-based approaches.

The approaches used in investigating glucose metabolism in the present thesis are assumed to be valid. However, this validity needs to be further assessed against experimental data.

1.3. Aims and objectives

The primary aim of this thesis is to use modelling techniques employing data collected during MTT and IVGTT to progress our understanding of pathology of type 2 diabetes. The secondary aim is to evaluate the domain of validity of ISM and, in part the minimal model. Type 2 diabetic subjects are studied at presentation as they present the end-point of the natural development of the disease prior to therapeutic intervention.

The aims of this thesis can be accomplished by achieving a subset of methodological and then clinical objectives. The methodological objectives are:

- To evaluate indices of pancreatic β-cell responsiveness with two reduced sampling schemes and compare them against indices obtained during the full sampling scheme

- To validate the insulin secretion model during OGTT and to compare the indices of pancreatic β-cell responsiveness during MTT and OGTT

- To investigate reproducibility of the quantified measures of the pancreatic β-cell responsiveness (M_1 and M_0) and glucose, insulin, and C-peptide responses during MTT and OGTT

- To evaluate and compare the performance of 1CMM and 2CMM for assessing insulin sensitivity in subjects with type 2 diabetes subjects during insulin-modified FSIVGTT and compare its performance with 1CMM

The clinical objectives are:

- To quantify the association between insulin resistance and pancreatic responsiveness with (i) HbA_{1C}, FPG, and FPI (ii) the responses of glucose and insulin to standardised meal

- To investigate the ability of IVGTT and MTT derived indices to explain the 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

1.4. Thesis overview

A review of the literature and associated approaches used are given in Chapter 2. The chapter starts with a background review to diabetes mellitus with more attention to type 2 diabetes and its main pathological factors. This is followed by a review of the main approaches developed and used to assess insulin sensitivity and pancreatic β-cell responsiveness with more concentration on the approaches used in the research course throughout this thesis.

In Chapter 3 the pancreatic β-cell responsiveness indices (M_I and M_0) are evaluated with two reduced sampling schemes (9 samples and 5 samples) and compared against indices obtained during the full sampling scheme.

Chapter 4 provides full details of the validation process of the insulin secretion model during OGTT in healthy subjects and subjects with T2D. It also includes the outcome of the comparisons between the β-cell responsiveness indices, and glucose, insulin, and C-peptide responses during MTT and OGTT.

The reproducibility of the pancreatic β-cell responsiveness indices (M_I and M_0) are investigated in Chapter 5. In addition, the reproducibility of glucose, insulin, and C-peptide responses to MTT and OGTT are also evaluated by assessing the reproducibility of glucose, insulin, and C-peptide incremental area under curve.

In Chapter 6, an evaluation of the performance of 1CMM and 2CMM in type 2 diabetes subjects is performed during insulin-modified FSIVGTT. The glucose clamp technique is used to assess the validity and performance of both 1CMM and 2CMM measurements, as the glucose clamp is considered the gold standard reference method for measuring insulin sensitivity.

Chapter 7 investigates the ability of the indices of insulin sensitivity and pancreatic β-cell responsiveness 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. A quantification of the association between insulin sensitivity and pancreatic β-cell responsiveness with (i) FPG, FPI, and glycated haemoglobin (ii) the responses of glucose and insulin to standardised meal are also considered.

An overall summary of the thesis and outlined achievements are given in Chapter 8, in addition to recommendations for any possibility of future work and research interest.

Appendices includes (I) tables relating to chapter 7, (II) list of publications derived from the work included in this thesis.

2. Background

2.1. Glucose Metabolism

Glucose is the main energy source for the body cells to carry on with its biological activities and survive. The blood glucose level has a normal range which is important to maintain. If the blood glucose level exceeds the normal level then the diabetes symptoms will starts to appear and if not treated or controlled the diabetes complications start to appear with blindness and death are among them.

2.1.1. Regulation of Blood Glucose

Glucose is the main energy-supplying molecule of the body. It is used by the body to produce ATP (adenosine triphosphate), which is the body ultimate source of energy (9). Normally the glucose used by the body is in the blood stream; otherwise it is converted to glycogen and stored by the liver if it is not needed immediately (10). Two hormones produced and secreted by the pancreas mainly control the glucose level in the blood stream; glucagon which increases blood glucose level and insulin, which decreases and adjusts blood glucose level. Blood glucose level controls secretion of glucagon and insulin via negative feedback systems.

Low blood glucose (hypoglycaemia) stimulates release of glucagon from alpha cells which acts on hepatocytes (liver cells) to accelerate the conversion of glycogen into glucose and to promote formation of glucose from lactic acids (11). As a result, the liver releases glucose into the blood more rapidly and blood glucose level rises. If the blood glucose continue to rise for any reason, or after food ingestion, high blood glucose (hyperglycaemia) stimulates release of insulin from beta cells (β-cells). Insulin acts on various body cells to accelerate facilitated diffusion of glucose into cells especially skeletal muscle fibres, and adipose tissues, and speeds up conversion of glucose into glycogen (glycogenesis) and slows and inhibits hepatic glucose production (glycogenolysis and gluconeogensis). As a result blood glucose levels falls to a normal level (10).

2.1.2. *Pathology of Glucose Metabolism*

2.1.2.1. Insulin resistance

Insulin resistance is a pathological condition and a metabolic feature of type 2 diabetes. It can be defined as a reduced response to a physiological amount of insulin (12-17). Scientists are still searching for the causes of insulin resistance, but they have identified two possible causes. The first could be a defect in insulin receptors in cells. There may not be enough receptors for insulin to bind to, or a defect in the receptors may prevent insulin from binding. Recognition of the insulin molecule by its receptor is a complex molecular event and is essential for signal transmission (18).

A second possible cause involves the process that occurs after insulin plugs into the receptor. Insulin may bind to the receptor, but the cells do not read the signal to metabolise the glucose. Scientists are studying cells to see why this might happen.

The cascade of insulin action in vivo involves many steps including transendothelial transport of hormone, binding to the insulin receptor, and activation of tyrosine kinase, followed by movement of GLUT4 transporters from the cell interior to the membrane so that glucose may enter the cell to be stored or oxidised (19). The insulin action might be delayed, the delay may be due to diffusion of insulin throughout the interstitium, and a decrease in capillary density in obesity could potentially account for insulin resistance because the time necessary for diffusion would be increased (19).

Current and previous researchers have showed some evidence for a relation between the insulin resistance and unhealthy life-style, endocrine abnormalities, and with several other abnormalities. Both the quantity and quality of food intake affect insulin binding and insulin action at a molecular level (20-23)

Obesity is the most common cause of insulin resistance in humans and with or without the presence of hyperglycaemia, it is almost certainly the most common state of insulin resistance (24-27). An important new potential mechanism of insulin resistance in obesity has been the observation that fat tissue itself may produce and secrete hormones or cytokines that affect metabolism and/or insulin sensitivity (28). In addition, prolonged exposure of β-cells to insulin leads to desensitisation and reduced

insulin-stimulated receptor autophosphorylation (29). On the other hand, exercise enhances insulin sensitivity and glucose disposal in normal physiology. This is associated with increased insulin binding to muscles (30). Almost all obese subjects are showing an exaggerated insulin response to glucose. Being overweight is considered as one of the most important prediabetic conditions (9).

2.1.2.2. β-cell dysfunction

β-cell dysfunction is the disability of the pancreas to secrete the body needs of insulin. It is defined as an inappropriate reduction in the rate of insulin secretion from the β-cells or the abnormality in the rate and pattern at which blood insulin concentration changes as a function of time (31). It might be due to the β cells themselves being not able to secrete insulin or the decrease in β-cell number because of an infection or an autoimmune disease. The pancreatic insulin secretion is regulated by many factors; with the plasma glucose level being the most important regulator.

The alteration in the level and pattern of the β-cell dysfunction can present in different ways. It could be the reduction in insulin release to glucose (32), change in the pulsatilitiy pattern and oscillatory of insulin secretion (33), defect in first phase insulin response to glucose stimulus (34), abnormality in the proinsulin to insulin conversion (35), and reduced release of islet amyloid polypeptide (36).

2.2. Diabetes

Diabetes mellitus is a group of metabolic disorders characterised by hyperglycaemia (elevation of glucose in the blood). These heterogeneous disorders usually result from defects in insulin secretion, and/or insulin action. The persistent hyperglycaemia of diabetes is associated with long-term complications, and dysfunction of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The aetiology and pathogenesis of diabetes are not fully understood and is believed to be a genetic inheritance with environmental factors. Several pathogenic processes ranging from autoimmune destruction of the β-cells of the pancreas with subsequent insulin deficiency to abnormalities that result in resistance to insulin action are involved in the development of diabetes.

The previous classification of diabetes was based on the extent to which a patient was dependent on insulin . Few years ago, both the reports of the American Diabetes Association (37) and the World Health Organisation (WHO) (1) recommended changing the classification to define four main subtypes of diabetes (Table 2-1) reflecting the heterogeneity of processes that lead to diabetes which hopefully will lead to more precise targeting of specific treatments and eventually to better outcomes.

Table 2-1. Etiologic classification of diabetes mellitus, adopted from Alberti et al (1).

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
A. Immune mediated
B. Idiopathic
II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III. Other specific types
A. Genetic defects of β-cell function
B. Genetic defects in insulin action
C. Diseases of the exocrine pancreas
D. Endocrinopathies
E. Drug- or chemical-induced
F. Infections
G. Uncommon forms of immune-mediated diabetes
H. Other genetic syndromes sometimes associated with diabetes
IV. Gestational diabetes mellitus (GDM)

Type 1 includes immune mediated and idiopathic forms of β-cell dysfunction which lead to absolute insulin deficiency. Type 2 diabetes is a disease of adult onset, which may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance. Type 3 disease covers a wide range of specific types of diabetes including the various genetic defects of β-cell function, genetic defects in insulin action, and diseases of the exocrine pancreas. Type 4 disease is gestational diabetes (GDM) which is only found during pregnancy. It normally vanishes after the delivery, but those women will have a higher chance to get Type 2 (37). The revised criteria for the diagnosis of diabetes are shown in Table 2-2. Type 2 will be discussed in more details as it is the scope of research in this thesis.

Table 2-2. Diabetes mellitus diagnostic criteria, adopted from the report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (37).

1. Symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl (11.1 mmol/l). 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.

or

2. FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.

or

3. 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization 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 third measure (OGTT) is not recommended for routine clinical use.

2.2.1. *Type I Diabetes Mellitus*

Previously called "insulin dependent diabetes mellitus" (IDDM), because there is an absolute chronic deficiency of insulin secretion, and regular injections of insulin are required to prevent death. Normally it affects children or adolescents only, although it can appear at any age. It is commonly developed in young people (under 20 years old) and persists throughout life. Type I appears to be an autoimmune disorder, one in which a person's immune system destroys the pancreatic beta cells. It is believed that both genetic factors and virus infections are responsible. Individuals at increased risk of developing type 1 of diabetes could be identified by using immunologic markers to islet antigens and serological evidence of an autoimmune pathologic process occurring in the pancreatic islets. Up to ten per cent of all diabetes diseases are of Type 1. Some of its first symptoms are increased needs of sleep, constant hunger and thirst, a bleary vision, and a loss of weight (1; 18; 37).

2.2.2. *Type 2 diabetes Mellitus*

Previously known as non-insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes is a heterogeneous disorder characterised by insulin deficiency due to β-cell failure associated with insulin resistance, which represents more than 90% of all cases of diabetes, and most often occurs in people over 40 years and overweight. About 150 million are estimated to have type 2 diabetes worldwide (38) and the numbers of type 2 diabetic patients are increasing each year as a result of several factors including increased obesity, civilised life style, and other environmental factors related to diet and nutrition (12; 38).

2.2.2.1. Pathophysoilogy of type 2 diabetes

The aetiology and pathogenesis of type 2 diabetes mellitus are not fully understood. It can be considered as being a complex interaction of genetic predisposition and environmental factors (7). The strong genetic component of type 2 diabetes is clearly found in certain families and ethnic groups such as Hispanic, and Pima Indians (39-42). There is a degree of agreement that the most important pathological factors of type 2 diabetes are β-cell dysfunction and insulin resistance (37). However a considerable

uncertainty exists about the sequence and the nature of the earliest biochemical changes and their relative contributions to the deterioration in glucose tolerance and development of type 2 diabetes. At the same time it is well founded that an intermediate stage of impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) predicts and precedes the progression of T2D (37).

It is well accepted that a substantial loss of β-cell function should be present for a hyperglycaemia to appear in type 2 diabetes, and it is implicated that insulin deficiency is the proximate cause of the progressive increase in plasma glucose levels (17; 31). The β-cell dysfunction could be reduction in insulin release to glucose (32), change in the pulsatile and oscillatory of insulin secretion (33), defect first phase insulin response to glucose stimulus (34), abnormality in the proinsulin to insulin conversion (35), and reduced release of islet amyloid polypeptide (36). However it is still a matter of controversy as to whether insulin resistance or β-cell dysfunction is the primary pathogenic defect in type 2 diabetes.

Many studies have focused on and reported the insulin resistance as the primary defect, where insulin resistance precedes and causes the hyperinsulinaemia and provides a stronger signal for β-cell stimulation (31; 43-45). The increase in plasma insulin is generally regarded as a compensation mechanism aiming to reverse the effect of insulin resistance (46). While the pancreatic β-cell decomposition is caused by the long exposure to high concentration of glucose (glucose toxicity) (47), and reflecting β-cell 'exhaustion' (48). Overt diabetes appears when the pancreas is not able to meet the body's demand for insulin in the face of increasing insulin resistance (49). The above aetiology hypothesis was supported by other studies which reported that the majority of patients with type 2 diabetes and subjects at risk for diabetes are insulin resistant (41; 50; 51), and are hyperinsulinaemic even before hyperglycaemia appears (52).

Others suggested the conception of a defect in insulin secretion to be the major early abnormality (53-55). In accordance with this concept, low early insulin response predicted diabetes in other studies (56), and the progression from normal glucose tolerance to impaired glucose tolerance (57), while insulin resistance predicted the transition from impaired glucose tolerance to type 2 diabetes (58; 59). Type 2 diabetes

can develop in individuals with normal insulin sensitivity with defective β-cell function (60), and individuals at high risk of diabetes demonstrated a diminished β-cell function while maintaining normal glucose tolerance (61-63).

Part of the United Kingdom Population Diabetes Study (UKPDS) findings and suggestions in individuals with established type 2 diabetes is that the onset of β-cell dysfunction is present early before the escalation of hyperglycaemia and could exist for years before diagnosis (64). This concept was supported by another longitudinal study on a group of high risk population with normal glucose tolerance, the study reported a progressive decrease in insulin secretion in subjects who developed hyperglycaemia, whereas insulin sensitivity was similar to those who retained normal glycemia (65).

Many other studies supported the idea that β-cell dysfunction together with insulin resistance predicts the development of type 2 diabetes (8; 65; 66), and the difficulty for these defects to be assessed in isolation. Thus, it is generally agreed that type 2 diabetes is a broad metabolic heterogeneous disorder and its development and progression is associated with and predicted by defects in both insulin secretion and insulin resistance, in addition to the genetic factor (56; 67). Other factors such as obesity (68), diet (69), physical activity (70), and many other known and unknown factors may interact and contribute to the development of type 2 diabetes.

2.2.2.2. Symptoms and risk factors

The main symptoms of the type 2 diabetes include polyuria, polydipsia, weight loss, sometimes with polyphagia, thirst, frequent urination, weakness and great tiredness, lack of ability to contrast, loss of co-ordination, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycaemia. Acute, life-threatening consequences of diabetes are hyperglycaemia with ketoacidosis or the nonketotic hyperosmolar syndrome (18; 37).

Although the major cause for diabetes is insufficient insulin produced by the pancreas or insulin resistance. There are some factors which increase the risk of diabetes incidences, the most important known risk factors are; obesity, stress, pregnancy, use of

certain drugs, including oral contraceptives, thiazide diuretics, cortisone or phenytoin, and family history of diabetes mellitus (18)(37).

2.2.2.3. Complications

Normally the diabetes complications are more dangerous than the disease itself. The most common and dangerous complications are cardiovascular disease, vision impairment, peripheral vascular disease, with gangrene in legs and feet, and sexual impotence in men (18). Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy and foot ulcers. Glycation of tissue proteins and other macromolecules are among the mechanisms believed to produce tissue damage from chronic hyperglycaemia. Hypertension, abnormalities of lipoprotein metabolism, and periodontal disease are often found in people with diabetes. In addition, the emotional and social impact of diabetes and the demands of therapy may cause significant psychosocial dysfunction in patients and their families (37).

2.3. Assessment of insulin sensitivity

Glucose tolerance is an expression of the efficiency with which homeostatic mechanisms restore glycaemia to basal levels after a perturbation. Insulin is a key regulator of glucose homeostasis. Insulin resistance (decreased sensitivity or responsiveness to the metabolic actions of insulin) is determined by both genetic and environmental factors and plays an important pathophysiological role in diabetes (5). The term "insulin resistance" refers to an impaired biological response to either exogenous or endogenous insulin. In addition, insulin resistance is associated with a number of other diseases, including obesity, hypertension, dyslipidemias, and coronary artery disease (5; 6). Therefore, it is of great interest to quantify insulin sensitivity and resistance in humans to investigate the pathophysiology and epidemiology of major public health problems and to follow the clinical course of patients on various therapeutic regimens. In addition, the ability to easily quantify insulin sensitivity in large numbers of subjects may be useful for investigating the role of insulin resistance in the pathophysiology of these major public health problems.

The glucose-insulin system is composed of a complex set of metabolic interactions and regulatory components. Even if all these were included in the description of a proposed system model, it would be still a kind of oversimplification because the system is embedded within the entire complex system, which is made up of energy metabolism and its hormonal and neural regulation. Many mathematical formulations for the glucose system have been made, from the comprehensive to the relatively simple.

Clinically, the most desired assessment of insulin sensitivity is following an oral glucose load, a surrogate for a more physiological meal (71). The homeostatic response includes an increase in the insulin levels and, therefore, also the insulin-dependent processes that lower glycaemia. Theoretically, the oral glucose tolerance test should yield an estimate of insulin sensitivity. Many mathematical formulations have been developed to estimate insulin sensitivity following an oral glucose load (72-74).

After oral glucose or meals, the increments in insulin do not depend entirely on glucose, but also on other factors such as gut hormones and neural stimulation. Thus the insulin response deviates from the purely glucose-dependent pattern. In addition, glucose concentrations also change in a manner that is partly dependent on insulin, but also

partly on gastric emptying and absorption (75; 76). Therefore, many attempts have been made to isolate the glucose-insulin relationship, as much as possible, from other factors.

A host of methods have been developed to assess insulin sensitivity (or insulin resistance) in vivo. These include the hyperinsulinaemic euglycaemic glucose clamp (77), minimal model analysis of a frequently sampled intravenous glucose tolerance test (FSIVGTT) (78), continuous infusion of glucose with model assessment (CIGMA) (79), and various indices derived from an oral glucose and meal tolerance tests (72; 80-83), and simple indices based on fasting glucose and insulin levels (84) including homeostasis model assessment (HOMA) (85), and QUICKI (86). A number of variations on each of these approaches are available. For example, the glucose clamp technique can be performed under other conditions such as hyperglycaemic with or without infusion of tracer-labelled glucose (87; 88). Similarly, minimal model analysis has been extended to analyse tolbutamide or insulin modified FSIVGTT (19; 89) with or without infusion of tracer-labelled glucose (90).

The hyperinsulinaemic euglycaemic glucose clamp is generally regarded as the "gold standard" reference method for assessing insulin sensitivity in humans because it directly measures metabolic actions of insulin under steady state conditions. However, the glucose clamp is not easily applied in large-scale investigations because intravenous (IV) infusion of insulin, frequent blood samples over a 3 to 6h period, and continuous adjustment of a glucose infusion are required for each subject studied (77).

Simple indices of insulin sensitivity based on fasting values such as fasting insulin value, 1/fasting insulin, and insulin-to-glucose ratio have been used as a surrogate measure for insulin resistance (84). In addition, the Bennett index (91), homeostasis model assessment (HOMA) (85) and QUICKI (86) are easily obtained during fasting (basal) states, and may be useful tools for large epidemiological studies. In the context of the current review, some of these methods will be explored with varying degree of depth.

2.3.1. *Hyperinsulinaemic euglycaemic glucose clamp*

The euglycaemic insulin clamp and the frequently sampled IV glucose tolerance test with the minimal model analysis are the standard methods of assessing insulin sensitivity (S_I). The former is considered to be the gold standard in the assessment of insulin resistance because it directly measures the effects of insulin to promote glucose utilisation under steady state conditions (77). It is a conceptually simple test, although technically, somewhat more complex.

The hyperinsulinaemic euglycaemic glucose clamp is performed by infusing insulin as a priming dose followed by a constant infusion rate to achieve and maintain a preset hyperinsulinaemic plateau. Simultaneously, glucose is monitored frequently and infused at variable rates to maintain near-constant glycaemia, which is equivalent to normal fasting glucose levels (or in the isoglycaemic case, the subject's own fasting glycaemia). When the glucose infusion rate has stabilised (2–3 h), this rate, divided by the incremental insulin level (subtracting basal insulin) and corrected for the ambient glucose concentration, is defined as clamp insulin sensitivity index (S_I). When a steady state is achieved, the exogenous glucose infusion rate equals the glucose disposal rate (M) (sum of suppression of endogenous glucose production and the stimulation of glucose disposal). If endogenous hepatic glucose production is completely inhibited by an intravenous infusion of insulin then the quantity of exogenous glucose required to maintain euglycaemia (the M value) is a reflection of the net sensitivity of target tissues (mainly skeletal muscle) to insulin (77).

S_I, during the clamp, was derived from the steady-state glucose infusion rate (M value) mainly during the 3rd hour of the clamp corrected for the ambient insulin and glucose concentrations

$$S_{I\text{-}Clamp} = \frac{M}{\Delta I \times G} \qquad (2\text{-}1)$$

where ΔI is the increment in insulin concentration from basal, and G is the clamped glucose concentration.

However, the clamp is a complicated method to implement because it requires simultaneous infusions of insulin, and glucose, multiple blood draws, and an experienced operator to adjust the glucose infusion appropriately over a 3- to 6-h time period. In addition, the clamp generates insulin levels above those usually experienced by subjects and may therefore fail to reveal potential abnormalities of processes regulated by lower insulin concentrations. The manner in which insulin sensitivity is determined during the hyperinsulinaemic euglycaemic clamp is based upon the assumption (unless appropriate tracer techniques are used) that endogenous glucose production is completely shut off by the insulin infusion. Moreover, during a clamp, insulin is administered as a constant infusion and, therefore, does not reflect the variations inherent in endogenous secretion (92).

2.3.2. *Insulin tolerance test*

The insulin tolerance test (ITT) was one among the first methods to assess the insulin sensitivity in vivo (93). The test consists of an intravenous insulin bolus injection and the slope of the decreased blood glucose concentration over the following 60 min is used as an index of insulin sensitivity. However the test does not provide a good quantitative measure as the hyperinsulinaemic euglycaemic glucose clamp. The rate and degree of plasma glucose fall in response to ITT are dependent not only on insulin sensitivity, but also on the presence and magnitude of the counterregulatory hormone response (including adrendin, glucagon, and cortisol), thus decreasing the value of ITT in assessing insulin sensitivity per se (92).

2.3.3. *HOMA*

The homeostatic model assessment (HOMA) focuses on and estimates insulin resistance function from basal fasting glucose and insulin levels. The HOMA method was first put forward in 1985 by Matthews *et al.* (85). This mathematical model is based on the theory of a negative feedback loop between the liver and β-cells that regulates both fasting glucose and insulin concentrations which can be used to estimate pancreatic β-cell function and degree of insulin resistance. Therefore, considering its simplicity, it may be a useful non-invasive tool for population studies.

The insulin resistance (R_{HOMA}) *is* calculated as:

$$R_{HOMA} = g * i/22.5 \quad (2\text{-}2)$$

where g and i is the fasting plasma glucose (mmol/l) and insulin (μU/ml), respectively. It is critical to note, however, that HOMA is an index of insulin resistance. Whereas insulin sensitivity (S_{IHOMA}) is calculated as exactly the inverse of the formula for resistance shown in equation (2-1):

$$S_{IHOMA} = 22.5/(g * i) \quad (2\text{-}3)$$

The basic rationale for the model is stated as: "The basal hyperglycaemia of diabetes may be considered as a compensatory response with a major role in maintaining sufficient insulin secretion, from a reduced β-cell capacity, to control hepatic glucose efflux"(85).

However it has sometimes been concluded that the HOMA index does not correlate well with other measures of insulin sensitivity (80).

2.3.4. *Minimal Model of Glucose Kinetics*

The minimal model (or thereafter one compartment minimal model, 1CMM) was developed to analyse data from frequently sampled intravenous glucose tolerance test (FSIVGTT) and to produce measures of peripheral insulin sensitivity (S_I) (94). It was termed the "minimal model," because it was the mathematical model with the fewest parameters that was found to provide a good fit to the data and the fits to data are remarkably good (94). It should be noted that the fits are obtained using, for example, non-linear least squares techniques: parameters are varied according to a defined strategy and are assigned a final value that minimizes the sum of squares of differences between the data and the glucose and insulin values predicted by the model (which is non-linear) for any parameter set. The goodness of fit and, therefore, the reliability of the parameters can then be evaluated statistically.

The FSIVGTT is constructed with a glucose bolus (300 mg/kg body weight as a 50% solution in water) injected at zero minute and about thirty samples withdrawn over more than three hours (95). The 1CMM works well in subjects with normal insulin secretion, because the model assumes that insulin action is zero at the start of the test and it reset to zero at each iteration loop for solving the equations for glucose disposal. In the case of impaired or poor insulin secretion in the presence of insulin resistance, the value of insulin action will be close to zero, which makes the estimation of S_I very sensitive to any small change in the plasma insulin leading to the test variability and poor quantification of insulin sensitivity (96). Thus, Beard et al, have injected tolbutamide intravenously 20 min after glucose injection to produce a large insulin secretory response (89).

This modification was not suitable for diabetic patients because most of the patients suffer from pancreatic dysfunction and do not have significant insulin secretion in type 1 diabetes or the combination of reduced insulin response and pancreatic dysfunction. To overcome this problem, in the modified FSIVGTT the tolbutamide injection was replaced by an insulin injection (0.05mU/kg) instead at time 20 min (16). This exogenous insulin simulates the function of the endogenous insulin by the pancreas and enables the model to make calculations with improved accuracy of the parameters estimation (19). Also different sampling techniques were used which reduced the numbers of samples to 13 samples and 12 samples (16; 95-97).

The modified intravenous glucose tolerance test (IVGTT) with insulin injection interpreted with the 1CMM of glucose kinetics is a powerful non-invasive tool to investigate glucose metabolism in physiological studies (98). The model analyses IVGTT data and provides two metabolic indices measuring glucose effectiveness (S_G) and insulin sensitivity (S_I) in a single individual. S_I and S_G are composite parameters, which measure the net effect of glucose and insulin respectively to promote glucose disappearance and inhibit endogenous glucose production (98). The 1CMM method has gained increasing popularity and is used by investigation around the world (99) because it is simple and non-invasive. The 1CMM is represented in Figure 2-1.

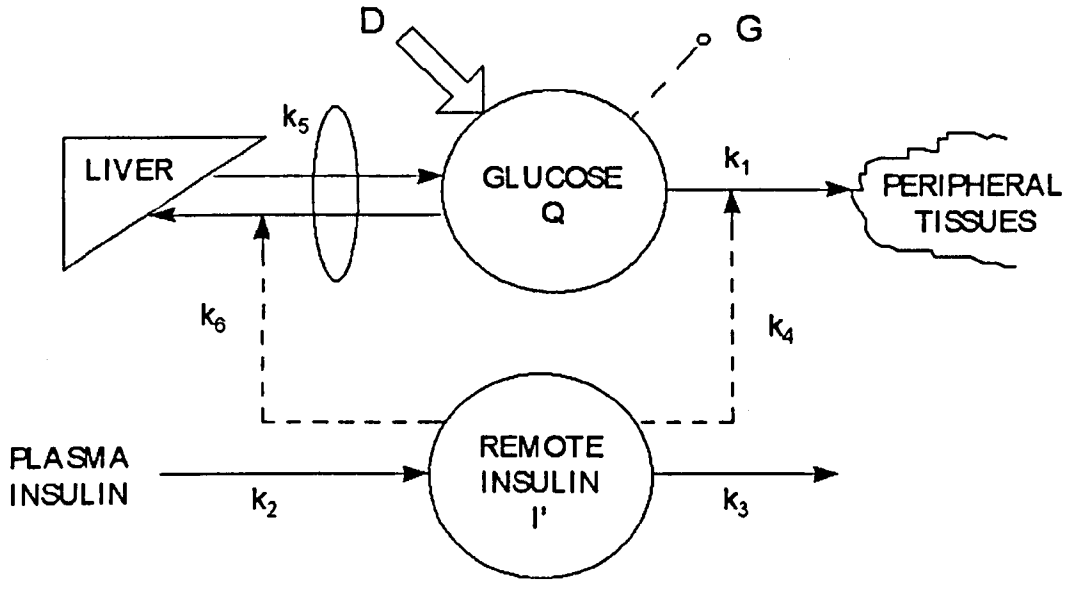

Figure 2-1. Minimal model of glucose kinetics (100). k_4 and k_6 relate to the efficiency of coupling of remote insulin with target biochemical processes. While k_1 and k_6 represent the effect of glucose to accelerate its utilisation.

The glucose space is represented as a single extracellular compartment (Q), the glucose in this space is determined by a balance between the net production of glucose by the liver and the utilisation of glucose by the peripheral tissues. The effects of glucose to accelerate its own uptake by the periphery and inhibit production are represented by rate coefficients k_1 and k_2 respectively. Insulin in the model (I) is envisaged to act on glucose metabolism not directly, but via a component remote from plasma (I'). This component represents the effect of insulin and account for the delayed insulin actions on glucose. The rate of metabolism of the remote insulin effect is envisioned as being independent of plasma insulin and determined by k_3. Independent metabolism of the insulin effect is consistent with insulin action continuing long after plasma insulin is normalised (100). G is the plasma glucose concentration, V is the distribution volume per unit body weight (ml/kg). The equations of the minimal model are:

$$Q(t) = -[S_G + X(t)]\, Q(t) + S_G\, Q_b \qquad (2\text{-}4)$$

$$Q(0) = Q_b \qquad (2\text{-}5)$$

$$dX(t)/dt = -p_2\, X(t) + p_3\, I(t) \qquad (2\text{-}6)$$

$$X(0) = 0 \quad (2\text{-}7)$$

$$G(t) = Q(t)/V \quad (2\text{-}8)$$

In equation (2-4) the rate of change in plasma glucose Q(t) is depending on S_G and the effect of glucose that is enhanced by remote insulin X(t). Q_b is baseline value for glucose compartment. Equation (2-5) describes the rate of change of insulin action (dX(t)/dt), p_3 describes how the increase in insulin action is dependent on the incremental insulin response while p_2 describes how the disappearance of insulin action is dependent on how much insulin action (X) was present at the time. S_G is taken directly from the first equation ($p_1 = k_1 + k_5$) (98). While S_I is calculated as the ratio between p_2 and p_3:

$$S_I = p_3/p_2 \quad (2\text{-}9)$$

where

$$p_2 = k_3 \text{ and } p_3 = k_2(k_4 + k_6) \quad (2\text{-}10)$$

The ICMM approach uses the computer to analyse the plasma glucose and insulin dynamics observed following glucose injection (101-103). It yields in vivo measurement of the relative contributions of the pancreas and tissues to glucose disposal. The insulin time course is part of the input to the computer program input, which then used to compare the prediction of the model with measured glucose level. This enables the program to estimate the equations parameters and by analysing frequently sampled intravenous glucose tolerance test (FSIVGTT) data the program provides values for the parameters of insulin sensitivity (S_I) and glucose effectiveness (S_G) (101).

The model assumes that the injected glucose is distributed rapidly in a single compartment. After injection of glucose, plasma glucose falls by two mechanisms: (1) a component of glucose disposal that is dependent only on plasma glucose concentration and independent of any increment in insulin level, (2) disposal which is dependent on the incremental insulin response. Glucose inhibits its own production and increases its own utilisation in proportion to its concentration in plasma. Insulin synergies these

effects of glucose and insulin. The insulin effect is proportional to its concentration in a remote compartment.

Finally, the assessment of an index of insulin sensitivity (S_I), by employing the minimal model kinetic analysis to data obtained from the FSIVGTT, appears to represent a more accurate means of quantifying insulin sensitivity (78). The S_I correlates well with the insulin-mediated glucose disposal rate (M), and S_I as determined by the euglycaemic hyperinsulinaemic clamp (16; 104-106). The dynamic and physiological nature of this test and the relative simplicity of its performance count among its attractive features. Differences and potential problems arise from the same source: the rapid dynamics may confound transients based on the distribution of glucose throughout the system and those due to glucose removal. Two-pool or higher order descriptions of glucose dynamics and the use of tracers were suggested (107; 108) as possible solutions to such difficulties.

2.3.5. *Two compartment minimal model of glucose kinetics*

Recent published reports and studies indicate that S_G is overestimated (109-113) and S_I is underestimated (109-112) during 1CMM attributing the main reason to the under modelling effect of using one compartment to represent the glucose pool (110; 113).

The new two compartment minimal model (2CMM) was first introduced in 1993 to measure hepatic glucose production during an isotopically labelled IVGTT by appending a second non-accessible compartment to the classic 1CMM. The new model was needed because, at that time, the available single compartmental minimal model specifically developed to interpret labelled IVGTT data, provided a non-physiological pattern of hepatic glucose production (114). In 1997 Vicini et al (109) validated the 2CMM to estimate S_I, S_G, and plasma clearance rate during an isotopically labelled FSIVGTT. Recently Cobelli et al (115) incorporated a priori knowledge on glucose exchange kinetics using Bayesian estimation to derive insulin sensitivity and glucose effectiveness with the 2CMM during standard IVGTT in healthy subjects.

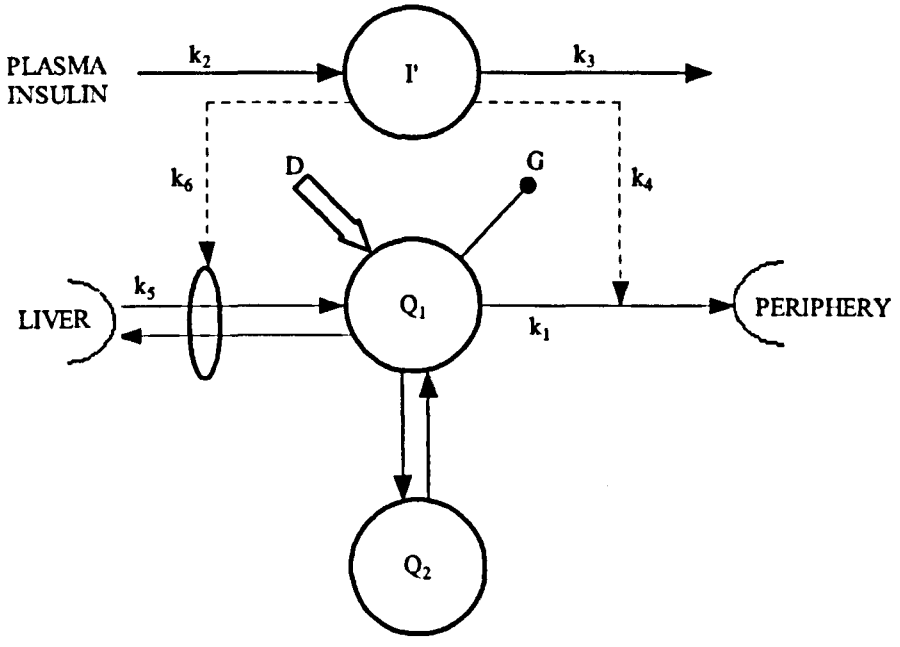

Figure 2-2. Two compartment minimal model (2CMM), (110).

The 2CMM appends a second non-accessible compartment to the 1CMM. The 2CMM was validated in normal subjects during standard IVGTT by applying a Bayesian approach to incorporate prior knowledge on k_{12} and k_{21} parameters (115). The model equations are as follows:

$$Q_1(t) = -[p_1 + k_{21} + X(t)]Q_1(t) + k_{12}Q_2(t) + p_1Q_{1b} \quad Q_1(0) = Q_{1b} + D \tag{2-11}$$

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

$$X(t) = -p_2X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \tag{2-13}$$

$$G(t) = Q_1(t)/V_1 \tag{2-14}$$

where Q_1 and Q_2 (mg/kg) denote the glucose masses in the accessible and non-accessible compartments, respectively, with subscript b denoting their basal

(end-test) steady-state values; V_1 is the volume of the accessible compartment (mg/kg); k_{12} and k_{21} are rate parameters describing glucose exchange kinetics (1/min); D, G, I, X, p_1, p_2, and p_3 are variables and parameters already defined for the 1CMM. The 2CMM parameters S_I^2 and S_G^2 are calculated as follows:

$$S_G^2 = p_1 V_1 \quad \text{(ml/min/kg)} \tag{2-15}$$

$$S_I^2 = \frac{p_3}{p_2} V_1 \quad \text{(ml/min/l per μU/ml)} \tag{2-16}$$

The 2CMM differs from 1CMM only in allowing an exchange of glucose between the accessible and the non-accessible compartment, see Figure 2-2. This added complexity brings a priori identifiably problems. The theoretical or a priori identifiably address the ability of getting unique solutions for the unknown parameters on the basis of the experiment-generated data. A Bayesian analysis using a priori information on the glucose exchange kinetics parameters k_{12} and k_{21} was applied to solve these problems and reach unique identifiability (115).

2.4. Assessment of pancreatic β-cell responsiveness

Type 2 diabetes results from varying forms and degrees of abnormality in β-cell function and insulin sensitivity. β-cell dysfunction predicted diabetes in many studies (56), and the progression from normal glucose tolerance to impaired glucose tolerance (57). In addition, the progression from IGT to T2D is characterised by progressive loss of β-cell function (116) and T2D is characterised by impaired pancreatic β-cell response to glucose. It is well founded that a substantial loss of β-cell function should be present for a hyperglycaemia to appear in type 2 diabetes, and it is implicated that insulin deficiency is the proximate cause of the progressive increase in plasma glucose levels (31).

Several methods with varying approaches and complexity were introduced to assess in vivo pancreatic β-cell responsiveness to glucose (117-119). The methods range from simple time series plots, and simple calculations such as the methods and indexes relating fasting insulin to fasting glucose (85; 119), the increase in plasma insulin or C-peptide in plasma insulin after oral glucose and meal tolerance test (119; 120), after IV glucose tolerance test (121) including the calculation of the acute insulin response to glucose ($AIR_{glucose}$) (122), and the increase in insulin or C-peptide after stimulation by glucagon (123), to moderate and sophisticated techniques and model-based approaches (77; 79; 124; 125). These approaches and methods have great value in the understanding and predicting the progression of the disease.

The hyperglycaemic glucose clamp is the gold standard method for assessing β-cell responsiveness in vivo (77). In spite of this, the clamp methods are not suitable for routine use and not feasible for investigating insulin secretion in large groups and population studies because it is costly and labour intensive. Other models and approaches were proposed and used after oral glucose tolerance test (126), after IV glucose tolerance test (127) and under other conditions (79; 85; 128). The insulin secretion model (ISM) was validated to assess pancreatic β-cell responsiveness during more physiological conditions (32). Among these method and approaches, some will be discussed and summarised in the following text.

2.4.1. *The hyperglycaemic clamp*

The hyperglycaemic clamp (77) is considered the gold standard which provides the most reliable and direct method for assessing pancreatic responsiveness. During the hyperglycaemic glucose clamp, plasma glucose is 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 employed 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 the early pancreatic response. Samples are withdrawn also at basal states before the glucose administration. The sampling schedule is usually every 1-5 minutes for the first 15-30 minutes then reduced and made every 10-30 minutes thereafter.

The time-secretion profile of insulin can be estimated by combining the hyperglycaemic clamp with the combined model which employs both insulin and C-peptide to calculate insulin secretion (129). The incremental area under the curve (0-10min) can be used to calculate and evaluate the first phase insulin secretion. However the use of the hyperglycaemic glucose clamp is limited due to the great investment of resources required and the complexity of the experimental interventions which make considerable demands on both labour and subjects.

2.4.2. *Minimal model of C-peptide secretion during IVGTT*

The minimal model of C-peptide secretion and kinetics builds upon the insulin minimal model (130; 131). However the C-peptide has the ability to reflect the pre-hepatic insulin secretion. In addition the C-peptide has been shown to exhibit a linear kinetics over a wide range of physiological plasma concentrations, and under fasting and postprandial conditions. The model provides two indices of first phase and second phase β-cell responsiveness (127). It yields a true pre-hepatic picture of the β-cell secretion and response to glucose stimuli.

2.4.3. HOMA

As described previously, the homeostasis model assessment (HOMA) is a simple computer-solved model, which takes advantages of the analysis of the fasting homeostatic state and provides reasonable measures of β-cell function as well as insulin sensitivity (85). The % β-cell function index is derived as:

$$\beta\text{-cell function (\%)} = 20 \times \text{insulin}/(\text{glucose}-3.5) \quad (2\text{-}17)$$

The accuracy and precision of estimates were assessed by comparison with independent measures of β-cell function (132) including the hyperglycaemic glucose clamp, and intravenous glucose tolerance test (85; 133). It is critical to take into account the degree of insulin resistance when assessing β-cell function with the HOMA model (134). However, because of its simplicity it forms a useful method to assess both insulin resistance and β-cell function in epidemiological studies (134; 135).

2.4.4. CIGMA

Continuous infusion of glucose with model assessment (CIGMA) is a model-based approach for assessing glucose tolerance and β-cell function (79). It consists of a continuous glucose infusion (5 mg glucose/kg ideal body weight per min) for 60 min, with measurement of plasma glucose and insulin concentrations. These are similar to the postprandial levels. These levels change slowly depending on the dynamic interaction between the insulin produced and its effect on glucose clearance. The glucose and insulin concentrations after 30 min can be compared with reference values and interpreted using a mathematical model of glucose and insulin homeostasis to assess insulin resistance and β-cell function.

The model is based on the available physiological data describing quantitatively the relationships between glucose and insulin (136; 137). The functions used in describing the model are drawn from experimental physiological data, which do not have simple mathematical formulations. β-cell responsiveness is defined as the ability of the pancreas to respond to glucose and is expressed as % of normal β-cell function.

CIGMA provides a near-physiological glucose load and the slowly changing glucose and insulin homeostasis can be easily assessed. It can be considered as a test of glucose tolerance and is analogous to the oral glucose tolerance test. The β-cell function measured by CIGMA correlated in a linear fashion with the steady state plasma insulin levels during hyperglycaemic clamp in diabetic but not normal (79). In addition CIGMA could be used with C-peptide instead of insulin measurements. It therefore assesses pancreatic secretion rather than the post-hepatic delivery rate which is more likely to cross-react with insulin assay than with C-peptide.

Theoretically the β-cell function values from CIGMA are independent of the glucose levels achieved because the model includes responses for different levels of glycaemia. However the model assumes that the assessment parameters are relevant to studied subjects, which apply more to diabetic than normal subjects. These assumptions include equality of liver and peripheral insulin resistance, and that the reduction in β-cell function in diabetes is a quantitative decrease. Therefore the model may not be suitable in special situations, such as in the case of a change in the shape of the β-cell dose response curve.

2.4.5. *Intravenous glucose tolerance test*

Insulin response to an intravenous glucose stimulus has been observed to have a biphasic pattern. In healthy humans, an intravenous glucose bolus results in an immediate and sharp and rapid insulin secretion (first phase insulin secretion with response to glucose injection; AIR_G). This peak in insulin secretion normally lasts for about 10 min and is followed by a second slow phase of insulin secretion which lasts for a longer duration. First phase insulin secretion has been observed during other techniques such as the hyperglycaemic glucose clamp (77; 138), glucagon injection (124), and stepped glucose injection (139).

There is no standard consensus as to how the first and second phase should be calculated. The first phase insulin secretion generally is defined as that secretion occurring 2-10 min after glucose injection. The second phase is usually defined as the overall secretion occuring during the rest of the experiment (130). The first and second phase insulin secretion during IVGTT can be measured by the minimal model of C-

peptide kinetics (140). The incremental area under the curve of insulin (or C-peptide) curve 2-10 min is usually used to calculate the first phase insulin secretion and assess the acute insulin response (123).

2.4.6. *Insulin Secretion Model*

The insulin secretion model (ISM) is a simple relatively non-invasive model-based approach developed several years ago to quantify pancreatic β-cell responsiveness during a meal tolerance test (MTT) (32). The model measures the prehepatic insulin secretion and assesses pancreatic β-cell responsiveness providing two indices of pancreatic responsiveness. Fasting (basal) pancreatic β-cell responsiveness (M_0; ability of fasting glucose to stimulate C-peptide secretion) and postprandial responsiveness (M_1; ability of postprandial glucose to stimulate C-peptide secretion). The model is able to quantify pancreatic responsiveness in healthy and disease states, and confirms significant differences between healthy subjects and subjects with newly diagnosed type 2 diabetes (141). The insulin secretion model is shown in Figure 2-3.

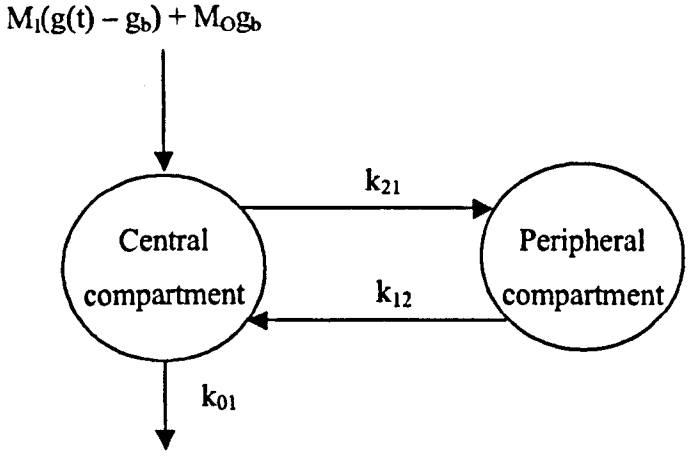

Figure 2-3. Insulin secretion model (ISM) (32).

Insulin and C-peptide are co-secreted in an equimolar ratio by β-cells, and this phenomenon has been exploited to assess prehepatic insulin secretion (130). The liver does not clear C-peptide to any significant extent. C-peptide kinetics have been shown to be linear over a physiological to supraphysiological range of plasma C-peptide concentration (32). Under steady state conditions a single measurement of plasma C-peptide is expected to provide a more reproducible index of insulin secretion than a single measurement of plasma insulin (130).

In the ISM a linear relationship between C-peptide secretion and plasma glucose was postulated and combined with the population model of C-peptide kinetics (32). This linear relationship was imposed from the time of meal ingestion until plasma glucose returned to its fasting concentration.

The population model of C-peptide kinetics was reported by Van Cauter et al in 1992 (142). It has been developed from the analysis of C-peptide decay curves obtained from experiments carried out in 200 adult subjects. It enables the parameters of the C-peptide kinetic to be approximated from a subject's height, weight, age, sex, and the classification of being normal, obese, and type 2 diabetic.

The model is described by a set of differential equations:

$$dc_1(t)/dt = -(k_{01} + k_{21})\, c_1(t) + k_{12}c_2(t) + u(t) \quad c_1(0) = u(0)/k_{01} \tag{2-18}$$

$$dc_2(t)/dt = k_{21}c_1(t) - k_{12}c_2(t) \quad c_2(0) = c_1(0)\, k_{21}/k_{12} \tag{2-19}$$

$$u(t) = \begin{cases} M_I(g(t) - g_b) + M_0 g_b \text{ if } M_I(g(t) - g_b + M_0 g_b > 0, \\ 0 \quad \text{otherwise}, 0 \le t \le t_{max} \end{cases} \tag{2-20}$$

where $c_1(t)$ is C-peptide concentration in the central (plasma) compartment, $c_2(t)$ is equivalent concentration in the peripheral compartment, k_{ij} are transfer rate constant per min, g(t) is plasma glucose concentration, g_b is fasting plasma glucose concentration, u(t) is secretion rate of C-peptide per unit volume of the central compartment and is

constrained to non-negative values and t_{max} is either 240 min or the time when plasma glucose returns to its fasting value.

M_I (postprandial sensitivity index) is the ability of postprandial glucose to stimulate β-cells. A change in plasma glucose by 1 mmol/L results in a change in the C-peptide secretion rate by M_I pmol/L min. M_0 (basal sensitivity index) is the ability of fasting glucose to stimulate β-cells. M_0 is numerically equal to the fasting C-peptide divided by the fasting plasma glucose concentration. The population model of C-peptide kinetics provides parameters k_{ij} of C-peptide kinetics from a subject's demographic data using a regression model and avoiding the need to assess C-peptide kinetics on an individual basis (142). The model uses the computer to analyse the plasma glucose and C-peptide during MTT. A sample of the graphs produced by the model is shown in Figure 2-4.

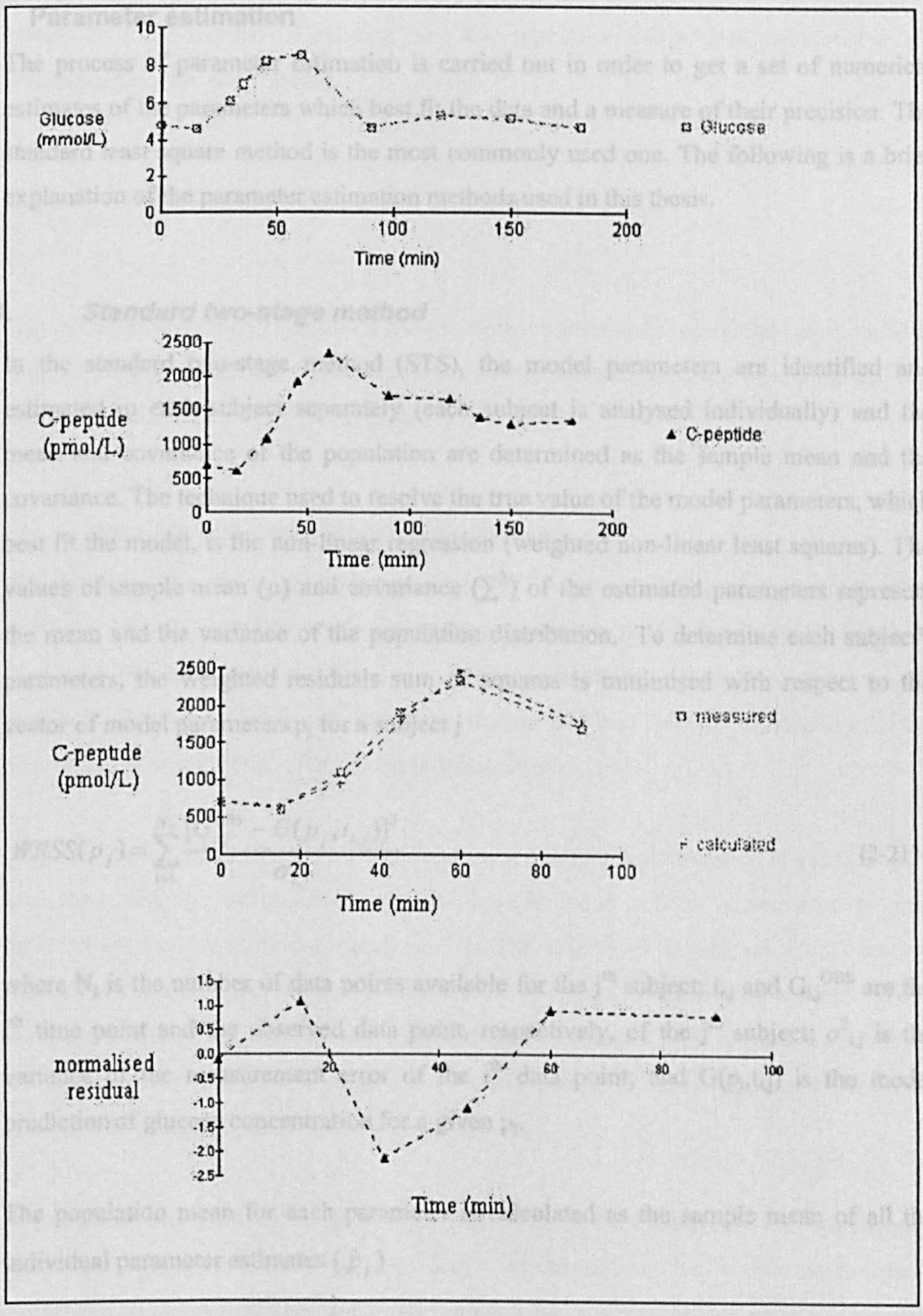

Figure 2-4. A sample of the graphs produced by the insulin secretion model.

2.5. Parameter estimation

The process of parameter estimation is carried out in order to get a set of numerical estimates of the parameters which best fit the data and a measure of their precision. The standard least square method is the most commonly used one. The following is a brief explanation of the parameter estimation methods used in this thesis.

2.5.1. Standard two-stage method

In the standard two-stage method (STS), the model parameters are identified and estimated in each subject separately (each subject is analysed individually) and the mean and covariance of the population are determined as the sample mean and the covariance. The technique used to resolve the true value of the model parameters, which best fit the model, is the non-linear regression (weighted non-linear least squares). The values of sample mean (μ) and covariance ($\sum^2$) of the estimated parameters represent the mean and the variance of the population distribution. To determine each subject's parameters, the weighted residuals sum of squares is minimised with respect to the vector of model parameters p_j for a subject j

$$WRSS(p_j) = \sum_{i=1}^{N_j} \frac{[G_{i,j}^{OBS} - G(p_j, t_{i,j})]^2}{\sigma_{i,j}^2} \qquad (2\text{-}21)$$

where N_j is the number of data points available for the j^{th} subject; $t_{i,j}$ and $G_{i,j}^{OBS}$ are the i^{th} time point and the observed data point, respectively, of the j^{th} subject; $\sigma^2_{i,j}$ is the variance of the measurement error of the i^{th} data point; and $G(p_j,t_{i,j})$ is the model prediction of glucose concentration for a given p_j.

The population mean for each parameter is calculated as the sample mean of all the individual parameter estimates ($\hat{p}_j$)

$$\mu_{STS} = \frac{1}{N}\sum_{j=1}^{N}\hat{p}_j \qquad (2\text{-}22)$$

where N is the number of subjects, and the population variance is calculated as the corresponding sample variance

$$\Sigma^2_{STS}=\frac{1}{N-1}\sum_{j=1}^{N}(\hat{p}_j-\mu_{STS})(\hat{p}_j-\mu_{STS})^T \qquad (2\text{-}23)$$

The STS method simply calculates the population statistics based on the best fit of each subject parameters independently. However, large between-subjects variability can lead to errors in the statistics for the population (143; 144).

2.5.2. *Iterative two-stage population analysis*

The iterative two-stage population analysis (ITS) is a methodology used to quantify between-subject variability relative to a given population model. It helps solving crucial problems in clinical studies such as parameter estimations with few sampling points, similarly in physiological and metabolic studies like the IVGTT moving out of the investigative stage into clinical and population studies (145).

The method calculates the population mean and the standard deviation at each iteration and then uses the information from the sample mean and the covariance as prior information for the individual analysis. The use of a prior in the population analysis should improve the precision of the individual estimates and provides a more reliable measure of the population parameters (144). Such estimates can be performed, despite the fact that the number of data points obtained from each individual may be less than the number of model parameters.

The ITS is based on the concepts of population prior knowledge and maximum a posteriori (MAP) probability empirical Bayesian estimation. The process starts with an initialisation step where the mean and the covariance are estimated as in the standard two-stage. The next step is to perform the parameter estimation on each subject j again, but this time minimising the following extended MAP Bayesian objective function with respect to p_j

$$MAP(p_j) = \sum_{i=1}^{N_i} \frac{[G_{i,j}^{OBS} - G(p_j, t_{i,j})]^2}{\sigma_{i,j}^2} + \sum_{i=1}^{N_p} \frac{[\mu_i(k) - p_{j,i}]^2}{\Sigma_{i,i}^2(k)} \quad (2\text{-}24)$$

where $p_{j,i}$ is the i^{th} element of the parameter vector for subject j and N_p is the number of elements in the vector p_j. The estimate $\hat{p}_j$ obtained by minimising this objective function is often called post hoc, or empirical Bayesian estimate. $\mu_i(k)$ is the value of the population mean at the k^{th} iteration of the method, and $\sum_{i,i}(k)$ is the i^{th} diagonal element of the population covariance matrix at the k^{th} iteration. The updated population mean of the parameter vector can then be calculated as:

$$\mu(k+1) = \frac{1}{N} \sum_{j=1}^{N} \hat{p}_j(k) \quad (2\text{-}25)$$

and the covariance is calculated as:

$$\Sigma^2(k+1) = \frac{1}{N} \sum_{j=1}^{N} \{V_j(k) + [\hat{p}_j(k) - \mu(k+1)][\hat{p}_j(k) - \mu(k+1)]^T\} \quad (2\text{-}26)$$

where V_j is the variance (precision) of the resulting estimate $\hat{p}_j$ of p_j.

This approach can minimise the sampling requirements from each individual dramatically. The iterative two-stage method assumes that the distribution of the population parameters is multivariate normal. However it is expected to perform well even if the assumptions of normality is not fully met (146; 147).

2.5.3. *Bayesian estimation*

Bayesian analysis is based on the idea that unknown quantities such as population mean and distribution have a probability distribution. This probability distribution for a population is based on the available prior knowledge about the population, then adding the knowledge which comes from the data set. The prior knowledge could be

summarised as the mean and the standard deviation or variance, which is constructing on a priori distribution. The data set is then used to refine and modify the prior probability distribution, which then results in the posterior distribution. The modified probability $\pi(p)$ is obtained according to the Bayes theorem:

$$\pi(p) = p(p|y) = \frac{p(y|p)p(p)}{p(y)} \qquad (2\text{-}27)$$

where $y = y_1 \ldots y_N$ is the vector of measurements, p(p) is the prior probability of parameters, p(y) is the prior probability of measurements, and p(y|p) is the conditional probability of measurements given data (148).

The great influence of the prior distribution on the results could be a major limitation of the Bayesian approach (149; 150).

3. Reduced sampling schemes for estimating pancreatic responsiveness during meal tolerance test

3.1. Introduction

Population studies for the assessment of β-cell function in subjects at risk of and with type 2 diabetes (T2D) are of primary importance to understand the disease aetiology and design preventative strategies. Most of the tools and models available for assessing the β-cell function are costly and labour and time consuming such as the hyperglycaemic clamp (79), and minimal model of C-peptide secretion during intravenous glucose tolerance test (123). Thus the in-depth examinations of β-cell function are limited to research studies.

The availability of a simple and easy model-based approach to quantify β-cell function will extend its use to population and clinical utilization which should help in predicting and understanding the progression of the disease.

The insulin secretion model (ISM) with the meal tolerance test (MTT) is a simple relatively non-invasive tool to investigate pancreatic responsiveness (32). It measures the prehepatic insulin secretion and assesses pancreatic β-cell responsiveness giving postprandial pancreatic β-cell responsiveness (M_1) and basal pancreatic β-cell responsiveness (M_0).

Different sampling schemes were used in the process of estimating pancreatic β-cell responsiveness with the insulin secretion model. The full sampling scheme employed in model development consisted of 14 samples over four hours at 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210 and 240 min as determined by clinical personnel when designing the experimental protocol.

A sampling scheme consisting of nine samples has also been used and will be used in the course of studies in this thesis (51) but its performance with the model is currently unknown. A reduced five sample scheme over two hours at 0, 30, 60, 90, and 120 min (151) will further reduce the time, labour and cost, facilitating a wide use of the model.

In the present study, the pancreatic β-cell responsiveness indices (M_1 and M_0) were evaluated with two reduced sampling schemes (nine samples and five samples) and compared against indices obtained during the full sampling scheme.

3.2. Methods

3.2.1. *Subjects and experimental design*

A total of twenty one subjects with newly presenting Type 2 diabetes participated in the study, see Table 3-1. Ethical approval for the studies was obtained from the South Glamorgan Local Research Ethics Committee, Cardiff, UK.

The subjects were admitted on the study day to the Diabetes Research Unit, Llandough Hospital (Penarth, UK). Each subject underwent the meal tolerance test to assess parameters of carbohydrate metabolism and to measure glucose, insulin, and C-peptide. The subjects were studied after an overnight fast for 12 hours.

Table 3-1. Demographic data for subjects participating in the study (N = 21).

	Sex (M/F)	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	16/5	52	1.71	87.9	30.4
SE		2	0.02	4.2	1.2

3.2.2. *Meal tolerance test*

The standard meal tolerance test (MTT) consisted of digesting 15g Weetabix, 10g skimmed milk, 250mL pineapple juice, 50g white meat chicken, 60g wholemeal bread, 10g polyunsaturated margarine (75g carbohydrates; total 500 Cal; calorie contribution: 58% carbohydrate, 23% fat and 19% protein) (49). The subjects were required to consume the whole meal within 10 minutes. In total 15 blood samples were taken over 240 minutes to measure plasma glucose, insulin and C-peptide, samples were taken at –30, 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210 and 240 minutes relative to meal ingestion. At each sample time the infusion is stopped and the first 2-ml blood withdrawn and discarded, prior to obtaining the sample for assay.

Blood was taken via an indwelling intravenous (IV) cannula which was inserted into the antecubital fossa vein and connected via a three-way tap to a slow running saline (0.154nmol/l) infusion to maintain the patency of vein.

3.2.3. *Sample Analysis*

Glucose was assayed using the glucose oxidase method (intra-assay CV < 2%). Insulin and C-peptide were assayed using a monoclonal antibodies method (intra-assay CV < 5% and < 6%, respectively). Insulin in plasma is measured by an enzyme immunoassay which does not cross- react with other insulin-like components, and C-peptide in plasma is also measured by an enzyme immunoassay which does not cross-react with insulin.

Following blood sampling the samples were separated as soon as possible. Blood was centrifuged (2000g, 5min) in a refrigerated (4°C) centrifuge and the plasma put into aliquots and frozen at -20° C immediately. Samples remained frozen until assay.

3.2.4. *Insulin secretion model*

The insulin secretion model was used to quantify pancreatic β-cell responsiveness from MTT data, providing fasting β-cell responsiveness (M_0; ability of fasting glucose to stimulate C-peptide secretion) and postprandial β-cell responsiveness (M_I; ability of postprandial glucose to stimulate C-peptide secretion) (32).

The package used to calculate M_0 and M_I was version 1.0 of CPR (Calculating Pancreatic Responsiveness; written by R. Hovorka and H.C. Subasinghe, MIM Centre, City University, London, UK, 1997).

The model parameters M_0 and M_I were estimated employing weighted non-linear regression analysis. The measured errors wereassumed to be uncorrelated, with zero mean and a constant coefficient of variance (CV = 6%). The precision of the parameters was obtained from the inverse of the Fisher information matrix and expressed as CV of the parameter estimates. The model is fully described in section (2.4.6).

3.2.5. *Sampling schemes*

3.2.5.1. Full sampling scheme

The full sampling scheme consisted of 14 samples at 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210 and 240 min relative to meal ingestion. The full sampling scheme was employed by the insulin secretion model to estimate $M_{1\text{-full}}$ and $M_{0\text{-full}}$. Parameter estimation results (estimates and precision) obtained with the full sampling scheme were used as the reference measurements.

3.2.5.2. Nine-sample scheme

The nine-sample is a reduced sampling scheme consisted of 9 samples at 0, 30, 60, 90, 120, 150, 180, 210 and 240 min relative to the meal ingestion. The nine-sample scheme was used to estimate the fasting ($M_{0\text{-}9}$) and postprandial ($M_{1\text{-}9}$) pancreatic β-cell responsiveness indices.

3.2.5.3. Five-sample scheme

A further reduced sampling scheme consisted of 5 samples at 0, 30, 60, 90, and 120 min relative to the meal ingestion. This sampling scheme was used to estimate the indices; $M_{1\text{-}5}$ and $M_{0\text{-}5}$.

3.2.6. *Statistical analysis*

The Pearson correlation coefficient was used to assess the relationship between indices. A paired sample t-test was applied to assess the difference between indices. Significance was declared at $P < 0.05$. The precision of estimation with respect to the reference measurement, and the agreement between variables was assessed by the Bland-Altman plots (plotting the difference between methods [Y-axis] against the reference method [X-axis]) (152). A 20% region of agreement was defined reflecting the day-to-day variability of fasting plasma glucose (FPG), insulin (FPI), and postprandial plasma glucose and insulin responses to a mixed meal. In subjects with type 2 diabetes, a 15% FPG within subjects variation was reported (153) and 20% for

FPI (154). The postmeal glucose and insulin variation were 15% and 20%, respectively (155). A 20% variation should not affect the discrepancy between healthy and type 2 diabetic subjects (32; 141).

3.3. Results

3.3.1. *Plasma glucose, insulin, and C-peptide*

Plasma glucose, insulin, and C-peptide profiles during MTT are shown in Figure 3-1. The data are presented as mean ± SE.

3.3.2. *Parameter estimates with nine-sample, five-sample and full sampling schemes*

Individual estimates of the pancreatic responsiveness indices with the full sampling ($M_{I\text{-}full}$ and $M_{0\text{-}full}$), and the reduced sampling schemes ($M_{I\text{-}9}$, $M_{0\text{-}9}$, $M_{I\text{-}5}$ and $M_{0\text{-}5}$) are given in Table 3-2. The parameters were estimated with good precision with the reduced sampling schemes (mean CV $\leq$ 9%, and < 6% for M_I and M_0, respectively), and with the full sampling scheme. The precision of the M_I estimates with the full sampling was slightly better than with the reduced schemes, see Table 3-2. Postparandial and fasting β-cell responsiveness with full sampling, nine-sample, and five-sample schemes are summarised in Figure 3-2.

3.3.3. *Evaluation of nine-sample indices*

$M_{I\text{-}9}$ was significantly correlated with $M_{I\text{-}full}$ (r_S = 0.99, P < 0.001). However it was significantly higher than $M_{I\text{-}full}$ (P < 0.05), see Table 3-3. The observed statistical significant does not imply the clinical significance as demonstrated in Figure 3-3, as most of the cases (19 out of 21) are lying within the predefined 20% clinical region of agreement. No systemic deviation or error trend was observed during the examination of the difference between $M_{I\text{-}9}$ and $M_{I\text{-}full}$. The scatter plot in Figure 3-5 shows the estimates' distribution around the equality line.

$M_{0\text{-}9}$ was significantly correlated with $M_{0\text{-}full}$ (r_S = 0.98, P < 0.001), and the individual estimates of $M_{0\text{-}9}$ were similar to $M_{0\text{-}full}$ (P = NS). All $M_{0\text{-}9}$ estimates lied within the predefined clinical region of agreement (Figure 3-3).

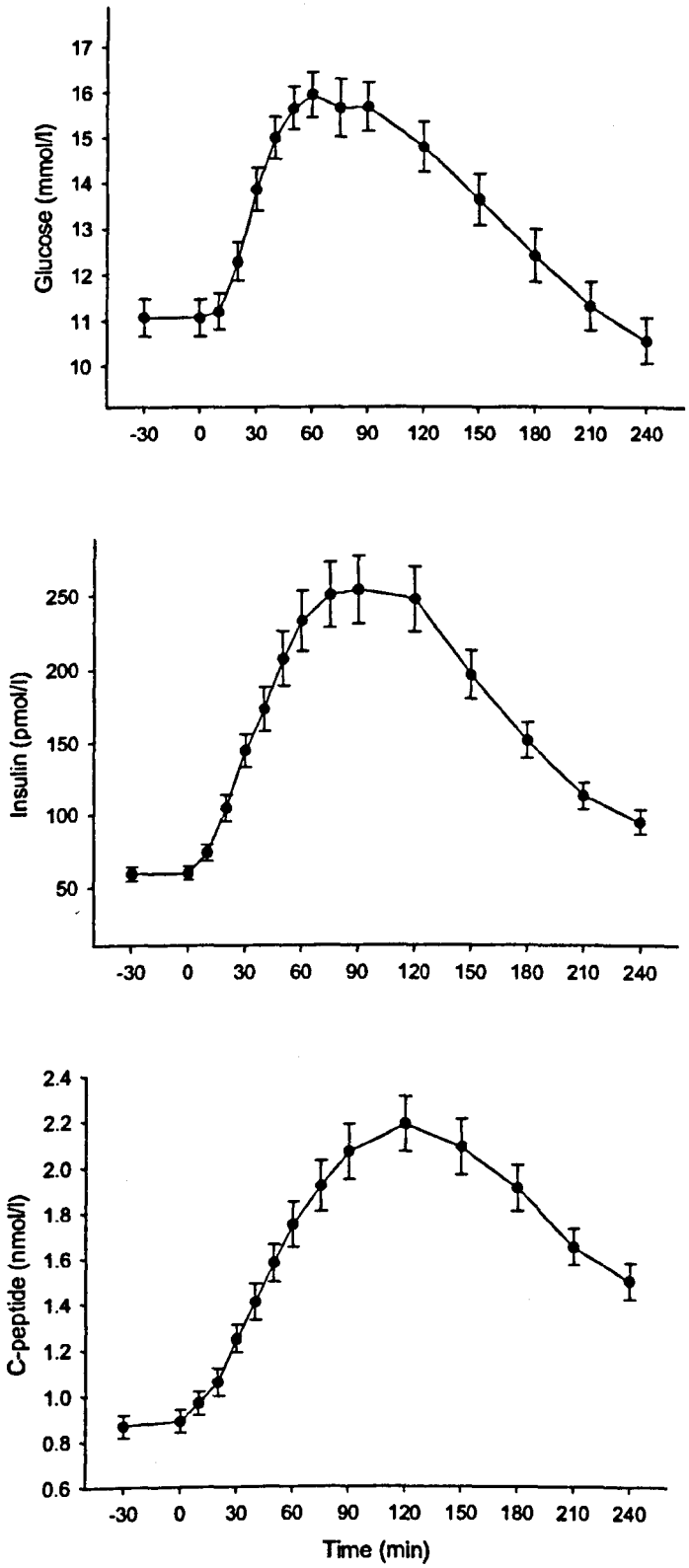

Figure 3-1. Plasma glucose, insulin, and C-peptide profiles during MTT in subjects with T2D (mean ± SE, N = 20).

Table 3-2. Parameter estimates with nine-sample, five-sample, and full sampling schemes in subjects with T2D (N = 21).

	Full-Sample				Nine-Sample				Five-Sample			
Sub. No.	$M_{I\text{-}full}$ (10^{-9} x min^{-1})	CV %	$M_{O\text{-}full}$ (10^{-9} x min^{-1})	CV %	$M_{I\text{-}9}$ (10^{-9} x min^{-1})	CV %	$M_{O\text{-}9}$ (10^{-9} x min^{-1})	CV %	$M_{I\text{-}5}$ (10^{-9} x min^{-1})	CV %	$M_{O\text{-}5}$ (10^{-9} x min^{-1})	CV %
606	21.2	8*	7.7	3	21.7	9	7.6	4	22.9	10	7.0	5
607	8.5	7	3.7	3	9.6	8	3.6	5	8.5	11	3.6	5
608	8.6	8	2.9	3	9.1	10	2.9	5	7.5	13	2.7	5
610	17.5	7	8.1	3	17.0	9	8.2	5	16.9	11	7.7	5
612	15.4	7	6.4	3	18.2	7	5.6	5	16.4	9	5.4	6
616	11.5	5	5.4	3	11.5	6	5.5	5	13.2	7	5.3	5
618	26.4	6	7.8	3	26.4	8	8.3	5	26.7	8	7.7	6
624	28.5	4	3.5	3	32.5	5	3.4	5	30.1	5	3.2	6
626	22.4	4	5.0	3	22.9	5	5.1	5	21.1	7	4.8	6
638	17.5	6	6.0	3	18.1	7	6.0	5	16.6	9	5.8	5
639	37.4	5	10.4	3	37.9	6	10.9	5	36.0	7	10.5	5
647	26.2	6	8.2	3	28.4	6	8.2	5	24.0	8	7.7	5
649	21.8	6	4.2	3	21.7	8	4.3	5	22.0	8	3.9	6
651	16.1	6	3.6	3	20.4	7	3.2	5	18.2	8	2.9	5
655	16.5	7	7.4	3	17.5	8	7.2	5	16.9	10	7.0	5
660	4.5	6	2.4	3	4.9	7	2.3	5	4.3	11	2.3	5
672	15.2	4	3.0	3	16.5	5	3.3	4	15.0	6	3.0	5
673	18.9	7	7.4	3	18.7	10	7.1	5	21.3	10	7.4	5
674	12.2	5	4.7	3	14.8	5	4.2	5	12.9	7	3.9	5
678	7.4	15	3.2	4	7.5	21	3.3	6	8.2	20	3.4	5
680	21.1	6	9.4	3	21.2	7	9.3	5	20.3	9	9.2	5
Mean	17.9	6	5.7	3	18.9	8	5.7	5	18.0	9	5.5	5
SE	1.7	1	0.5	0	1.8	1	0.5	0	1.7	1	0.5	0

*Precision of parameters estimates expressed as coefficient of variation (CV)

3.3.4. *Evaluation five-sample indices*

$M_{I\text{-}5}$ was significantly correlated with $M_{I\text{-}full}$ (r_S = 0.99, P < 0.001), and was not statistically different (P = NS). The individual estimates of $M_{I\text{-}5}$ and $M_{I\text{-}full}$ were quite similar as the differences between individual estimates were quite well within the agreed level of variation, see Figure 3-4.

$M_{0\text{-}5}$ was significantly correlated with $M_{0\text{-}full}$ (r_S = 0.99, P < 0.001), but it was significantly lower than $M_{0\text{-}full}$ (P < 0.05). However $M_{0\text{-}5}$ were not clinically different from the full sampling estimates. The Bland-Altman plot in Figure 3-4 shows no systematic deviation in the parameter estimates with the five-sample scheme. All individual estimates of $M_{0\text{-}5}$ were within the clinically predefined 20% region of agreement. The tight association and agreement between the five-sample indices with the full sampling indices are shown in Figure 3-6.

Table 3-3. Paired sample t-test and Pearson correlation with Bonferroni adjustment between pancreatic responsiveness indices (M_I and M_0) with nine-sample, five-sample and full sampling schemes in subjects with T2D (N = 21).

	Pearson correlation		T-test	
	Correlation	P-value	t	P-value
$M_{I\text{-}full}$ - $M_{I\text{-}9}$	0.99	0.000	-3.46	0.012
$M_{0\text{-}full}$ - $M_{0\text{-}9}$	0.98	0.000	0.83	0.416
$M_{I\text{-}full}$ - $M_{I\text{-}5}$	0.99	0.000	-0.69	0.501
$M_{0\text{-}full}$ - $M_{0\text{-}5}$	0.99	0.000	4.15	0.003

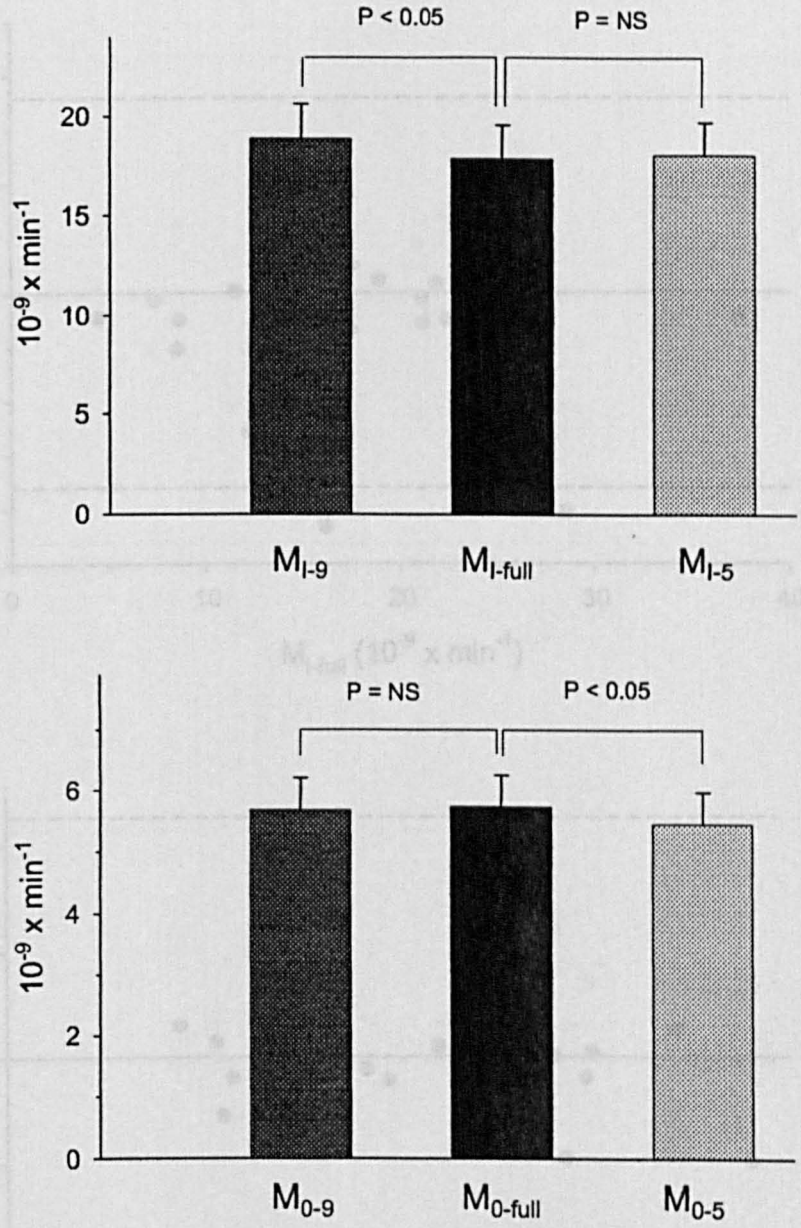

Figure 3-2. Postparandial ($M_{I\text{-}full}$, $M_{I\text{-}9}$, and $M_{I\text{-}5}$) and fasting ($M_{0\text{-}full}$, $M_{0\text{-}9}$, and $M_{0\text{-}5}$) β-cell responsiveness with full sampling, nine-sample, and five-sample schemes in subjects with T2D (mean ± SE, N= 21).

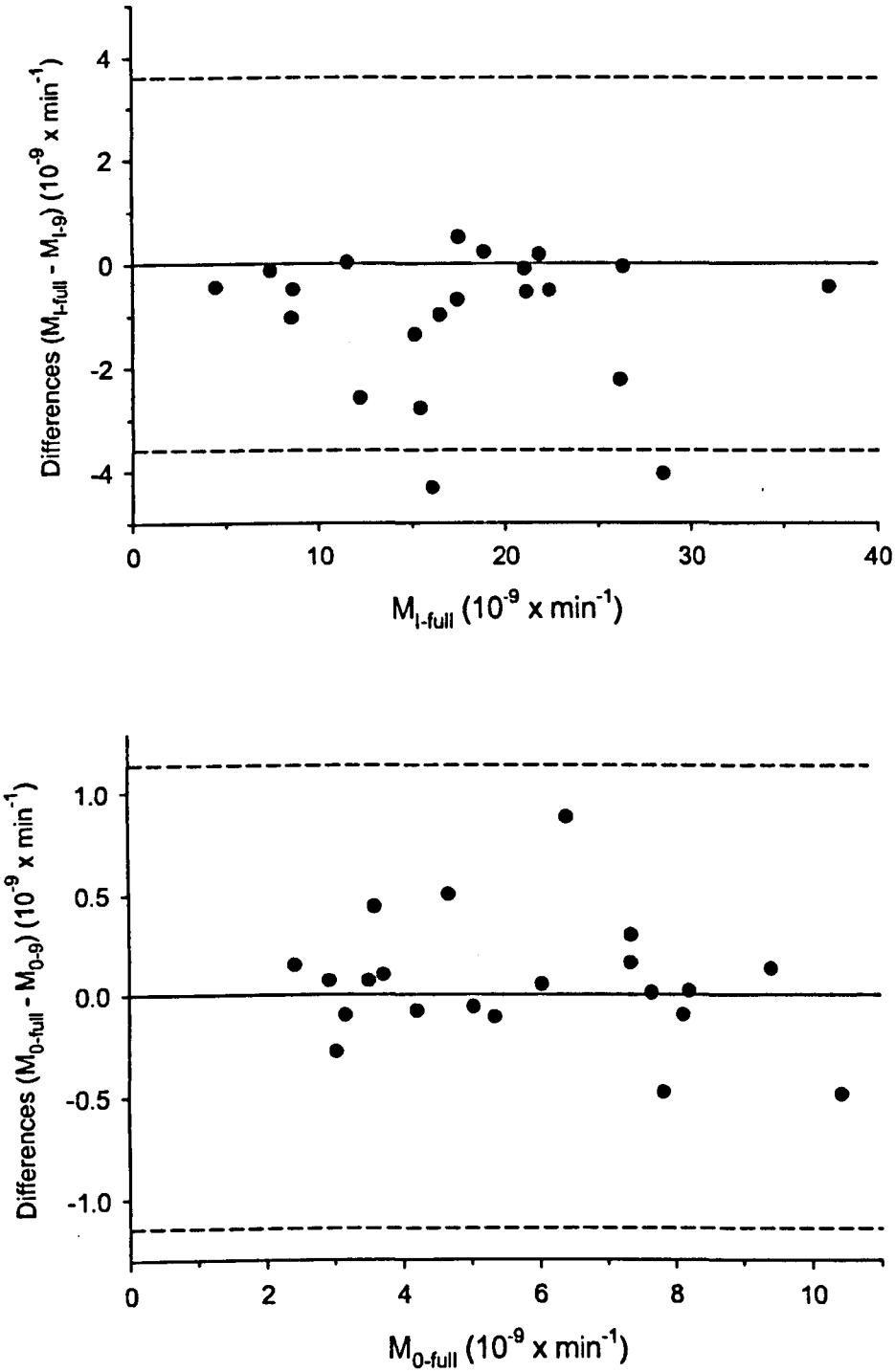

Figure 3-3. Comparison of postprandial (top) and fasting (bottom) β-cell responsiveness with full sampling and nine-sample indices in subjects with T2D. Differences between methods (y-axis) against the full sampling index (x-axis) are plotted. Dashed lines indict the region of agreement.

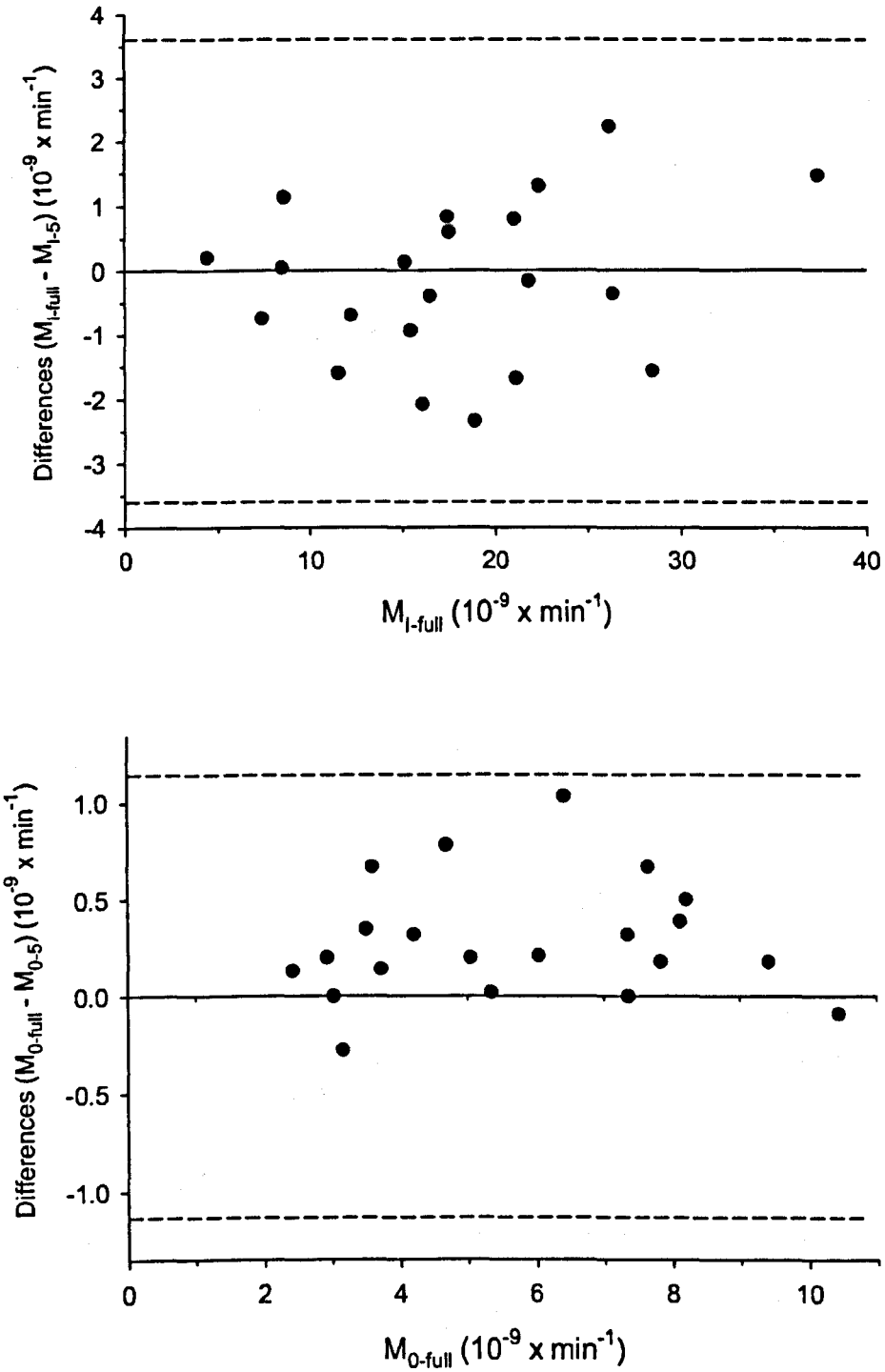

Figure 3-4. Comparison of postprandial (top), and fasting (bottom) β-cell responsiveness with full sampling and five-sample indices in subjects with T2D. Differences between methods (y-axis) against the full sampling index (x-axis) are plotted. Dashed lines indict the region of agreement.

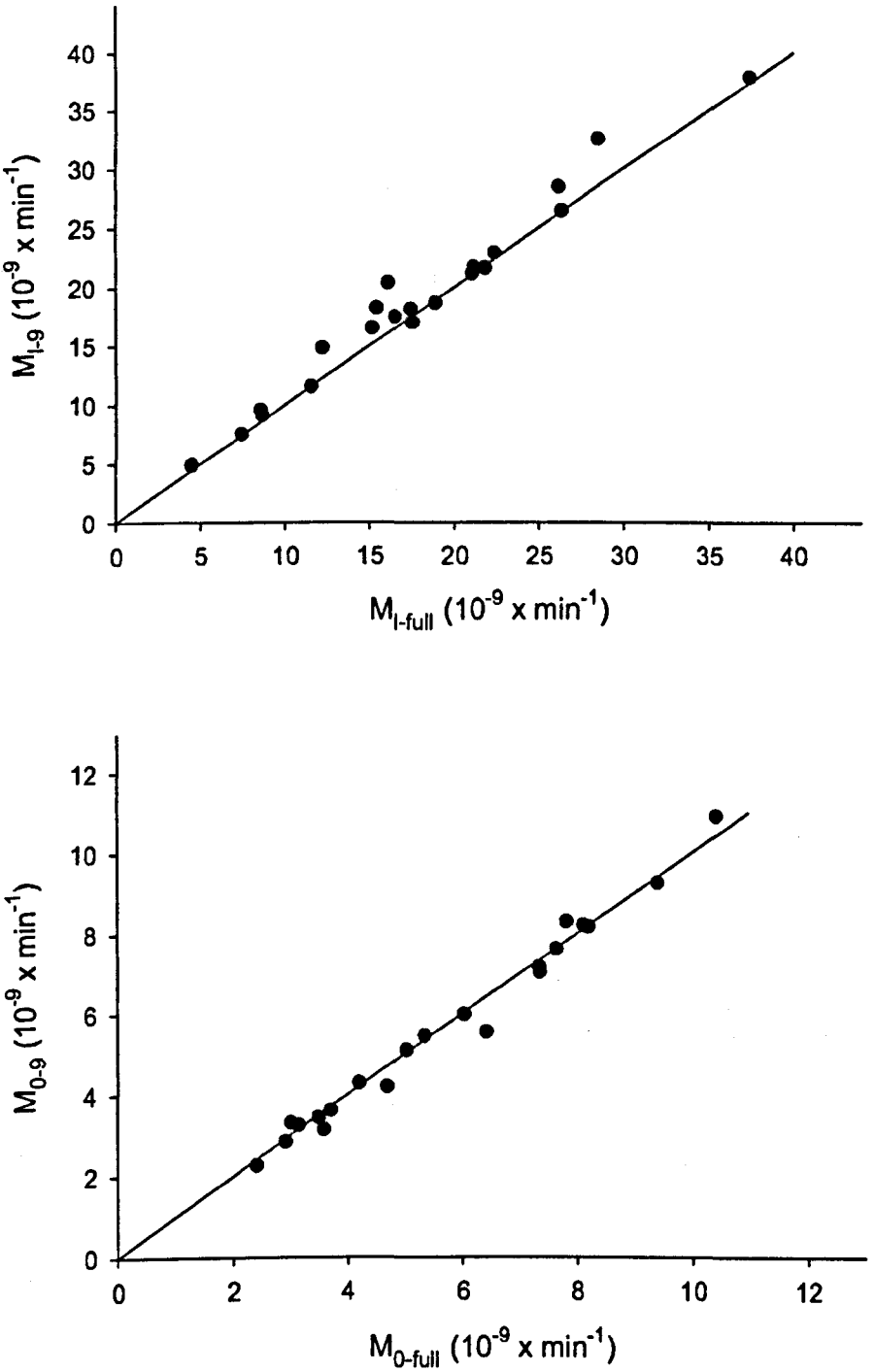

Figure 3-5. Relationship between individual estimates of postprandial pancreatic β-cell responsiveness indices with full sampling ($M_{I\text{-}full}$), and nine-sample ($M_{I\text{-}9}$) schemes (top panel), and between fasting pancreatic β-cell responsiveness indices with full sampling ($M_{0\text{-}full}$), and nine-sample ($M_{0\text{-}9}$) schemes (bottom panel), (N = 21). A unity line is shown.

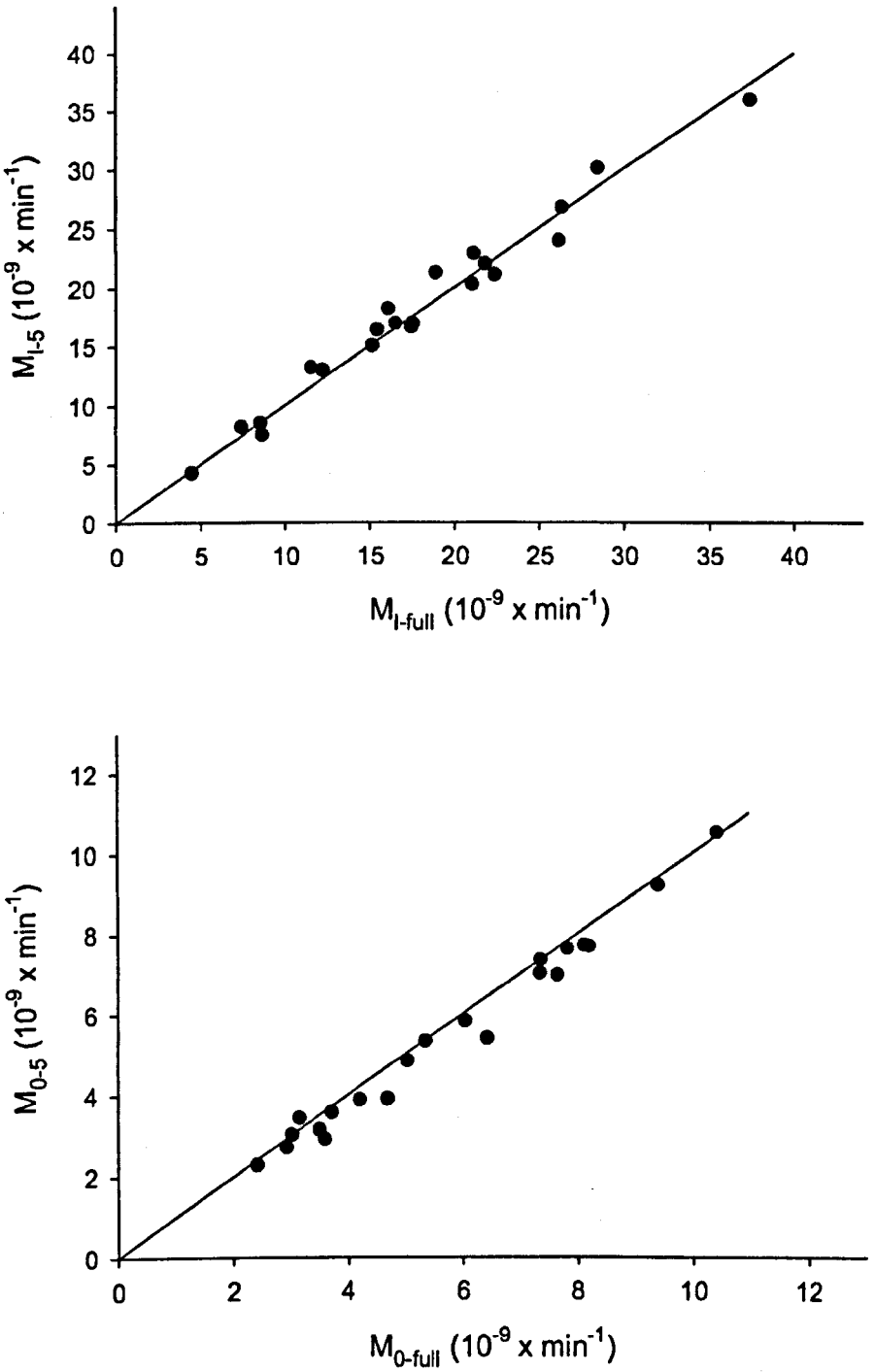

Figure 3-6. Relationship between individual estimates of postprandial pancreatic β-cell responsiveness indices with full sampling ($M_{I\text{-}full}$), and five-sample ($M_{I\text{-}5}$) schemes (top panel), and between fasting pancreatic β-cell responsiveness indices with full sampling ($M_{0\text{-}full}$), and five-sample ($M_{0\text{-}5}$) schemes (bottom panel), (N = 21). A unity line is shown.

3.4. Discussion

In the present study, ISM indices of pancreatic β-cell responsiveness were successfully estimated and evaluated during MTT with nine-sample and five-sample schemes. Reduction in sample number from 14 to 9 and 5 did not have any significant effect on the clinical utility and the calculation and precision of the β-cell responsiveness indices M_1 and M_0. The reduced sample schedules did not produce any observed clinical bias on the calculated value of M_1 and M_0.

To derive reliable estimates of β-cell responsiveness during MTT, a rich data set was initially used with 14 samples taken over four hours. However a sampling scheme with nine samples is in use (49), and a standard five sample scheme (151) is widely adopted, reducing the cost, time and labour.

A 20% clinical region of agreement was defined depending on reported variation in FPG, FPI, and postprandial glucose and insulin responses. Ollerton et al (153) reported a ±15% FPG day-to-day variability. They suggested that 14% of the variability is due to the biological variability in 193 subjects with newly diagnosed type 2 diabetes (153). In eight subjects with mild diabetes Wolever et al (154) reported about 20% fasting and postmeal plasma insulin within-subject variability. They reported 11% as an overall mean plasma glucose within-subject variation after meal (154).

Nine-sample scheme

ISM pancreatic β-cell responsiveness parameters ($M_{1\text{-}9}$ and $M_{0\text{-}9}$) during MTT were estimated with good precision with the nine-sample scheme. $M_{1\text{-}9}$ was significantly correlated with $M_{1\text{-}full}$, but was statistically higher than $M_{1\text{-}full}$ ($P < 0.05$). However $M_{1\text{-}9}$ was not clinically different from the $M_{1\text{-}full}$ and the difference between the index measurements with the two schemes was within the predefined clinical accepted range. No systematic bias was observed in calculating M_1 from nine-sample scheme as demonstrated by the Bland-Altman plot. $M_{0\text{-}9}$ was well correlated with and identical to $M_{0\text{-}full}$.

Five-sample scheme

The five-sample scheme was sufficient and rich enough to get reliable estimates of both $M_{1\text{-}5}$ and $M_{0\text{-}5}$. The model was able to fit the data and to provide pancreatic β-cell

responsiveness parameters during MTT with good precision (mean CV ≤ 9%). $M_{1\text{-}5}$ was significantly correlated with and identical to $M_{1\text{-}full}$ and the differences between the individual estimates of indices were within the predefined variation level.

$M_{0\text{-}5}$ was significantly correlated with $M_{0\text{-}full}$, but was statistically lower ($P < 0.05$). However it was not clinically different, the differences between the individual estimates were all within the clinically accepted range. The $M_{0\text{-}5}$ individual estimates are in agreement with the reported range in subjects with newly diagnosed T2D (32; 141). The $M_{0\text{-}5}$ estimates were not biased, or different from $M_{0\text{-}full}$ as documented by the Bland-Altman plot. No systemic trend or deviation was observed (see Figure 3-4).

3.5. Summary

Nine-sample and five-sample reduced sampling schemes were proposed and successfully evaluated against the full sampling scheme. Reduction in sample number did not have any significant effect on the calculation and precision of the β-cell responsiveness indices providing accurate estimates of both M_1 and M_0.

4. Validation of the insulin secretion model during OGTT in healthy subjects and subjects with type 2 diabetes

4.1. Introduction

Type 2 diabetes (T2D) is characterised by various degree of β-cell defect (64), and it is well accepted that a substantial loss of β-cell function should be present for a hyperglycaemia to appear in type 2 diabetes (60). In addition, individuals at high risk of diabetes demonstrated a diminished β-cell function while maintaining normal glucose tolerance (62; 63; 155).

The availability of β-cell function indices is important for understanding and identifying with other factors, such as insulin sensitivity the aetiology of type 2 diabetes. A reliable quantification of β-cell functions and insulin resistance could make it possible to predict the disease progression. Various methods and approaches have been developed to assess pancreatic β-cell function including the hyperglycaemic clamp (77), minimal model of C-peptide secretion during intravenous glucose tolerance test (123), combined model of insulin and C-peptide secretion (156), Low-dose insulin and glucose-infusion test (157), Homeostasis Model Assessment (HOMA) (85), and Continuous Infusion of Glucose with Model Assessment (CIGMA) (79), in addition to several other simple one measurement and simple mathematical calculations.

The insulin secretion model (ISM) is a non-invasive model-based method developed to quantify pancreatic β-cell responsiveness during a meal tolerance test (MTT) (32). The model calculates two indices of pancreatic responsiveness. It measures the prehepatic insulin secretion and assesses pancreatic β-cell responsiveness giving fasting pancreatic β-cell responsiveness (M_0; ability of fasting glucose to stimulate C-peptide secretion) and postprandial responsiveness (M_1; ability of postprandial glucose to stimulate C-peptide secretion). The model is able to quantify pancreatic responsiveness in healthy and disease states, and confirms significant differences between healthy subjects and subjects with newly diagnosed type 2 diabetes (141).

MTT is used because it results in a typical postprandial exposure of the pancreas to glucose and gut and vagal hormones.(158). However the oral glucose tolerance test

(OGTT) is the most widely used test because of its simplicity, its ease of implementation and the fact that it is easy to standardised. In addition OGTT is the standard diagnostic test of type 2 diabetes and impaired glucose tolerance (IGT) approved by the World Health Organisation (WHO) (1).

The insulin secretion model was developed and validated during MTT (32). In the present study we validated the insulin secretion model during OGTT in healthy subjects and subjects with T2D. Comparisons between the ISM estimates, and glucose, insulin, and C-peptide responses during MTT and OGTT were carried out.

MTT has the advantage of increased the stimulatory effect of incretin as a result of the meal composition (see Methodology). However, both MTT and OGTT represent a physiological stimulation and represent a typical postprandial exposure of pancreas to glucose and gut and vagal hormones, and OGTT is expected to perform in a comparable manner to MTT.

4.2. Methods

4.2.1. *Subjects and experimental design*

Two groups of subjects were studied, namely a healthy group and a T2D group having subjects with newly presenting type 2 diabetes (Table 4-1, and 4-2, respectively). Each group underwent a meal tolerance test and an oral glucose tolerance test on two occasions one week apart. The subjects were admitted on the study day to the Diabetes Research Unit, Llandough Hospital, Penarth, UK. The subjects were studied after an overnight 12 hour fast. The ethics approval for the studies was obtained from the South Glamorgan Local Research Ethics Committee, Cardiff, UK.

Table 4-1. Demographic data for healthy subjects (N = 9).

	Sex (M/F)	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	9/0	28	1.80	78.1	24.2
SE		2	0.02	1.5	0.5

Table 4-2. Demographic data for subjects with newly presenting T2D (N = 20).

	Sex (M/F)	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	17/3	55	1.71	83.1	28.6
SE		2	0.02	2.3	0.8

4.2.1.1. Meal tolerance test

The meal tolerance test consisted of digestion of 15g Weetabix, 10g skimmed milk, 250mL pineapple juice, 50g white meat chicken, 60g wholemeal bread, 10g polyunsaturated margarine (75g carbohydrates; total 500 Cal; calorie contribution: 58% carbohydrate, 23% fat and 19% protein) (49). The subjects were required to consume the whole meal within 10 min.

Samples were taken over 240 minutes in addition to another two samples withdrawn at fasting states to measure glucose, IRI and C-peptide. The samples were taken at -30, 0, 30, 60, 90, 120, 150, 180, 210, and 240 min in T2D group. The sampling in the healthy

group had an additional five samples at 10, 15, 20, 25, and 45 min, relative to meal ingestion. At each sample time the infusion is stopped and the first 2-ml blood withdrawn and discarded, prior to obtaining the sample for assay.

4.2.1.2. Oral glucose tolerance test

The oral glucose tolerance test consisted of 75g glucose syrup taken by mouth. Samples were taken over 240 min in addition to another two samples withdrawn at fasting states to measure glucose, IRI and C-peptide. The samples were taken at -30, 0, 30, 60, 90, 120, 150, 180, 210, and 240 min in T2D group. The sampling in the healthy group had an additional five samples at 10, 15, 20, 25 and 45 min, relative to glucose intake. At each sample time the infusion is stopped and the first 2-ml blood withdrawn and discarded, prior to obtaining the sample for assay.

4.2.1.3. Assay methods

Following blood sampling, the samples were separated as soon as possible. Blood was centrifuged (2000g, 5min) in a refrigerated (4°C) centrifuge and the plasma put into aliquots and frozen at -20° C immediately.

Glucose was assayed using the glucose oxidase method (intra-assay CV < 2%). Insulin and C-peptide were assayed using a conventional radioimmunoassay (intra-assay CV < 6%).

4.2.2. *Insulin secretion model*

The model provides two indices, M_1 (postprandial responsiveness index) represents the ability of postprandial glucose to stimulate the β-cell. M_0 (fasting responsiveness index) represents the ability of fasting glucose to stimulate β-cells. The model is comprehensively described in Chapter 2.

4.3. Data analysis

4.3.1. *Model validation and parameter estimation during OGTT*

To assess the adequacy of the ISM with OGTT in healthy subjects and subjects with T2D, we evaluated practical (a posteriori) identifiability, and goodness of fit (143). The following criteria were used to assess model validity:

- coefficient of variation (CV) measured as the fractional standard deviation (FSD) for the assessment of precision of parameter estimates

- distribution of normalised residual for the assessment of model ability to fit data (goodness of fit) considering the measurement errors, and to detect any systematic deviation between the data and the model prediction

- runs test to assess the distribution of the residuals and check for presence of model misfit.

The package used to calculate M_0 and M_1 was version 1.0 of CPR (Calculating Pancreatic Responsiveness; written by R. Hovorka and H.C. Subasinghe, MIM Centre, City University, London, UK 1997). The model parameters M_0 and M_1 were estimated employing weighted non-linear regression analysis. The measured errors were assumed to be uncorrelated, with zero mean and a constant coefficient of variance (CV = 6%). The precision of parameters was obtained from the inverse of the Fisher information matrix and expressed as CV of parameter estimates (32).

4.3.2. *Incremental area under curve*

In addition to the parameters obtained from the insulin secretion model, we evaluated an incremental area under the curve (AUC) from 0-90 minutes for glucose (AUC_G), insulin (AUC_I), and C-peptide (AUC_C) during both MTT and OGTT. The incremental response was used to eliminate the effect of the basal values on the analysis. The calculation was done over 0-90 min because glucose concentrations reach the basal level by 90 min in the majority of subjects. Fasting plasma glucose (FPG), insulin (FPI), and C-peptide

(FPC) were calculated as the mean of fasting values (at –30 and 0 min) during both MTT and OGTT

4.3.3. *Statistical analysis*

A Spearman correlation analysis with Boniferroni correction was carried out to assess relationships between indices. Wilcoxon signed ranks test was applied to assess the difference between the model indices and glucose, insulin, and C-peptide responses during MTT and OGTT.

4.4. Results

4.4.1. Healthy subjects

4.4.1.1. Plasma glucose, insulin, and C-peptide

Table 4-3 shows the individuals, fasting values of glucose, insulin, and C-peptide (HbA_{1C} is not available). No significant difference in fasting values (FPG, FPI, and FPC) was observed between the two test days (P = NS). Plasma glucose, insulin, and C-peptide profiles during OGTT and MTT are shown in Figure 4-1. During MTT and OGTT, the glucose and insulin levels reached their peak values at 30–60 minutes. However the glucose returned to the fasting level more quickly during MTT at 90 min. The glucose levels during OGTT remained elevated for longer and returned to fasting levels at 120–150 minutes.

Table 4-3. Fasting plasma glucose (FPG), insulin (FPI), and C-peptide (FPC) in healthy subjects.

Subject No.	FPG (mmol/l)	FPI (pmol/l)	FPC (nmol/l)
1	5.4*	73*	0.407*
2	5.5	20	0.232
3	5.2	35	0.360
4	5.5	44	0.338
5	4.9	34	0.258
6	5.2	24	0.465
7	5.6	40	0.288
8	5.9	59	0.257
9	5.2	39	0.283
Mean	5.4	41	0.321
SE	0.1	6	0.026

* Mean of fasting values on two study days

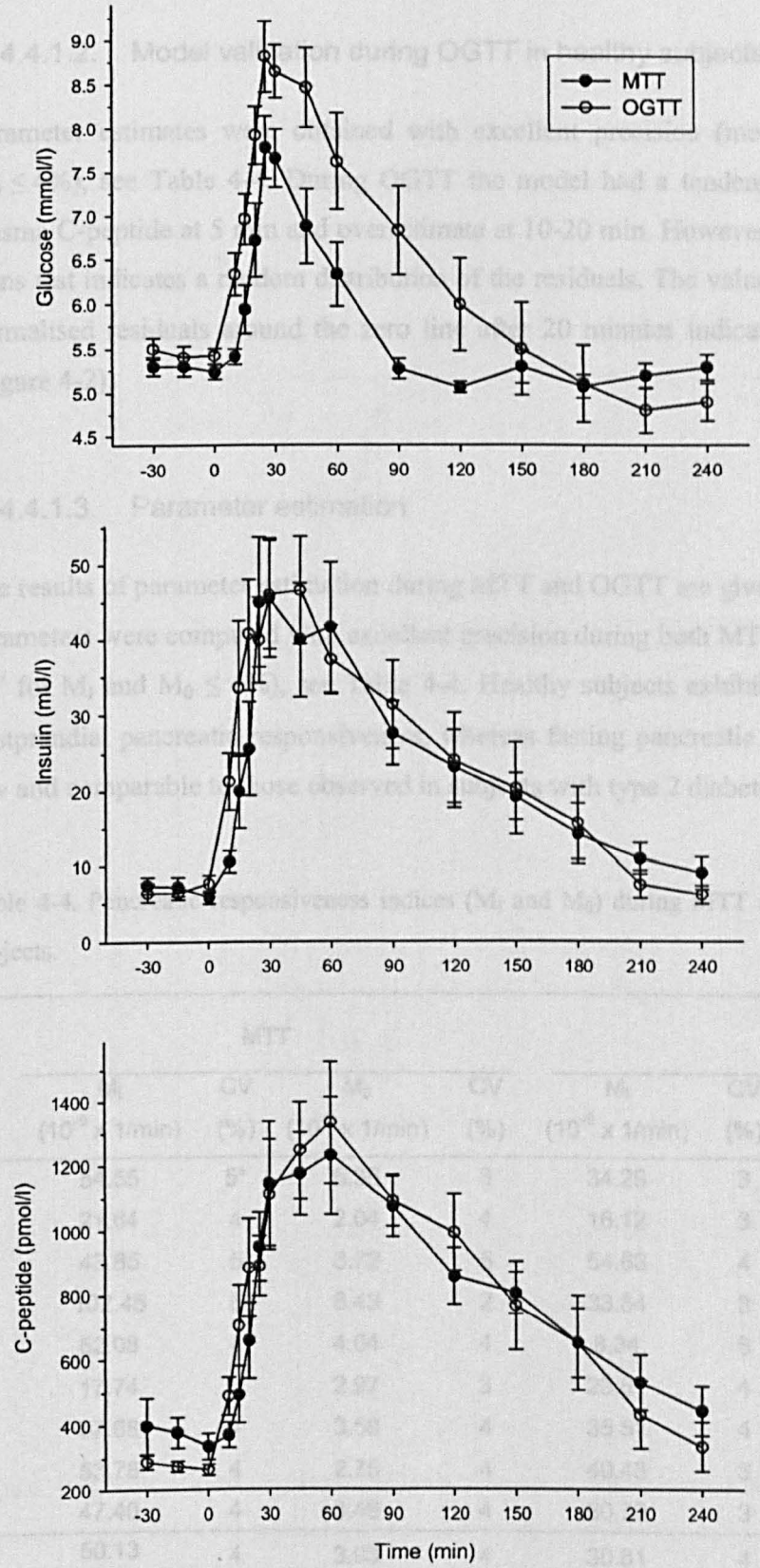

Figure 4-1. Plasma glucose, insulin, and C-peptide profiles in healthy subjects during OGTT and MTT (mean ± SE, N=9).

4.4.1.2. Model validation during OGTT in healthy subjects

Parameter estimates were obtained with excellent precision (mean CV for M_I and $M_0 \leq 4\%$), see Table 4-4. During OGTT the model had a tendency to underestimate plasma C-peptide at 5 min and overestimate at 10-20 min. However the non-significant Runs test indicates a random distribution of the residuals. The value and distribution of normalised residuals around the zero line after 20 minutes indicate a good model fit (Figure 4-2).

4.4.1.3. Parameter estimation

The results of parameter estimation during MTT and OGTT are given in Table 4-4. The parameters were computed with excellent precision during both MTT and OGTT (mean CV for M_I and $M_0 \leq 4\%$), see Table 4-4. Healthy subjects exhibited a wide range of postprandial pancreatic responsiveness, whereas fasting pancreatic responsiveness was low and comparable to those observed in subjects with type 2 diabetes (32).

Table 4-4. Pancreatic responsiveness indices (M_I and M_0) during MTT and OGTT in healthy subjects.

Subject No.	MTT				OGTT			
	M_I (10^{-9} x 1/min)	CV (%)	M_0 (10^{-9} x 1/min)	CV (%)	M_I (10^{-9} x 1/min)	CV (%)	M_0 (10^{-9} x 1/min)	CV (%)
1	54.55	5*	6.36	3	34.29	3	3.22	4
2	21.64	4	2.04	4	16.12	3	3.55	4
3	43.85	5	3.72	5	54.63	4	4.88	5
4	102.45	5	6.43	2	33.64	3	2.23	5
5	52.08	4	4.04	4	8.34	6	2.28	5
6	17.74	5	2.97	3	23.61	4	3.21	4
7	57.68	4	3.59	4	35.51	4	3.95	4
8	53.78	4	2.75	4	40.43	3	2.61	6
9	47.40	4	3.46	4	30.70	3	4.16	3
Mean	50.13	4	3.93	4	30.81	4	3.34	4
SE	8.11	0	0.51	0	4.53	0	0.30	0

*Precision of parameter estimate expressed as coefficient of variation

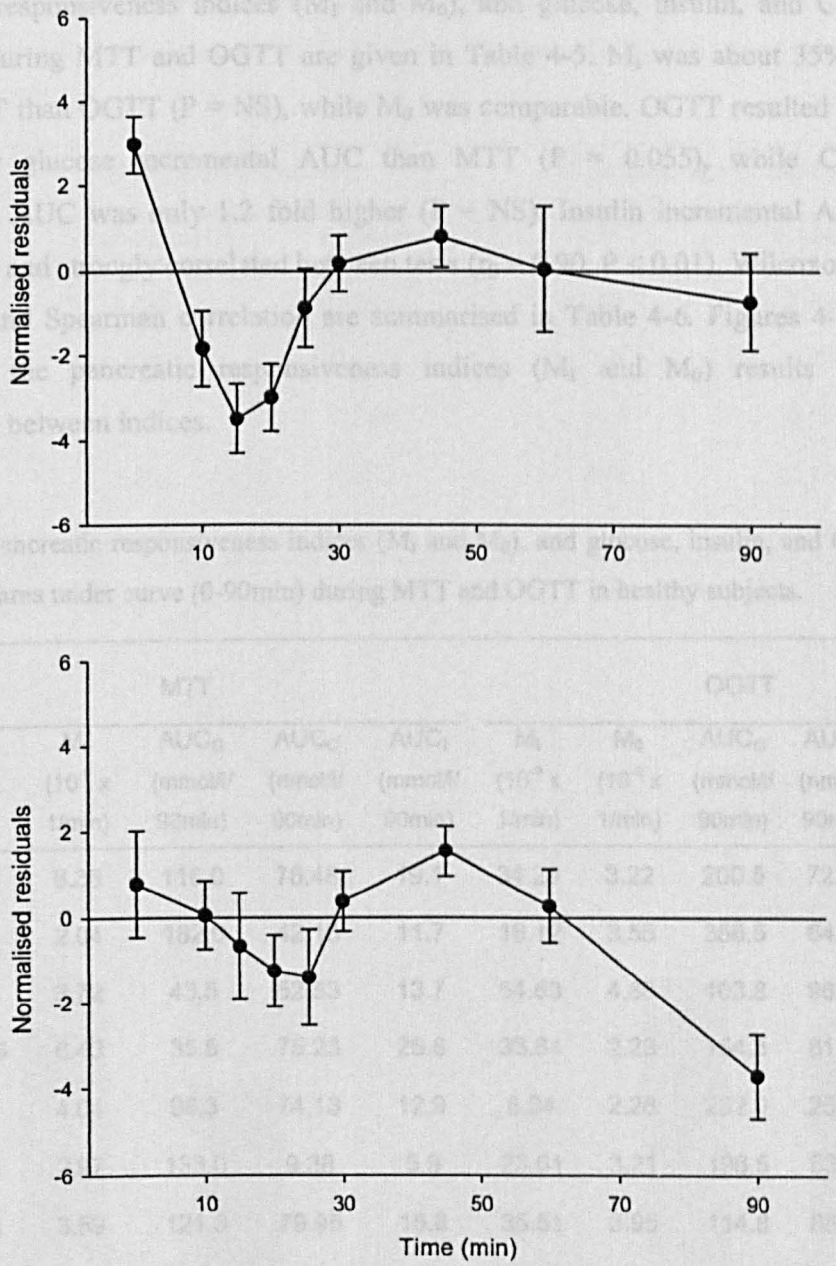

Figure 4-2. Normalised residual (difference between calculated and measured C-peptide concentration divided by measurement error) vs. time in healthy subjects during OGTT (top panel) and during MTT (bottom panel), (mean ± SE).

4.4.1.4. Comparability of pancreatic responsiveness during MTT and OGTT

Pancreatic responsiveness indices (M_I and M_0), and glucose, insulin, and C-peptide responses during MTT and OGTT are given in Table 4-5. M_I was about 35% higher during MTT than OGTT (P = NS), while M_0 was comparable. OGTT resulted in a two fold higher glucose incremental AUC than MTT (P = 0.055), while C-peptide incremental AUC was only 1.2 fold higher (P = NS). Insulin incremental AUC was comparable and strongly correlated between tests ($r_S = 0.90$, $P < 0.01$). Wilcoxon signed ranks test and Spearman correlation are summarised in Table 4-6. Figures 4-3 to 4-6 summarise the pancreatic responsiveness indices (M_I and M_0) results and the relationship between indices.

Table 4-5. Pancreatic responsiveness indices (M_I and M_0), and glucose, insulin, and C-peptide incremental area under curve (0-90min) during MTT and OGTT in healthy subjects.

Subject No.	MTT					OGTT				
	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/ 90min)	AUC_C (nmol/l/ 90min)	AUC_I (mmol/l/ 90min)	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/ 90min)	AUC_C (nmol/l/ 90min)	AUC_I (mmol/l/ 90min)
1	54.55	6.36	116.0	76.48	19.1	34.29	3.22	200.5	72.83	23.3
2	21.64	2.04	182.0	42.13	11.7	16.12	3.55	356.5	64.10	12.3
3	43.85	3.72	43.5	52.53	13.7	54.63	4.88	103.8	96.50	19.7
4	102.45	6.43	35.5	75.23	26.6	33.64	2.23	154.3	81.88	20.2
5	52.08	4.04	98.3	74.13	12.9	8.34	2.28	232.0	25.65	11.8
6	17.74	2.97	133.0	9.38	5.9	23.61	3.21	198.5	53.73	8.4
7	57.68	3.59	121.3	79.95	15.8	35.51	3.95	114.8	88.50	17.2
8	53.78	2.75	95.0	79.08	18.3	40.43	2.61	194.0	99.15	22.0
9	47.4	3.46	59.8	31.20	8.0	30.7	4.16	176.8	48.00	2.9
Mean	50.13	3.93	98.3	57.79	14.7	30.81	3.34	192.3	70.04	15.3
SE	8.11	0.51	15.6	8.49	1.9.	4.53	0.30	24.8	8.16	2.3

Table 4-6. Wilcoxon signed ranks test and Spearman correlation with Bonferroni adjustment between pancreatic responsiveness indices (M_I and M_0) and AUC_G, AUC_C, and AUC_I during MTT and OGTT.

	Spearman correlation		Wilcoxon signed ranks test	
	Correlation	P-value	test	P-value
M_I	0.35	0.356	-2.037	0.190
M_0	-0.28	0.460	-0.296	0.767
AUC_G	0.62	0.385	-2.547	0.055
AUC_C	0.62	0.385	-1.481	0.139
AUC_I	0.90	0.005	-0.533	0.594

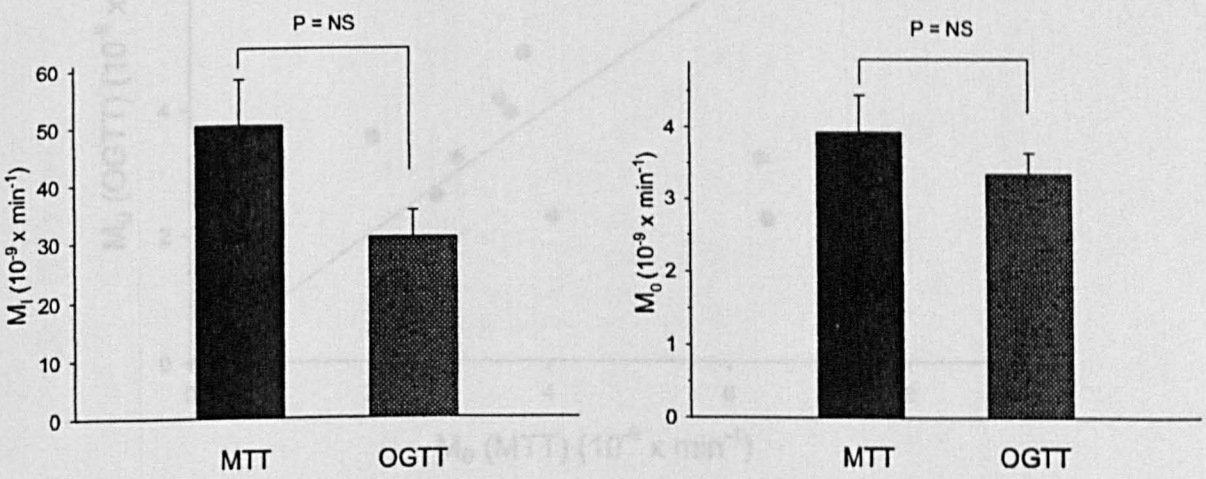

Figure 4-3. Pancreatic responsiveness indices (M_I and M_0) during MTT and OGTT in healthy subjects, (mean ± SE, N = 9).

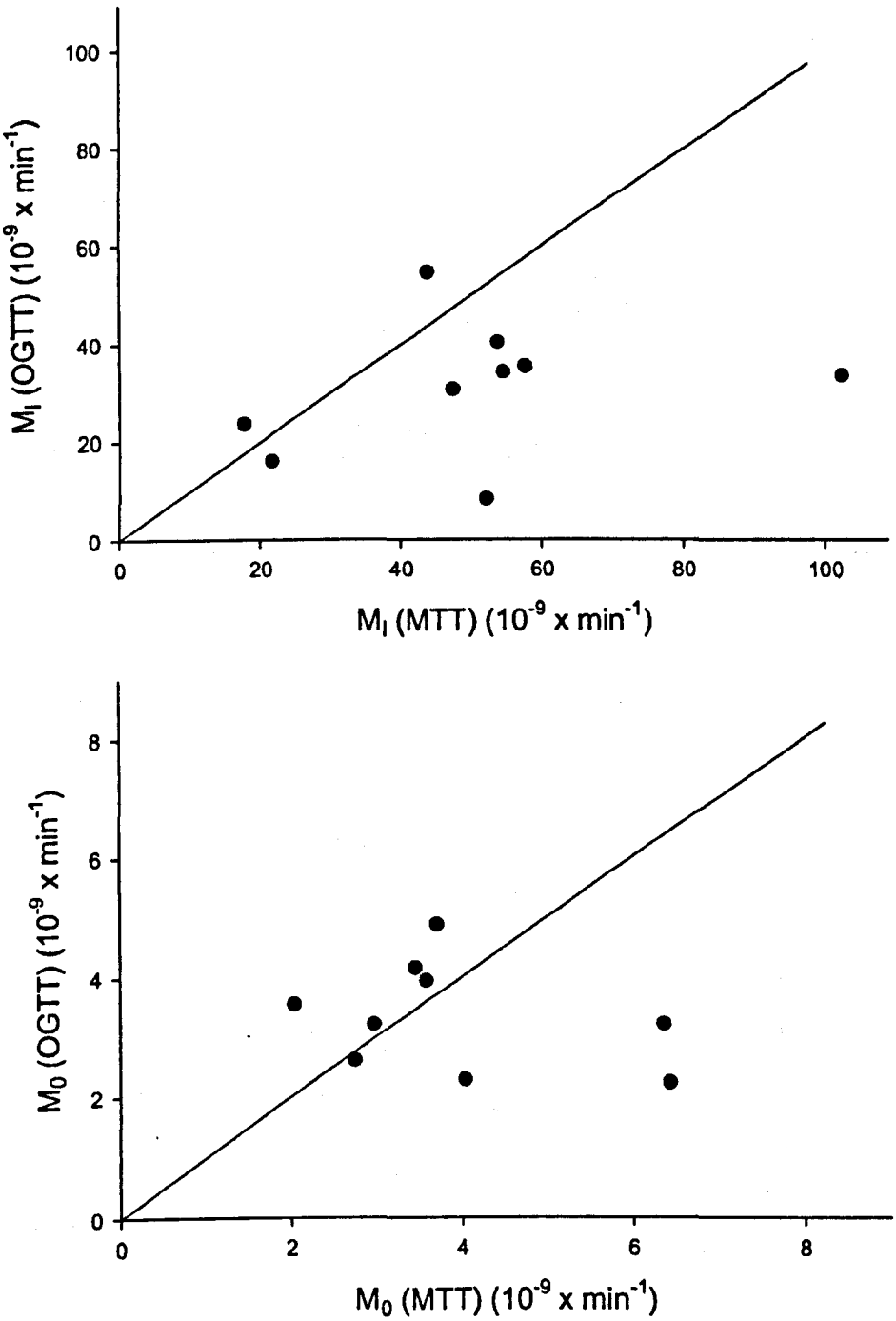

Figure 4-4. Relationship between individual estimates of postprandial pancreatic β-cell responsiveness indices during MTT and OGTT (top panel), and between fasting pancreatic β-cell responsiveness indices during MTT and OGTT (bottom panel) in healthy subjects, (N = 9). A unity line is shown.

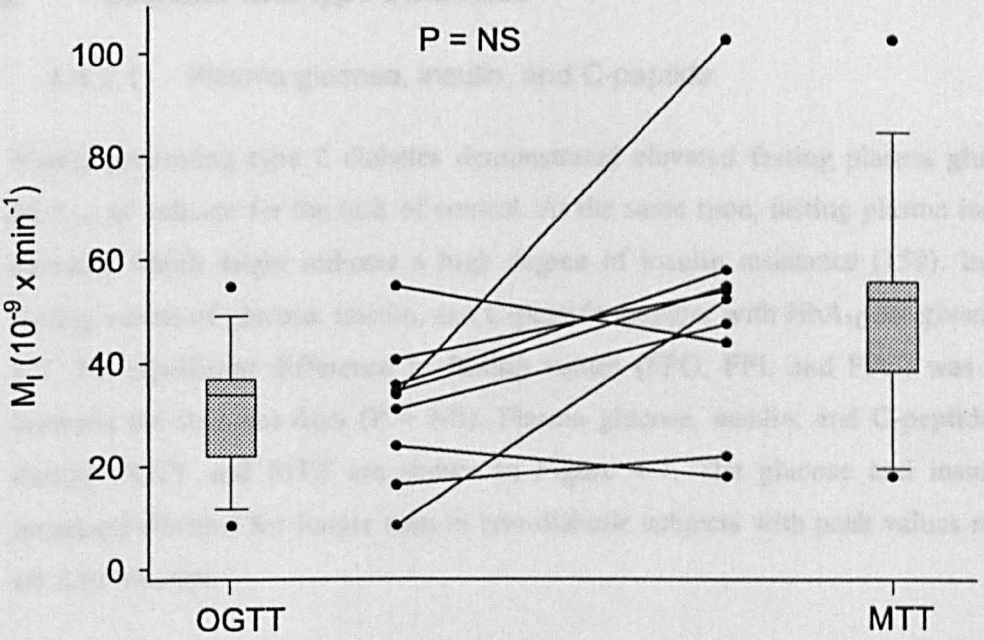

Figure 4-5. Postprandial pancreatic β-cell responsiveness during OGTT and MTT in healthy subjects (N =9).

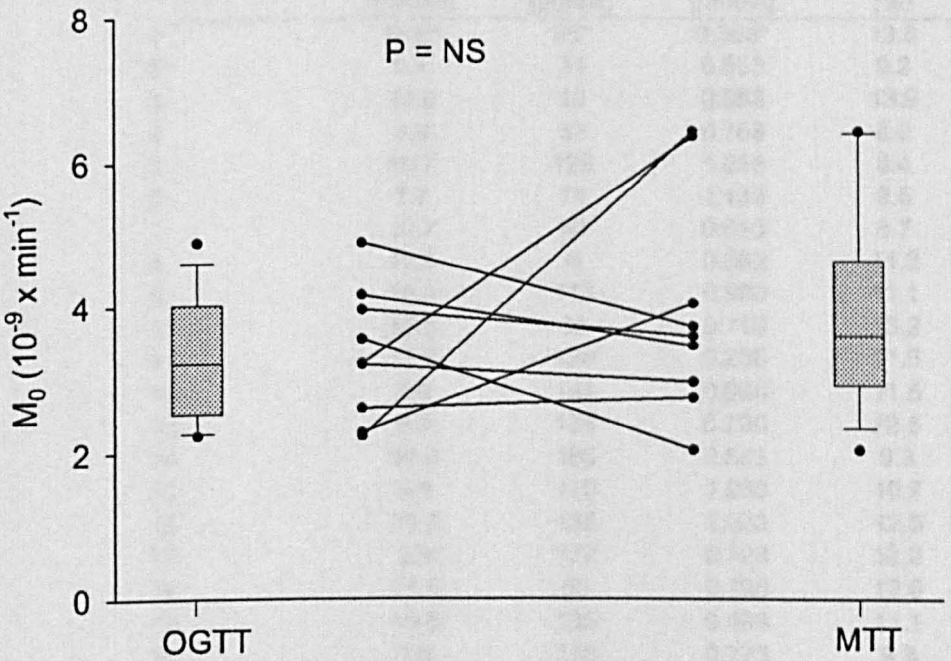

Figure 4-6. Fasting pancreatic β-cell responsiveness during OGTT and MTT in healthy subjects (N =9).

4.4.2. *Subjects with type 2 diabetes*

4.4.2.1. Plasma glucose, insulin, and C-peptide

Newly presenting type 2 diabetes demonstrated elevated fasting plasma glucose and HbA_{1C} as indicate for the lack of control. At the same time, fasting plasma insulin was elevated which might indicate a high degree of insulin resistance (159). Individuals fasting values of glucose, insulin, and C-peptide together with HbA_{1C} are given in Table 4-7. No significant difference in fasting values (FPG, FPI, and FPC) was observed between the two test days (P = NS). Plasma glucose, insulin, and C-peptide profiles during OGTT and MTT are shown in Figure 4-7. The glucose and insulin levels remained elevated for longer than in non-diabetic subjects with peak values reached at 60–120 minutes.

Table 4-7. Fasting plasma glucose (FPG), insulin (FPI), C-peptide (FPC), and glycated haemoglobin (HbA_{1C}) in subjects with type 2 diabetes.

Subject No.	FPG (mmol/l)	FPI (pmol/l)	FPC (nmol/l)	HbA_{1c} (%)
1	14.6*	36*	0.368*	13.8
2	9.1	21	0.653	9.2
3	14.6	12	0.563	13.9
4	9.9	32	0.768	8.0
6	10.7	129	1.095	8.4
6	7.7	78	1.133	9.5
7	10.7	50	0.613	8.7
8	12.6	6	0.363	11.2
9	15.8	113	0.990	11.1
10	15.5	95	0.710	13.2
11	11.6	128	0.258	11.6
12	9.9	164	0.880	11.5
13	6.9	138	0.700	12.5
14	10.0	155	0.583	9.3
15	5.9	170	1.280	10.7
16	10.3	138	1.023	12.5
17	9.9	122	0.723	12.0
18	11.9	53	0.705	12.6
19	10.8	135	0.483	11.1
20	7.6	156	0.773	9.8
Mean	10.8	97	0.733	11.0
SE	0.6	13	0.061	0.4

* Mean of fasting values on two study days

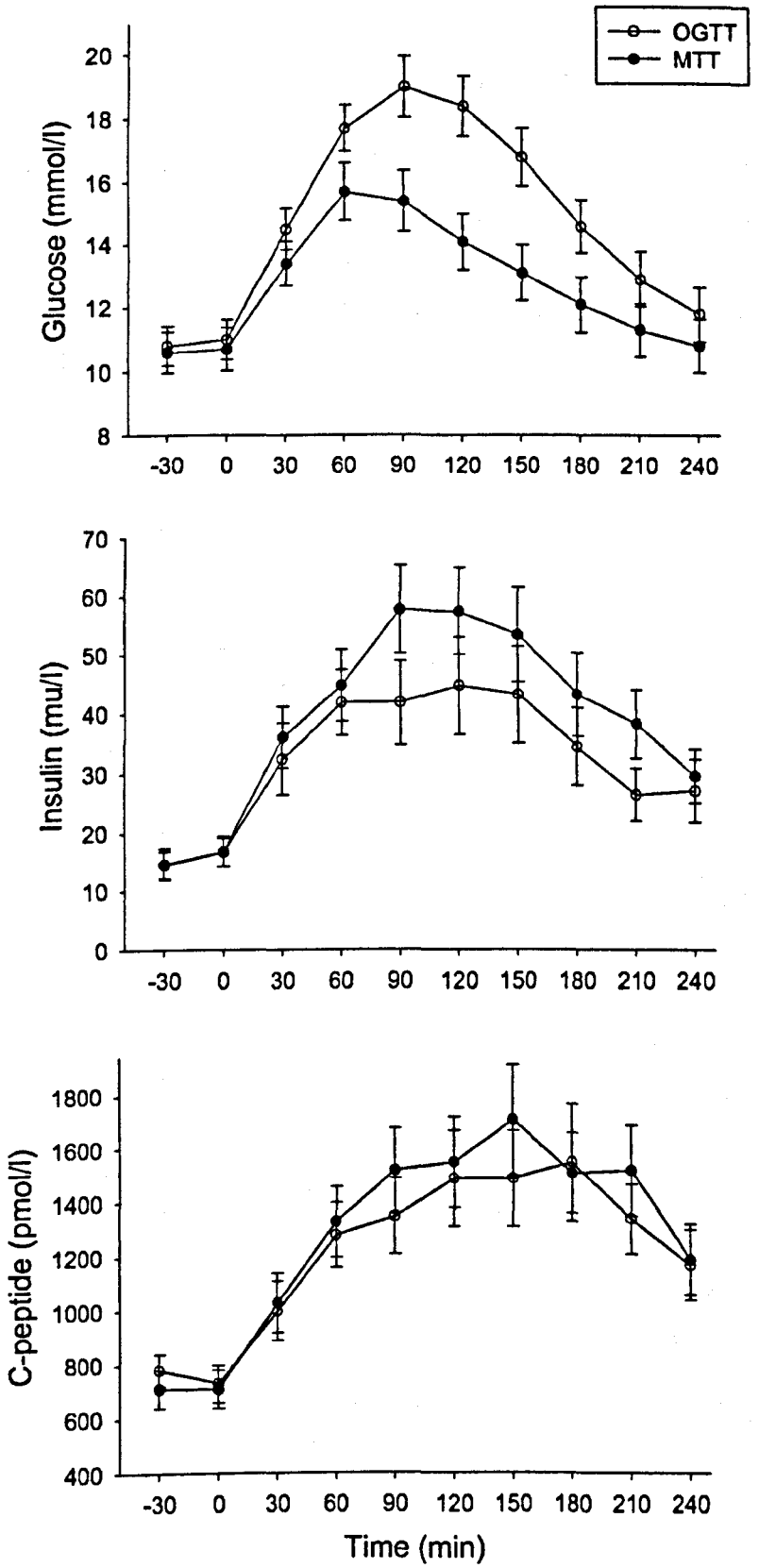

Figure 4-7. Plasma glucose, insulin, and C-peptide profiles during MTT and OGTT in subjects with T2D (mean ± SE, N=20).

4.4.2.2. Model validation during OGTT in subjects with type 2 diabetes

Parameter estimates were obtained with good precision (mean CV of parameter estimates for M_1 and $M_0 \leq 10\%$ and 5%, respectively), see Table 4-8. The magnitude and distribution of normalised residuals around the zero line indicate a good model fit (Figure 4-8). The Runs test was not significant indicating a random distribution of the residuals.

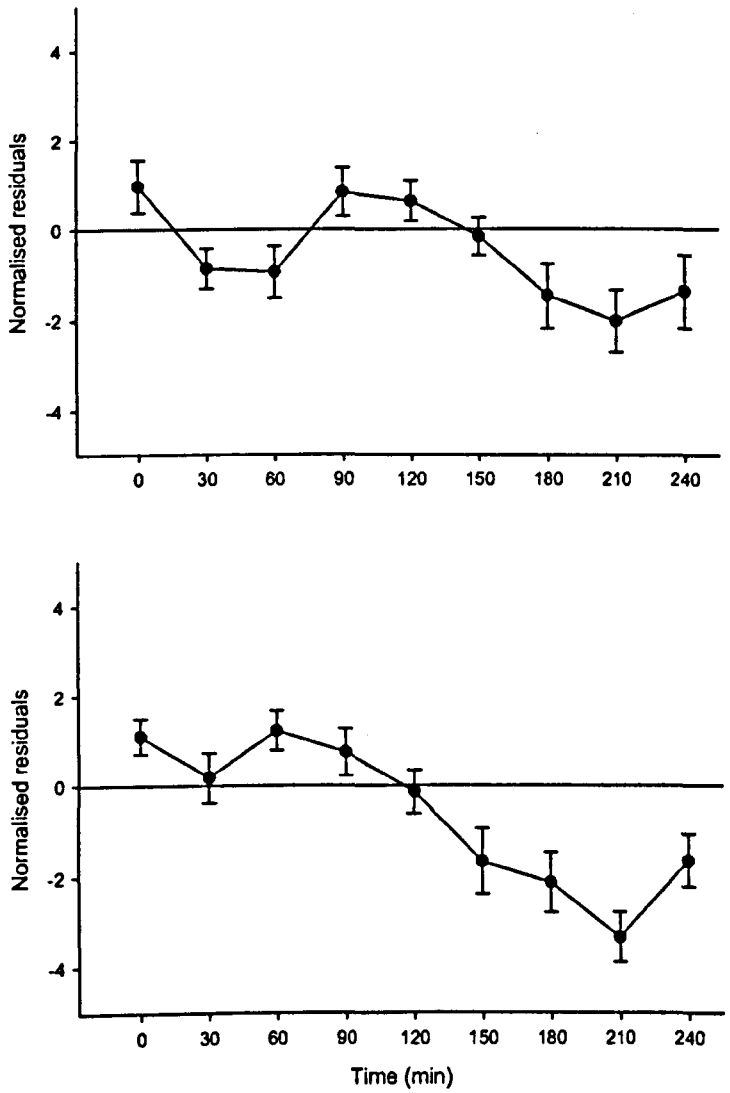

Figure 4-8. Normalised residual (difference between calculated and measured C-peptide concentration divided by measurement error) in subjects with T2D during OGTT (top panel) and MTT (bottom panel), (mean ± SE).

4.4.2.3. Parameter estimation

Parameters were estimated with good precision during both MTT and OGTT (mean CV of parameter estimates for M_1 and $M_0 \leq 10\%$ and 5%, respectively). Individual estimates of pancreatic responsiveness indices during MTT and OGTT together with their precision are presented in Table 4-8.

Table 4-8. Pancreatic responsiveness indices (M_1 and M_0) during MTT and OGTT in subjects with T2D.

Subject No.	MTT				OGTT			
	M_1 (10^{-9} x 1/min)	CV (%)	M_0 (10^{-9} x 1/min)	CV (%)	M_1 (10^{-9} x 1/min)	CV (%)	M_0 (10^{-9} x 1/min)	CV (%)
1	7.12	6*	1.42	5	3.83	5	1.23	4
2	14.92	6	3.80	6	8.61	7	4.74	5
3	6.31	10	2.73	4	2.15	15	2.63	4
4	43.39	7	4.19	6	12.83	6	4.69	5
5	35.75	8	6.99	4	14.09	10	7.13	5
6	23.18	10	9.14	5	19.46	7	9.80	5
7	12.84	8	4.07	5	6.71	7	3.29	5
8	9.53	7	1.84	5	4.63	6	1.81	5
9	8.18	9	4.68	4	3.29	15	3.72	4
10	7.09	12	2.67	5	2.22	20	2.63	5
11	1.69	15	0.97	4	3.17	7	1.50	5
12	26.86	7	7.05	5	3.74	9	1.84	6
13	6.08	11	3.81	6	7.52	8	7.93	4
14	12.19	7	4.67	5	1.88	15	3.27	5
15	29.08	7	14.33	5	12.75	7	13.67	4
16	16.31	7	5.40	5	5.47	13	5.63	5
17	8.53	9	4.44	5	5.78	12	5.13	4
18	7.04	6	2.53	6	3.94	13	3.97	5
19	15.93	7	2.81	4	8.83	8	3.01	4
20	11.88	10	6.25	5	12.88	7	7.16	5
Mean	15.19	9	4.69	5	7.19	10	4.74	5
SE	2.46	1	0.68	0	1.09	1	0.70	0

*Precision of parameter estimate expressed as coefficient of variation

4.4.2.4. Comparability of pancreatic responsiveness during MTT and OGTT

M_I was two fold higher during MTT than OGTT (P = 0.001), see Table 4-9 and Fig 4-9. The two indices were correlated (r_S = 0.62; P < 0.05). M_0 was identical and highly correlated during MTT and OGTT (r_S = 0.70; P < 0.01). Glucose response during OGTT was higher than during MTT (P = 0.001), however the insulin and C-peptide responses were slightly higher during MTT (P = NS). Pancreatic responsiveness indices (M_I and M_0), and glucose, insulin, and C-peptide response during MTT and OGTT are presented in Table 4-11.

Table 4-9. Wilcoxon signed ranks test and Spearman correlation with Bonferroni adjustment between pancreatic responsiveness indices (M_I and M_0) and AUC_G, AUC_C, and AUC_I during MTT and OGTT.

	Spearman correlation		Wilcoxon signed ranks test	
	Correlation	P-value	test	P-value
M_I	0.62	0.020	-3.696	0.001
M_0	0.70	0.005	-0.672	0.502
AUC_G	0.48	0.155	-3.696	0.001
AUC_C	0.44	0.275	-0.859	0.390
AUC_I	0.49	0.135	-1.549	0.121

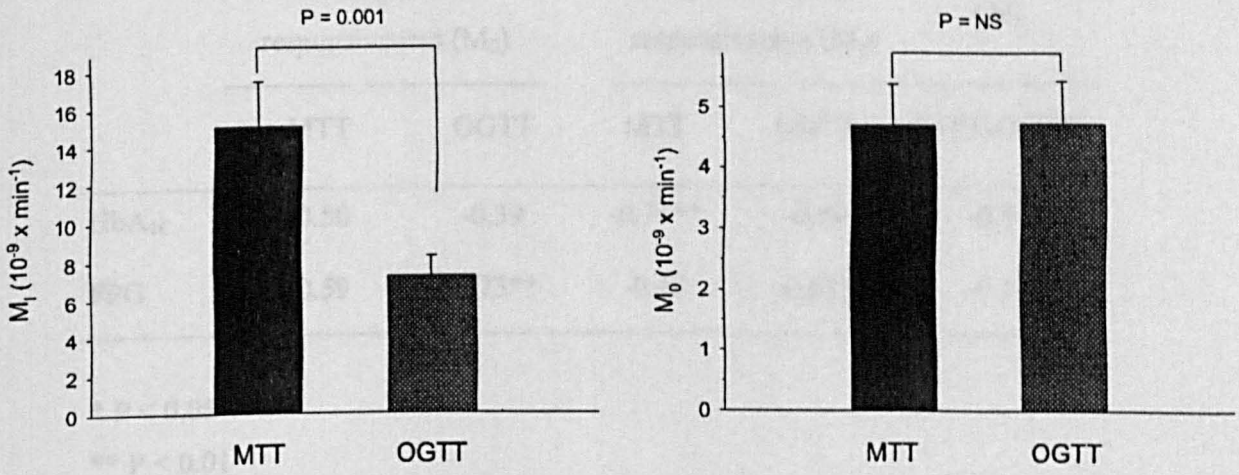

Figure 4-9. Pancreatic responsiveness indices (M_I and M_0) during MTT and OGTT in subjects with T2D, (mean ± SE, N = 20).

4.4.2.5. Correlation between β-cell responsiveness and glucose control

The β-cell response during OGTT represents the effect of postprandial glucose on insulin secretion, while it represents the effect of postprandial glucose and the effect due to the fat and protein contents of the meal during MTT. The difference between M_I during MTT and OGTT (ΔM_I) theoretically could represent the β-cell response to the non-carbohydrate meal contents during MTT and could account for the effect of fat and protein on insulin secretion (incretin effect).

Postprandial β-cell responsiveness (M_I) during MTT and OGTT was strongly correlated with HbA_{1C} and FPG. However ΔM_I was correlated with HbA_{1C} ($r_S = 0.51$, P was not significant after conservative adjustment) but not with FPG. Fasting β-cell responsiveness during MTT and OGTT was strongly correlated with FPG. Table 4-10 shows the results of the Spearman correlation analysis between glucose control (HbA_{1C} and FPG) and fasting and postprandial β-cell responsiveness during MTT and OGTT. The correlations between glucose control and the difference between M_I during MTT and OGTT are also given.

Table 4-10. Spearman correlation with Bonferroni adjustment between pancreatic responsiveness indices (M_I and M_0) during MTT and OGTT and ΔM_I (difference between M_I during MTT and OGTT) with FPG and HbA_{1C} in subjects with newly presenting type 2 diabetes.

	Fasting β-cell responsiveness (M_0)		Postprandial β-cell responsiveness (M_I)		ΔM_I
	MTT	OGTT	MTT	OGTT	(MTT-OGTT)
HbA_{1C}	-0.50	-0.39	-0.71**	-0.59	-0.51
FPG	-0.59	-0.73**	-0.48	-0.63*	-0.15

* $P < 0.05$

** $P < 0.01$

Table 4-11. Pancreatic responsiveness indices (M_I and M_0), and glucose, insulin, and C-peptide incremental area under curve (0-90min) during MTT and OGTT in subjects with T2D.

Subject No.	MTT					OGTT				
	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/ 90min)	AUC_C (nmol/l/ 90min)	AUC_I (mmol/l/ 90min)	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/ 90min)	AUC_C (nmol/l/ 90min)	AUC_I (mmol/l/ 90min)
1	7.12	1.42	294	23.55	3.1	3.83	1.23	444	21.90	1.9
2	14.92	3.80	405	44.25	12.6	8.61	4.74	552	34.50	4.5
3	6.31	2.73	320	19.20	4.0	2.15	2.63	465	18.30	1.7
4	43.39	4.19	159	64.05	22.7	12.83	4.69	327	62.25	19.5
5	35.75	6.99	198	57.30	18.5	14.09	7.13	434	66.45	8.6
6	23.18	9.14	195	44.40	12.7	19.46	9.80	387	53.40	25.4
7	12.84	4.07	330	60.90	16.2	6.71	3.29	279	25.80	2.7
8	9.53	1.84	242	22.65	3.2	4.63	1.81	428	38.70	7.6
9	8.18	4.68	579	51.30	9.3	3.29	3.72	560	20.85	3.4
10	7.09	2.67	272	10.95	4.7	2.22	2.63	360	8.25	1.4
11	1.69	0.97	230	9.00	6.6	3.17	1.50	582	17.85	7.9
12	26.86	7.05	305	71.10	27.0	3.74	1.84	468	6.00	22.1
13	6.08	3.81	216	3.75	11.8	7.52	7.93	372	22.50	2.9
14	12.19	4.67	344	48.00	21.8	1.88	3.27	437	15.60	2.5
15	29.08	14.33	279	79.20	29.1	12.75	13.67	509	92.55	31.3
16	16.31	5.40	422	71.10	10.2	5.47	5.63	536	43.80	14.9
17	8.53	4.44	293	27.00	5.0	5.78	5.13	398	29.85	3.2
18	7.04	2.53	437	22.80	4.2	3.94	3.97	414	16.35	18.0
19	15.93	2.81	198	31.65	6.8	8.83	3.01	281	33.30	6.2
20	11.88	6.25	278	40.95	14.9	12.88	7.16	372	39.30	7.6
Mean	15.19	4.69	285	40.16	12.2	7.19	4.74	430	33.38	9.7
SE	2.46	0.68	18	5.05	1.8	1.09	0.70	20	4.85	2.0

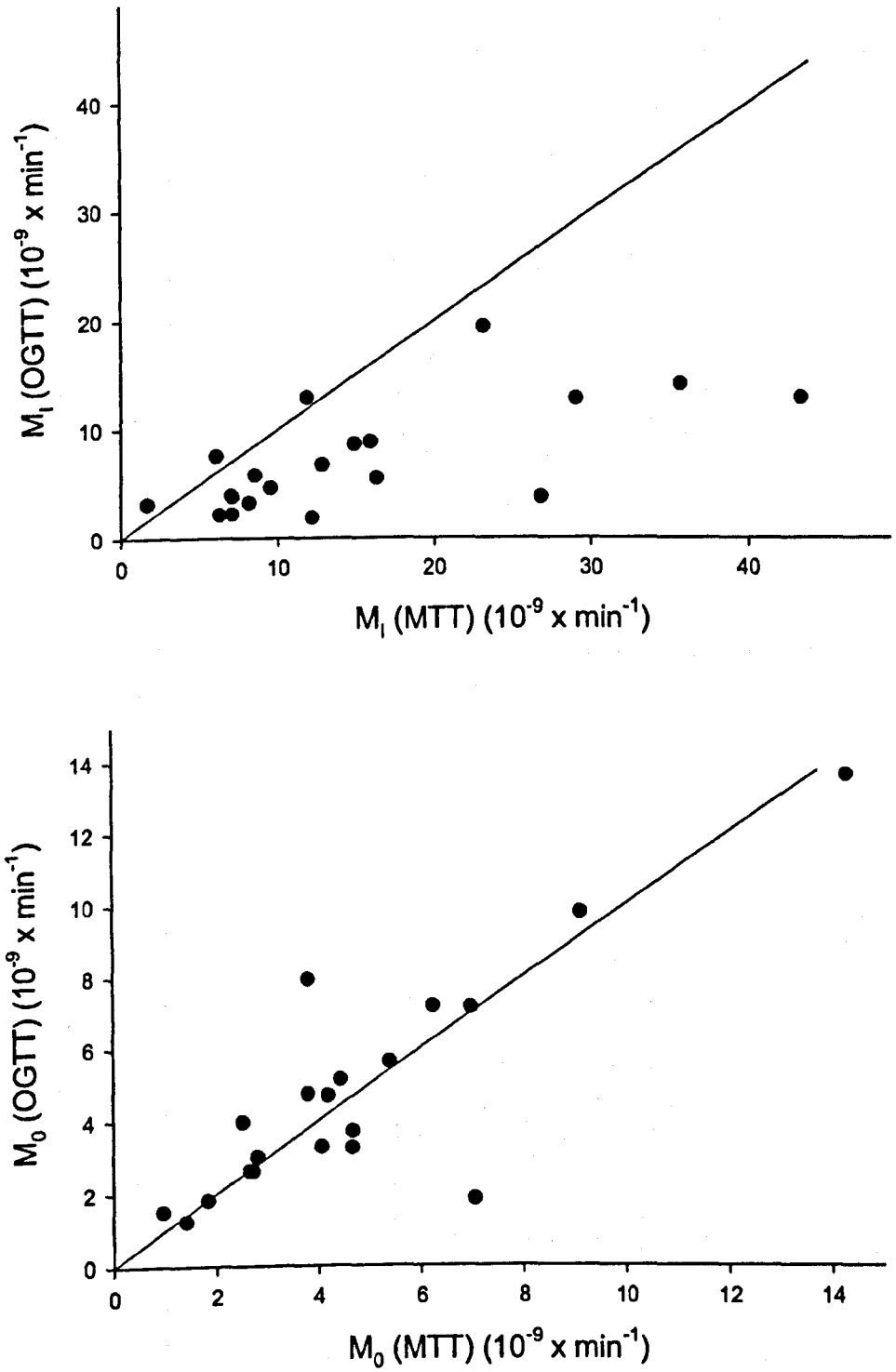

Figure 4-10. Relationship between individual estimates of postprandial pancreatic β-cell responsiveness indices during MTT and OGTT (top panel), and between fasting pancreatic β-cell responsiveness indices during MTT and OGTT (bottom panel), (N = 20). A unity line is shown.

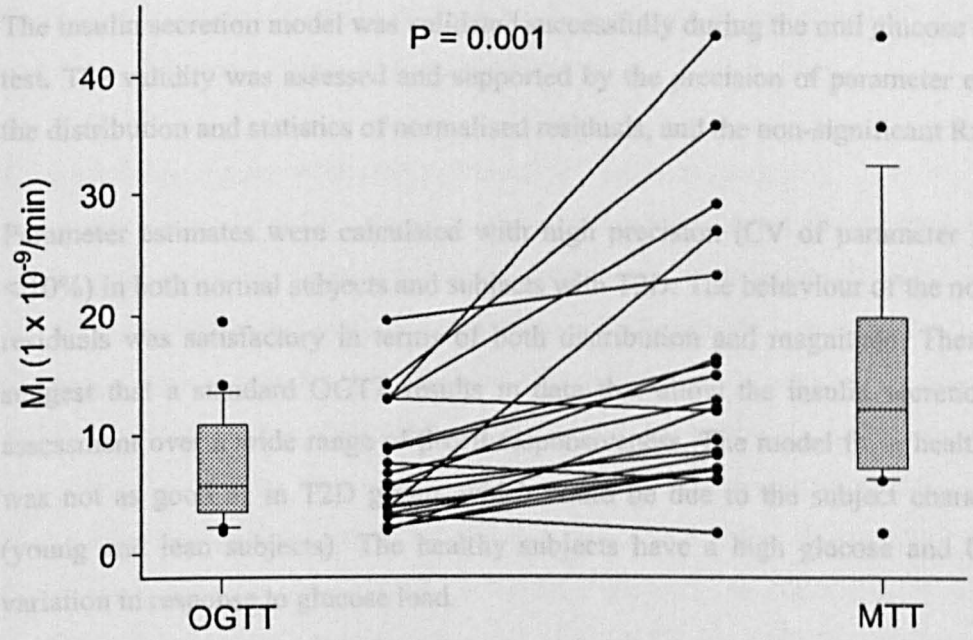

Figure 4-11. Postprandial pancreatic β-cell responsiveness during OGTT and MTT in subjects with T2D (N =20).

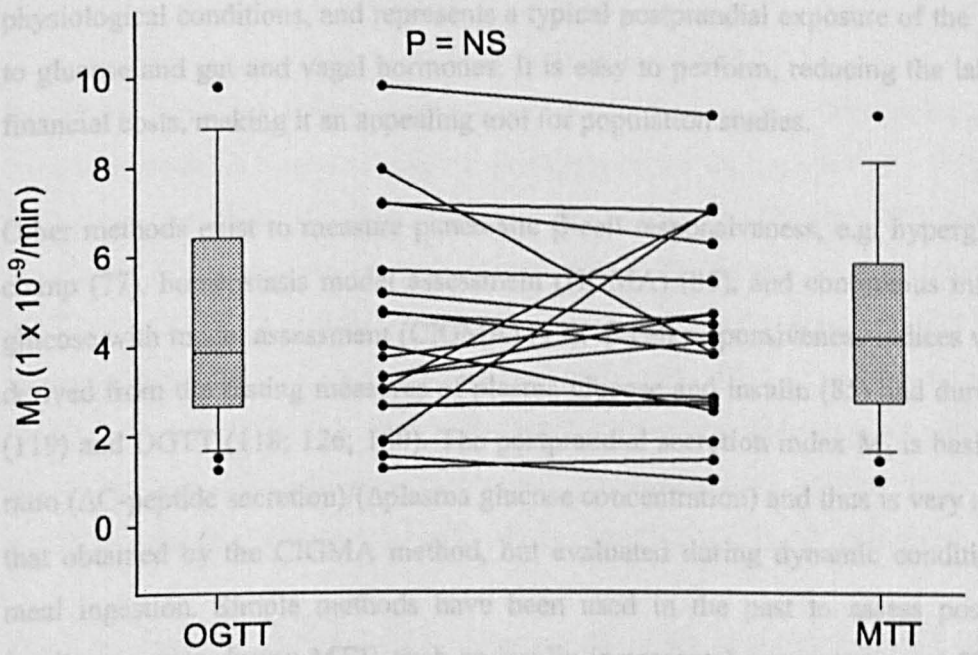

Figure 4-12. Fasting pancreatic β-cell responsiveness during OGTT and MTT in subjects with T2D (N =20).

4.5. Discussion

The insulin secretion model was validated successfully during the oral glucose tolerance test. The validity was assessed and supported by the precision of parameter estimates, the distribution and statistics of normalised residuals, and the non-significant Runs test.

Parameter estimates were calculated with high precision (CV of parameter estimates < 10%) in both normal subjects and subjects with T2D. The behaviour of the normalised residuals was satisfactory in terms of both distribution and magnitude. These results suggest that a standard OGTT results in data that allow the insulin secretion model assessment over a wide range of β-cell responsiveness. The model fit in healthy group was not as good as in T2D group, which could be due to the subject characteristics (young and lean subjects). The healthy subjects have a high glucose and C-peptide variation in response to glucose load.

β-cell responsiveness indices were proposed during IVGTT (121) and clamp (77), however the oral load (OGTT and MTT) has some advantages with respect to IVGTT or clamp approaches. It allows an accurate assessment of the β-cell function under physiological conditions, and represents a typical postprandial exposure of the pancreas to glucose and gut and vagal hormones. It is easy to perform, reducing the labour and financial costs, making it an appealing tool for population studies.

Other methods exist to measure pancreatic β-cell responsiveness, e.g. hyperglycaemic clamp (77), homeostasis model assessment (HOMA) (85), and continuous infusion of glucose with model assessment (CIGMA) (79). β-cell responsiveness indices were also derived from the fasting measures of plasma glucose and insulin (85) and during MTT (119) and OGTT (118; 126; 160). The postprandial secretion index M_1 is basically the ratio (ΔC-peptide secretion)/(Δplasma glucose concentration) and thus is very similar to that obtained by the CIGMA method, but evaluated during dynamic conditions after meal ingestion. Simple methods have been used in the past to assess postprandial insulin secretion during MTT, such as insulin incremental concentration at 30 min, or insulin incremental concentration at 30 min over glucose incremental concentration at 30 min. However, methodological considerations (the effect of the measurement error and the inter-subject variability in insulin and C-peptide kinetics) suggest the superiority

of the model-based method over a simple one or two concentration-point assessment. Furthermore, the model-based method has been shown to be reproducible in subjects with Type 2 diabetes as reported in Chapter 5.

Comparability of pancreatic responsiveness in healthy subjects

In healthy subjects, OGTT resulted in a two fold higher glucose incremental AUC than MTT, while C-peptide incremental AUC were only 1.2 fold higher (P = NS). Marena et al (161) observed higher glucose response to OGTT than MTT, and similar insulin and C-peptide responses in healthy subjects. This could be explained by the fat content of MTT stimulating the secretion of incretin hormones, which in turn stimulate insulin secretion (75; 76; 162). As a result of high C-peptide secretion during MTT with respect to the glucose concentration, MTT resulted in 1.5 fold higher postprandial pancreatic β-cell responsiveness than OGTT. In healthy subjects, no correlation was observed between glucose and C-peptide responses across MTT and OGTT. Explaining the lack of correlation between M_I's. Robert et al (163) also reported poor correlation ($r_S = 0.15$) between 2hour glucose values after OGTT and standard meal in health pregnant women.

Comparability of pancreatic responsiveness in subjects with T2D

In subjects with T2D, glucose response during OGTT was higher than during MTT (P = 0.001), whereas the insulin response was slightly higher during MTT (P = NS). Marena et al (161) also observed a higher glucose response to OGTT than MTT, and similar insulin and C-peptide responses in subjects with T2D. M_I was two fold higher during MTT than OGTT (P = 0.001). The two indices were correlated. The higher insulin response during MTT is explained by the stimulatory effect of incretin hormones on insulin secretion (162), as a result of the meal composition which contain carbohydrate, fat, and protein. Glucagon-like peptide and gastric inhibitory polypeptide are glucose and fat dependent gut hormones (164). M_0 was identical and highly correlated.

The β-cell response during OGTT represents the effect of the postprandial glucose on insulin secretion, while it represents the effect of postprandial glucose and the effect due to the fat and protein contents of the meal during MTT. It is suggested that the difference between M_I during MTT and OGTT (ΔM_I) represents the effect of fat and protein on insulin secretion (incretin effect). The effect of incretin on insulin secretion

and postprandial glucose control could be a good explanation for the correlation between ΔM_I and HbA_{1C} and the lack of correlation with FPG. HbA_{1C} was more strongly correlated with the postprandial than with fasting β-cell responses during MTT and OGTT. On the other hand FPG was strongly correlated with the fasting β-cell responsiveness during MTT and OGTT. This suggests that ΔM_I is a clinically relevant index of the incretin effect and that it could be used in clinical studies to characterise the pharmacodynamics of the incretin effect as compared to the measurement of incretin hormones, which characterise the pharmacokinetics properties.

4.6. Summary

The insulin secretion model was validated during OGTT in healthy subjects, and subjects with T2D. The model is able to assess pancreatic β-cell responsiveness from MTT as well as OGTT data. However the postprandial β-cell responsiveness during MTT is higher than that measured during OGTT, most likely due to the stimulatory effect of incretin hormones on insulin secretion during MTT. The meal tolerance test provides more physiological challenge to the β-cell than OGTT, enhances β-cell responsiveness compared to OGTT, and facilitates more comprehensive assessment of β-cell function.

5. Reproducibility of pancreatic β-cell responsiveness in healthy subjects and subjects with type 2 diabetes

5.1. Introduction

Pancreatic β-cell responsiveness can be quantified by a model-based method during meal tolerance test (MTT) (32). In the previous chapter this model (insulin secretion model; ISM) was validated also to quantify pancreatic β-sell responsiveness during oral glucose tolerance test (OGTT).

ISM measures the prehepatic insulin secretion and assesses pancreatic β-cell responsiveness giving postprandial pancreatic β-cell responsiveness (M_I; ability of postprandial glucose to stimulate C-peptide secretion) and basal pancreatic β-cell responsiveness (M_0; ability of basal glucose to stimulate C-peptide secretion). However these quantified measures were not studied for reproducibility.

In the present study we investigated the reproducibility of the quantified measures of the pancreatic β-cell responsiveness (M_I and M_0) and glucose, insulin, and C-peptide responses during both MTT and OGTT. In part one, we assessed the reproducibility during MTT and OGTT in healthy subjects. In the second part and due to the limitation of data availability, we investigated the reproducibility during MTT but not OGTT in subjects with type 2 diabetes (T2D).

5.2. Methods

5.2.1. *Subjects and experimental design*

Two groups of subjects participated in the current study namely: healthy group, and T2D group.

Healthy group. Healthy male subjects (N=9, age 27.6 ± 2.3 year, BMI 24.2 ± 0.5 kg/m^2; mean ± SE) participated in the study see Table (5-1). Each subject in this group underwent MTT and OGTT twice on four separate occasions, one week apart. Part of the data obtained from healthy subjects was used in the previous chapter.

Table 5-1. Demographic data for healthy subjects participating in the study (N = 9).

	Sex (M/F)	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	9/0	28	1.80	78.1	24.2
SE		2	0.02	1.5	0.5

T2D group. Twelve male subjects with T2D (N = 12, age 46.3 ± 3.0 year, BMI 29.2 ± 1.6 kg/m^2; mean ± SE) participated in the study, see Table 5-2. Each subject underwent MTT twice in two separate occasions one day apart.

Table 5-2 Demographic data for subjects with T2D (N = 9).

	Sex (M/F)	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	12/0	46	1.70	85.0	29.2
SE		3	0.04	7.2	1.6

5.2.1.1. Meal tolerance test

The meal tolerance test consisted of digestion of 15g Weetabix, 10g skimmed milk, 250mL pineapple juice, 50g white meat chicken, 60g wholemeal bread, 10g polyunsaturated margarine (75g carbohydrates; total 500 Cal; calorie contribution: 58%

carbohydrate, 23% fat and 19% protein) (49). The subjects were required to consume the whole meal within 10 min.

Samples were taken over 240 minutes in addition to one sample withdrawn in the fasting states to measure plasma glucose, insulin, and C-peptide. The samples were taken at -30, 0, 30, 60, 90, 120, 150, 180, 210, and 240 min in T2D group. The sampling in the healthy group had an additional six samples at –15, 10, 15, 20, 25 and 45 min, relative to meal ingestion. At each sample time the infusion was stopped and the first 2-ml blood withdrawn and discarded, prior to obtaining the sample for assay.

5.2.1.2. Oral glucose tolerance test

The oral glucose tolerance test consisted of 75g glucose syrup taken by mouth. Samples were taken over 240 min in addition to another two samples withdrawn in the fasting state to measure glucose, insulin, and C-peptide. The samples were taken at -30, -15, 0, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min relative to glucose intake. At each sample time the infusion was stopped and the first 2-ml blood withdrawn and discarded, prior to obtaining the sample for assay.

5.2.1.3. Assay methods

Glucose was assayed using the glucose oxidase method (intra-assay CV < 2%). Insulin and C-peptide were assayed using conventional radioimmunoassay (intra-assay CV < 6%).

5.2.1.4. Insulin secretion model

The insulin secretion model was used to quantify pancreatic responsiveness using MTT or OGTT data, providing basal sensitivity (M_0; ability of basal glucose to stimulate C-peptide secretion) and postprandial sensitivity (M_I; ability of postprandial glucose to stimulate C-peptide secretion) (32). The package used to calculate M_0 and M_I was version 1.0 of CPR (Calculating Pancreatic Responsiveness; written by R. Hovorka and H.C. Subasinghe, MIM Centre, City University, London, UK 1997). The model

parameters M_0 and M_1 were estimated employing weighted non-linear regression analysis. The measured errors were assumed to be uncorrelated, with zero mean and a constant coefficient of variance (CV = 6%). The precision of the parameters was obtained from the inverse of the Fisher information matrix and expressed as CV of parameter estimates. For more detailed description see Chapter 2.

5.2.2. *Data analysis*

5.2.2.1. Incremental area under curve

In addition to the parameters obtained from the insulin secretion model, we evaluated an incremental area under the curve from 0-90 minutes for glucose (AUC_G), insulin (AUC_I), and C-peptide (AUC_C) during both MTT and OGTT.

5.2.2.2. Statistical analysis

Reproducibility was assessed by ANOVA allowing for effects due to subjects, giving estimates of within subject CV, within subject variation as % of total variation and 95% range for difference between duplicate measurements. Comparison was made by Spearman correlation analysis and Wilcoxon signed ranks test. The results are expressed as mean ± SE unless stated otherwise.

5.3. Results

5.3.1. Healthy group

5.3.1.1. Plasma glucose, insulin, and C-peptide

Plasma glucose, insulin and C-peptide profiles during the repeated OGTT and MTT are shown in Figure 5-1 and 5-2 respectively.

5.3.1.2. Parameter estimation during OGTT and MTT

Parameter estimates were obtained with high precision during OGTT and MTT (CV of parameters estimates for M_1 and $M_0 \leq 6\%$). The individual estimates of the pancreatic responsiveness indices during OGTT and MTT together with their precision are summarised in Tables 5-3 and 5-4, respectively.

Table 5-3. Pancreatic responsiveness indices (M_1 and M_0) during OGTT at occasions 1 and 2 in healthy subjects.

Subject No.	M_1^1 (10^{-9} x 1/min)	CV %	M_1^2 (10^{-9} x 1/min)	CV %	M_0^1 (10^{-9} x 1/min)	CV %	M_0^2 (10^{-9} x 1/min)	CV %
1	34.29	3*	33.62	4	3.22	4	6.72	3
2	16.12	3	27.42	4	3.55	4	3.81	4
3	54.63	4	30.65	5	4.88	5	4.76	4
4	33.64	3	25.98	3	2.23	5	2.93	4
5	8.34	6	55.64	4	2.28	5	5.52	5
6	23.61	4	22.92	4	3.21	4	3.69	3
7	35.51	4	42.95	3	3.95	4	2.38	4
8	40.43	3	28.07	4	2.61	6	1.86	4
9	30.70	3	39.5	4	4.16	3	4.38	4
Mean	30.81	4	34.08	4	3.34	4	4.01	4
SE	4.53	0	3.45	0	0.30	0	0.51	0

*Precision of parameter estimate expressed as coefficient of variation

Table 5-4. Pancreatic responsiveness indices (M_I and M_0) during MTT at occasions 1 and 2 in healthy subjects.

Subject No.	M_I^1 (10^{-9} x 1/min)	CV %	M_I^2 (10^{-9} x 1/min)	CV %	M_0^1 (10^{-9} x 1/min)	CV %	M_0^2 (10^{-9} x 1/min)	CV %
1	54.6	5*	56.6	5	6.36	3	7.16	3
2	21.6	4	38.5	4	2.04	4	3.65	4
3	43.9	5	59.1	3	3.72	5	2.63	5
4	102.5	5	53.7	3	6.43	2	2.01	5
5	52.1	4	18.0	6	4.04	4	2.4	4
6	17.7	5	44.1	5	2.97	3	3.96	3
7	57.7	4	34.2	4	3.59	4	2.63	5
8	53.8	4	72.4	4	2.75	4	2.79	3
9	47.4	4	60.4	4	3.46	4	4.55	3
Mean	50.1	4	48.6	4	3.9	4	3.5	4
SE	8.1	0	5.5	0	0.51	0	0.53	0

*Precision of parameter estimate expressed as coefficient of variation

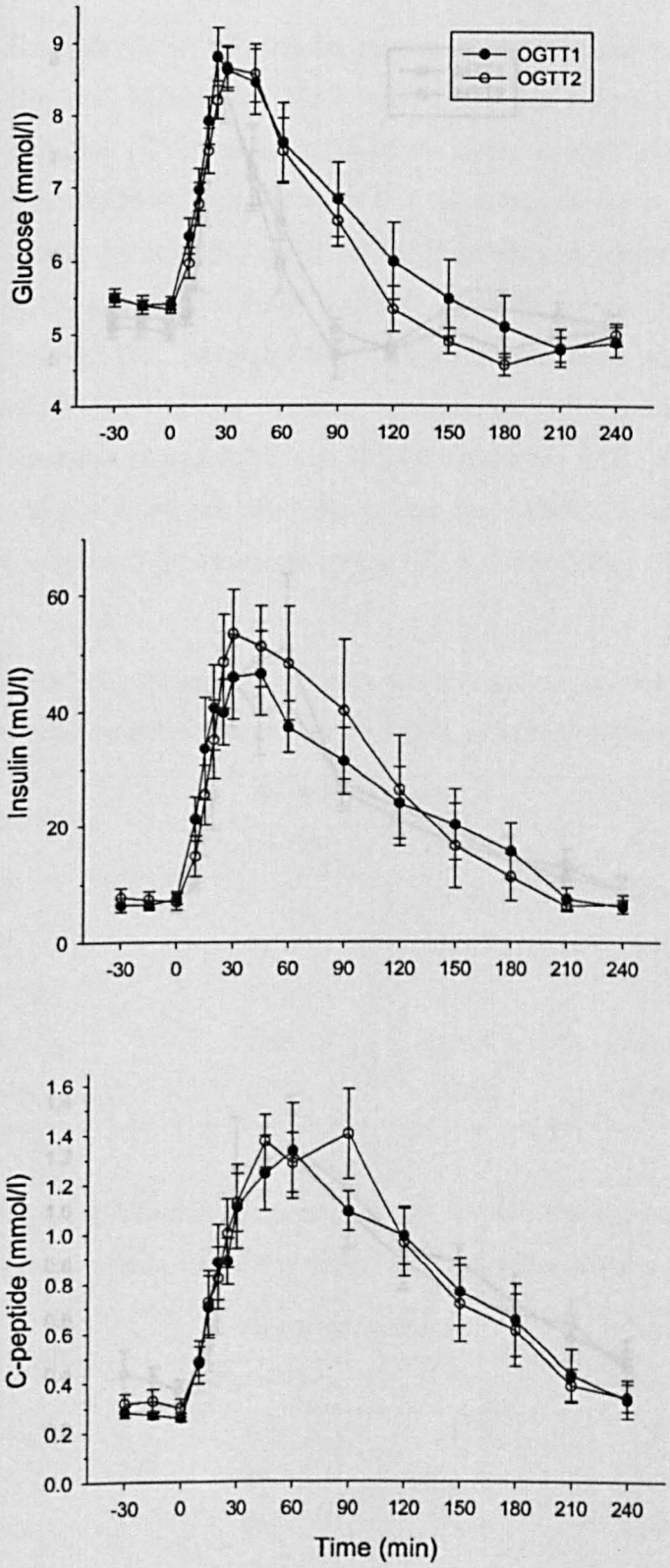

Figure 5-1. Plasma glucose, insulin, and C-peptide profiles (mean ± SE, N = 9) in healthy subjects during OGTT on two separate occasions.

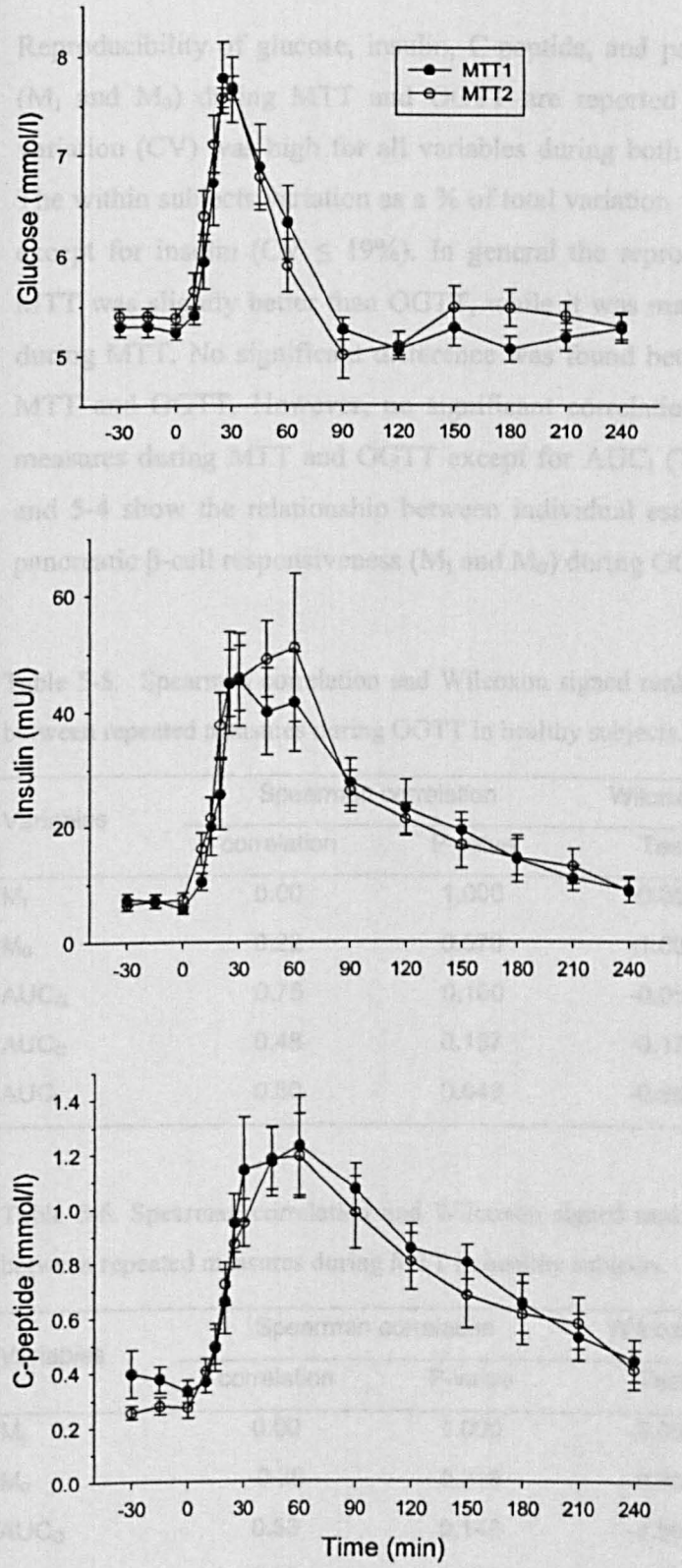

Figure 5-2. Plasma glucose, insulin, and C-peptide profiles (mean ± SE, N = 9) in healthy subjects during MTT on two separate occasions.

5.3.1.3. Reproducibility

Reproducibility of glucose, insulin, C-peptide, and pancreatic responsiveness indices (M_I and M_0) during MTT and OGTT are reported in Table 5-7. Within subjects variation (CV) was high for all variables during both MTT and OGTT (CV $\geq$ 26%). The within subjects variation as a % of total variation was also quite high (CV $\geq$ 31%) except for insulin (CV $\leq$ 19%). In general the reproducibility of parameters during MTT was slightly better than OGTT, while it was markedly better for M_I and AUC_C during MTT. No significant difference was found between repeated measures during MTT and OGTT. However, no significant correlation was found between repeated measures during MTT and OGTT except for AUC_I (Table 5-5 and 5-6). Figures 5-3 and 5-4 show the relationship between individual estimates of repeated measures of pancreatic β-cell responsiveness (M_I and M_0) during OGTT and MTT.

Table 5-5. Spearman correlation and Wilcoxon signed ranks test with Bonferroni corrections between repeated measures during OGTT in healthy subjects.

Variables	Spearman correlation		Wilcoxon signed ranks test	
	correlation	P-value	Test	P-value
M_I	0.00	1.000	-0.059	0.953
M_0	0.22	0.576	-1.007	0.314
AUC_G	0.75	0.100	-0.059	0.953
AUC_C	0.48	0.187	-0.178	0.859
AUC_I	0.80	0.048	-0.889	0.374

Table 5-6. Spearman correlation and Wilcoxon signed ranks test with Bonferroni corrections between repeated measures during MTT in healthy subjects.

Variables	Spearman correlation		Wilcoxon signed ranks test	
	correlation	P-value	Test	P-value
M_I	0.00	1.000	-0.059	0.953
M_0	-0.38	0.318	-0.356	0.722
AUC_G	0.53	0.145	-1.599	0.110
AUC_C	0.20	0.606	-0.296	0.767
AUC_I	0.85	0.019	-0.889	0.374

Table 5-7. Reproducibility of pancreatic responsiveness indices (M_I and M_0) and glucose, insulin, and C-peptide responses during MTT and OGTT in healthy subjects. Mean ± SE values are reported.

	MTT					OGTT				
	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/90min)	AUC_C (nmol/l/90min)	AUC_I (mmol/l/90/min)	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/90min)	AUC_C (nmol/l/90min)	AUC_I (mmol/l/90/min)
Day 1	50.13±8.11	3.62±0.45	98.25±15.59	57.79±8.49	14.7±2.1	32.81±4.53	3.34±0.30	192.33±24.77	70.04±8.16	15.3±2.3
Day 2	48.57±5.49	3.53±0.53	76.69±9.66	59.02±6.04	16.5±2.7	34.08±3.45	4.00±0.51	185.11±20.72	72.72±2.09	18.1±2.9
Mean	49.35±5.28	3.48±0.45	87.47±11.05	58.40±5.41	15.6±2.2	32.45±2.78	3.67±0.31	188.72±18.12	71.38±3.86	16.7±2.4
Within subjects CV	36%	34%	36%	34%	26%	42%	33%	30%	37%	29%
Within subjects variation as % of total variation	39%	35%	31%	43%	16%	66%	45%	35%	72%	19%
95% range for difference between duplicate measurements	±17.58	±1.23	±26.6	±19.60	±0.64	±13.04	±1.10	±54.50	±25.84	±0.72

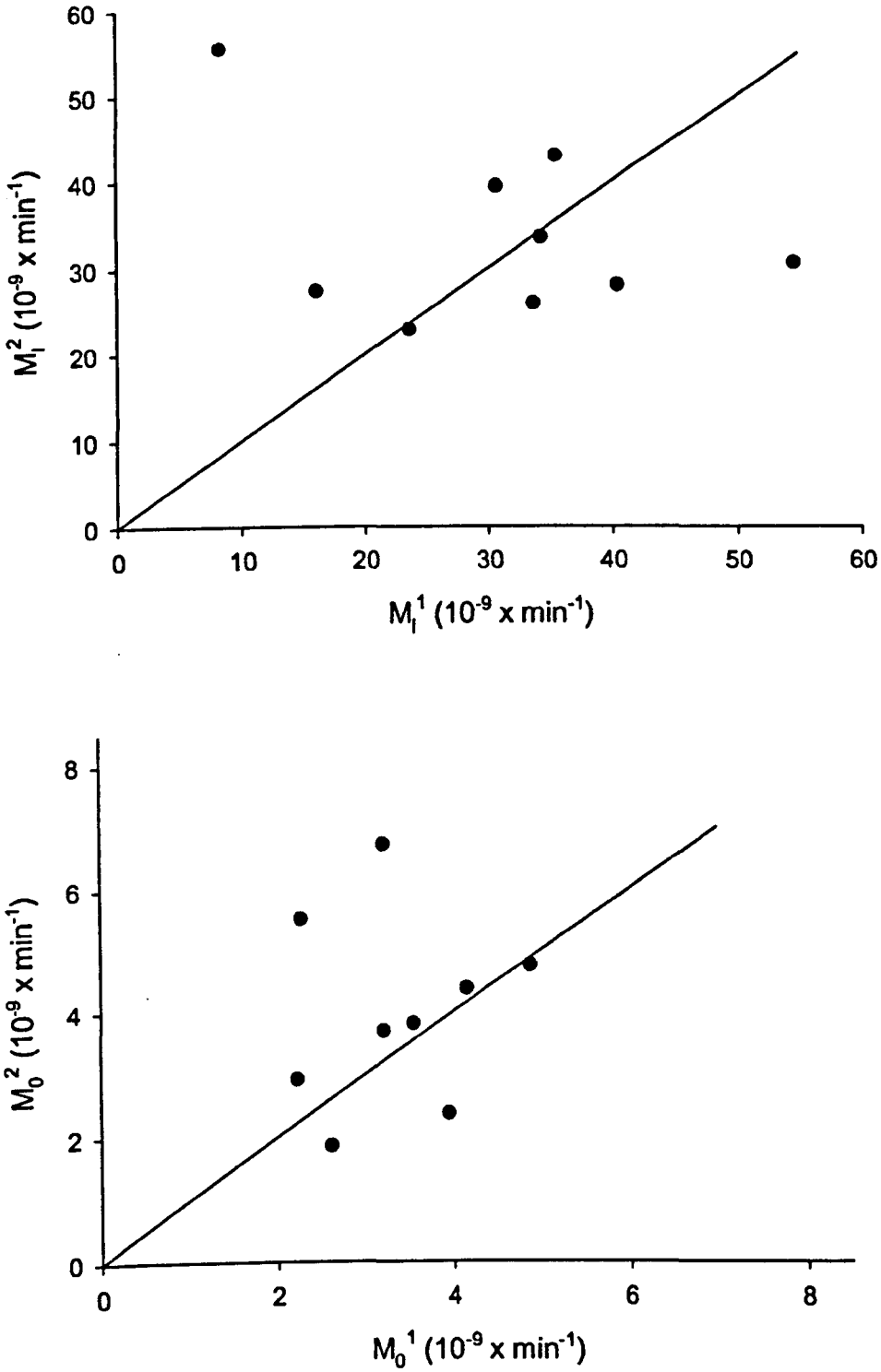

Figure 5-3. Relationship between individual estimates of repeated measures of pancreatic β-cell responsiveness indices (M_I) top panel, and (M_0) bottom panel during OGTT in healthy subjects (N = 9).

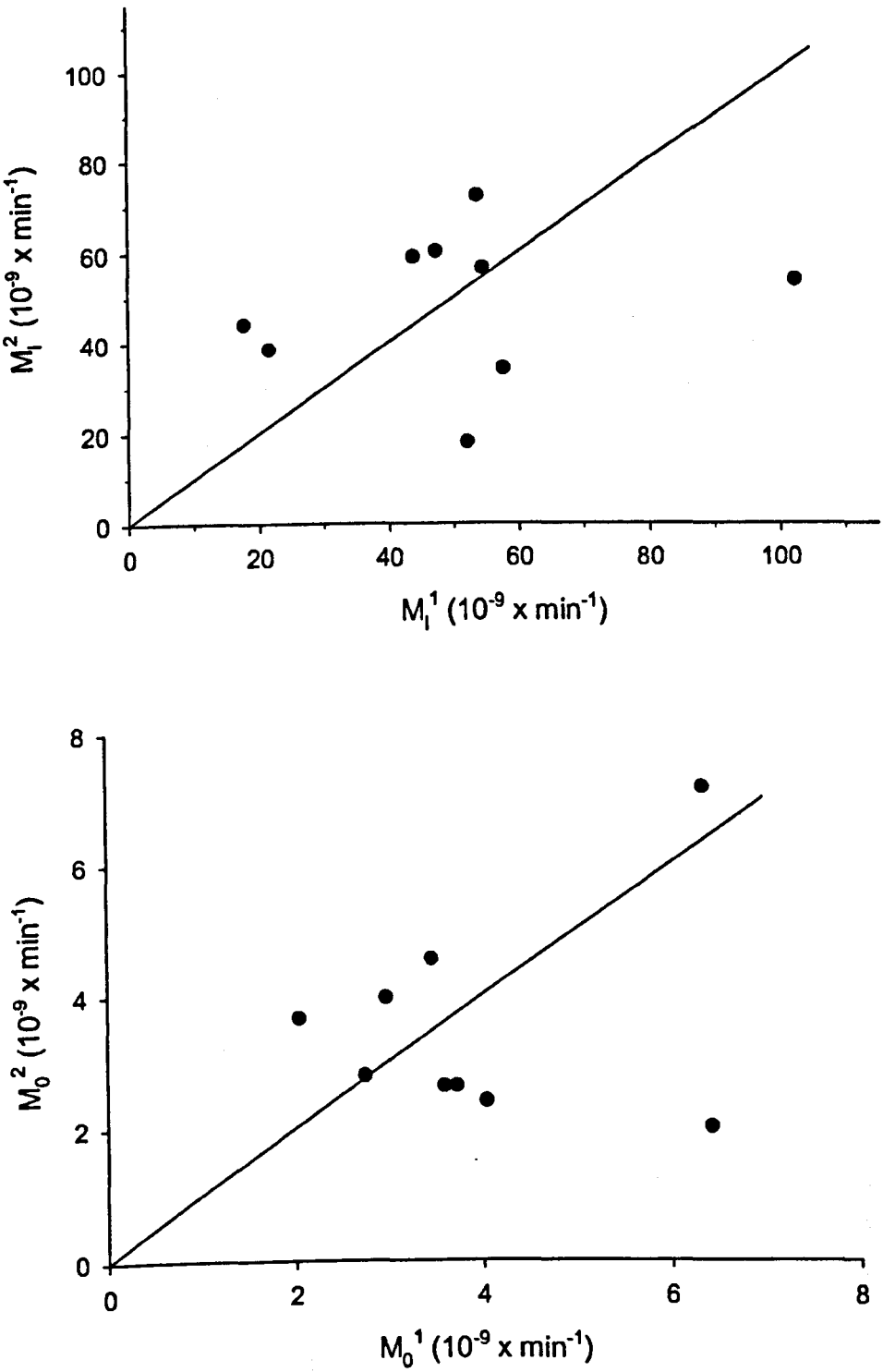

Figure 5-4. Relationship between individual estimates of repeated measures of pancreatic β-cell responsiveness indices (M_I) top panel, and (M_0) bottom panel during MTT in healthy subjects (N = 9).

5.3.2. *Type 2 diabetes group*

5.3.2.1. Plasma glucose, insulin, and C-peptide

The repeated measures of plasma glucose, insulin and C-peptide concentration during MTT are shown in Figure 5-5.

5.3.2.2. Parameter estimation during MTT

Parameter estimates were obtained with high precision during MTT. M_0 was estimated with excellent precision on both occasions (CV ≤ 7%). M_1 was estimated with good precision in both occasions (CV ≤ 23%), except in one subject (CV = 27 and 86% on occasion 1 and 2, respectively). The individual estimates of the pancreatic responsiveness during MTT together with their precision are summarised in Table 5-8.

Table 5-8. Pancreatic responsiveness indices (M_1 and M_0) during MTT on occasions 1 and 2 in subjects with T2D.

Subject No.	M_1^1 (10^{-9} x 1/min)	CV %	M_1^2 (10^{-9} x 1/min)	CV %	M_0^1 (10^{-9} x 1/min)	CV %	M_0^2 (10^{-9} x 1/min)	CV %
1	57.61	5*	44.02	5	4.90	6	9.01	5
2	12.92	23	42.61	6	12.37	3	9.26	4
3	38.39	10	47.81	7	15.28	4	11.21	5
4	56.56	8	50.24	6	19.39	5	15.00	5
5	8.97	27	2.51	86	6.10	5	7.35	5
6	28.14	7	27.09	7	8.59	5	10.12	4
7	20.61	7	28.80	8	7.28	5	9.62	5
8	51.15	12	41.09	10	18.17	4	16.53	4
9	26.66	5	24.76	6	6.50	5	9.09	4
11	2.83	9	13.33	6	1.37	5	4.45	5
12	22.78	5	20.85	6	10.44	5	7.55	7
13	12.50	8	13.69	7	5.25	5	4.63	5
Mean	28.26	11	29.73	13	9.64	5	9.49	5
SE	5.42	2	4.46	7	1.62	0	1.03	0

*Precision of parameter estimate expressed as coefficient of variation

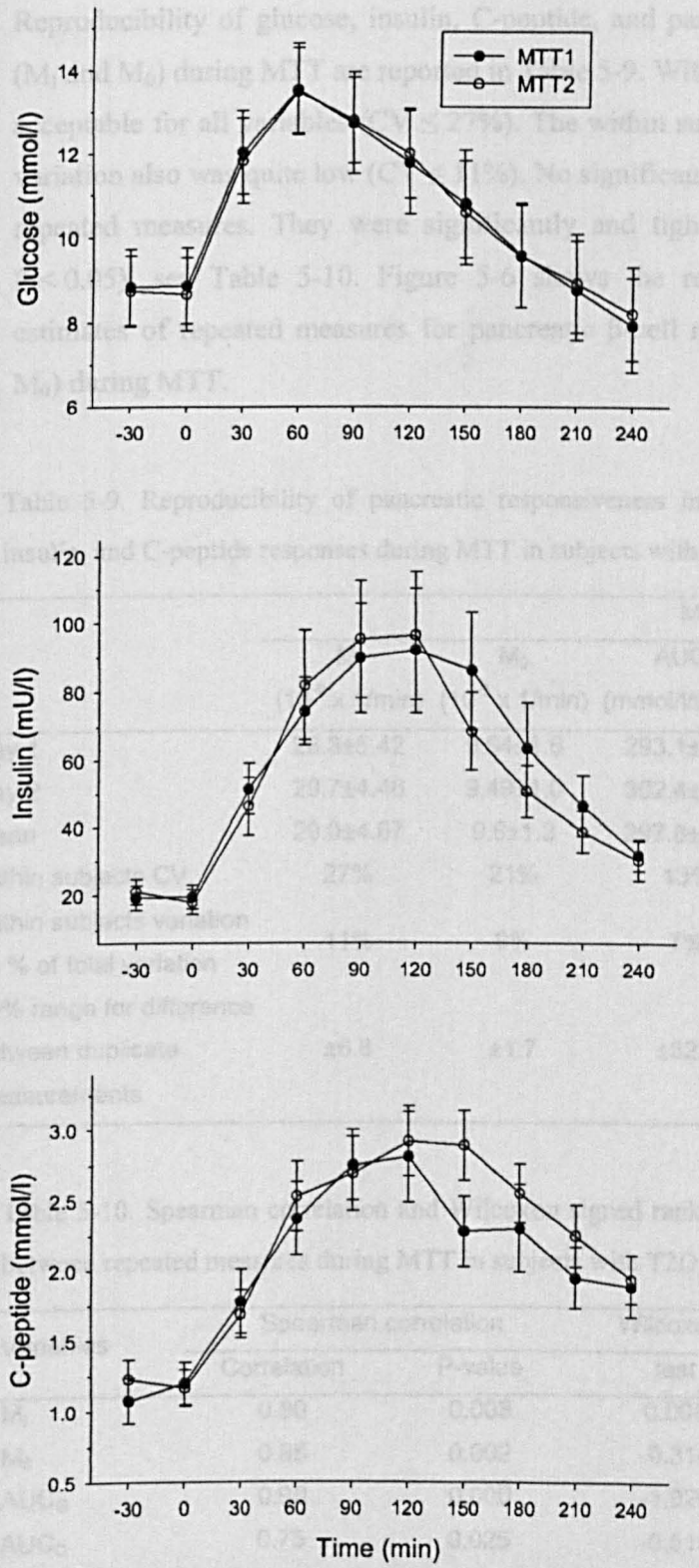

Figure 5-5. Plasma glucose, insulin, and C-peptide profiles during MTT at two separate occasions in subjects with T2D (mean ± SE, N=12).

5.3.2.3. Reproducibility

Reproducibility of glucose, insulin, C-peptide, and pancreatic responsiveness indices (M_I and M_0) during MTT are reported in Table 5-9. Within subjects variation (CV) was acceptable for all variables (CV ≤ 27%). The within subjects variation as a % of total variation also was quite low (CV ≤ 11%). No significant difference was found between repeated measures. They were significantly and tightly correlated ($r_S > 0.75$, and $P < 0.05$), see Table 5-10. Figure 5-6 shows the relationship between individual estimates of repeated measures for pancreatic β-cell responsiveness indices (M_I and M_0) during MTT.

Table 5-9. Reproducibility of pancreatic responsiveness indices (M_I and M_0) and glucose, insulin, and C-peptide responses during MTT in subjects with T2D (Mean ± SE).

	MTT				
	M_I (10^{-9} x 1/min)	M_0 (10^{-9} x 1/min)	AUC_G (mmol/l/90min)	AUC_C (nmol/l/90min)	AUC_I (mmol/l/90/min)
Day 1	28.3±5.42	9.64±1.6	293.1±31.6	75.8±10.7	21.9±3.5
Day 2	29.7±4.46	9.49±1.0	302.4±28.5	79.3±9.2	23.5±4.7
Mean	29.0±4.67	9.6±1.3	297.8±29.0	77.6±9.6	22.7±4.0
Within subjects CV	27%	21%	13%	17%	21%
Within subjects variation as % of total variation	11%	9%	7%	7%	3%
95% range for difference between duplicate measurements	±6.6	±1.7	±32.7	±10.9	±0.7

Table 5-10. Spearman correlation and Wilcoxon signed ranks test with Bonferroni corrections between repeated measures during MTT in subjects with T2D.

Variables	Spearman correlation		Wilcoxon signed ranks test	
	Correlation	P-value	test	P-value
M_I	0.80	0.008	0.000	0.100
M_0	0.85	0.002	-0.314	0.754
AUC_G	0.90	0.000	-1.020	0.308
AUC_C	0.75	0.025	-0.510	0.610
AUC_I	0.92	0.000	-0.314	0.754

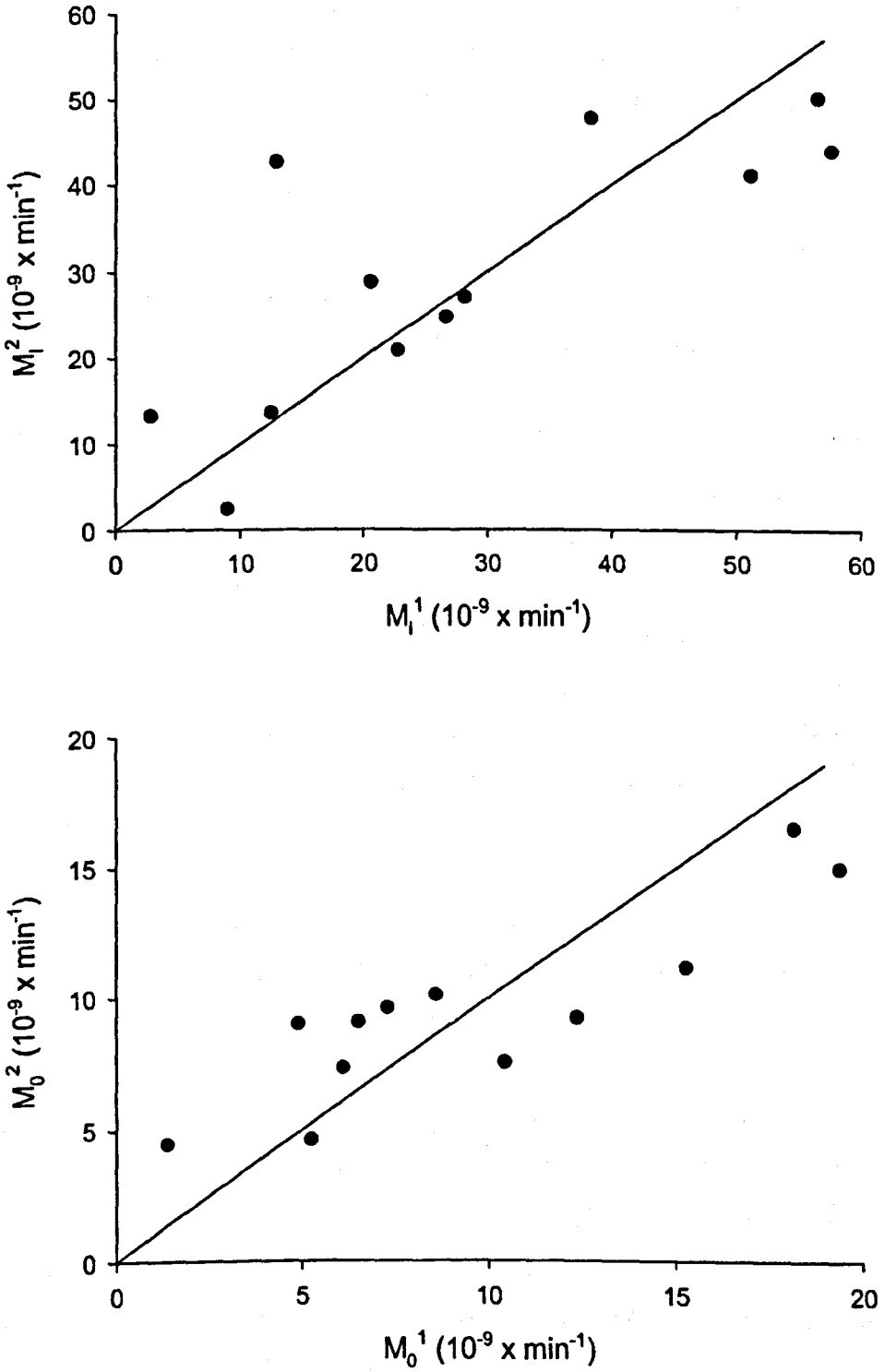

Figure 5-6. Relationship between individual estimates of repeated measures of pancreatic β-cell responsiveness indices (M_I) top panel, and (M_0) bottom panel during MTT in subjects with T2D (N = 12).

5.4. Discussion

We investigated the reproducibility of pancreatic β-cell responsiveness indices, M_I and M_0 and the reproducibility of glucose, insulin, and C-peptide to oral challenge tests in healthy subjects and subjects with T2D. In the first part we investigated the reproducibility of M_I, M_0, and glucose, insulin, and C-peptide incremental area under curve 0-90 min during MTT and OGTT in healthy subjects. In the second part and due to the data limitation, we investigated the same parameters during MTT but not OGTT in subjects with T2D. The glucose, insulin and C-peptide responses were studied with the incremental area under the curve to eliminate the effect of the basal values (165).

Healthy group

We observed a lack of reproducibility of glucose and C-peptide profiles during MTT and OGTT (within subject CV ≥ 30%). Slightly smaller CV values (21% ≤ CV ≤ 29%) were reported for the repeated 2hr glucose during OGTT in healthy subjects (166). In accordance with other studies (167; 168), incremental area under curve 0-90 minutes of insulin but not glucose or C-peptide were reproducible during MTT and OGTT. McDonald et al (169) reported a lack of reproducibility for the 1h 2h and 3h glucose concentration after a 100mg oral glucose load repeated six times over a period of one year in healthy subjects.

As a result of the lack of reproducibility of glucose and C-peptide, M_I and M_0 showed weak reproducibility during MTT and OGTT. Although they were not significantly different, no significant correlation was observed.

In the present study, glucose, and C-peptide, but not insulin, responses failed to show significant correlation between the repeated measures during MTT and OGTT ($r_S \geq 0.78$, $P \leq 0.07$ for insulin). The lack of correlation between glucose AUC during OGTT in healthy subjects was reported by Ganda et al (168). The authors reported a significant correlation between insulin AUC 0-60 min ($r_S = 49$, $P < 0.05$). Harding et al (167) reported insulin AUC to be more reproducible than glucose AUC in a large group of subjects with a high proportion of women of which about 15% exhibited abnormal glucose tolerance.

Several reports indicated poor reproducibility of the OGTT in healthy subjects and subjects with T2D (37; 166; 170; 171). This was explained in part by considerable broad within-subject variation after oral glucose and meal tolerance tests (155; 172-174). The inter-subject variability in healthy young and lean subjects was reported to be higher (155). The level of reproducibility is also affected by changes in digestion or absorption from time to time and uncontrolled environmental factors (175; 176). Other studies suggested that day-to-day variations in glucose tolerance could result from variation in cortisol secretion in response to stress (167).

T2D group

Pancreatic β-cell responsiveness indices M_I and M_0 were reproducible during MTT in subjects with T2D, in line with reproducible glucose and C-peptide profiles (within subject variation as % of total variation was 11 and 9% for M_I and M_0, respectively). The repeated measures were not significantly different and were strongly correlated ($r_S \geq 0.78$, $P < 0.05$). In subjects with T2D who underwent MTT on two separate occasions (73g carbohydrate, 20g lipid, and 31g protein for a total of 596 kcal), Le Floch et al (176) reported significant correlations between and sufficient reproducibility of glucose and insulin AUC 0-180 min ($r_S = 0.64$ and 0.87 and $P < 0.01$ and < 0.001 for glucose and insulin, respectively).

In the present study the within subject variation as a % of total variation was quite low. CV was also good (CV ≤ 21%) for glucose, insulin, and C-peptide. Wolever et al (154) reported similar CV values for glucose and insulin values over two hours (measurement were taken at 0, 15, 30, 45, 60, 90, and 120 min) after repeated mixed meal test in subjects with T2D.

5.5. Summary

Reproducibility of pancreatic β-cell responsiveness to MTT and OGTT was investigated. In healthy subjects the indices of pancreatic β-cell responsiveness failed to show sufficient reproducibility due to lack of reproducibility of glucose and C-peptide to MTT and OGTT. Insulin responses to MTT and OGTT were reproducible. In subjects with T2D, M_I and M_0 were sufficiently reproducible in line with reproducible glucose, insulin, and C-peptide responses to MTT.

6. Evaluation of two compartment minimal model performance in type 2 diabetes during insulin-modified FSIVGTT

6.1. Introduction

The one compartment minimal model (1CMM) of glucose kinetics is a powerful non-invasive tool to investigate glucose metabolism in physiological studies in different pathophysiological states (178-180). It provides two metabolic indices measuring glucose effectiveness (S_G) and insulin sensitivity (S_I) in a single individual. S_G and S_I are composite parameters, which measure the net effect of glucose and insulin respectively to promote glucose disappearance and inhibit endogenous glucose production (181). The 1CMM method has gained increasing popularity and is widely used in clinical and epidemiological studies with more than 450 papers up to 2002, because it is simple and relatively non-invasive (100). The 1CMM is represented in Figure 6-1. Recent published reports and studies indicate that S_G is overestimated (109-111; 113; 182-184) and S_I is underestimated (109-111; 184) during 1CMM attributing the main reason to the under modelling effect of using one compartment to represent the glucose pool (113; 180; 185; 186).

The new two compartment minimal model (2CMM) was first introduced in 1993 to measure hepatic glucose production during labelled IVGTT by appending a second non-accessible compartment to the classic 1CMM. The new model was needed because at that time the available single compartmental minimal model specifically developed to interpret labelled IVGTT data, provided a non-physiological pattern of hepatic glucose production (114). In 1997 Vicini et al (107) validated the 2CMM to estimate S_I, S_G, and plasma clearance rate during an isotopically labelled FSIVGTT. Recently Cobelli et al (115) incorporated a priori knowledge on glucose exchange kinetics using Bayesian estimation to derive insulin sensitivity and glucose effectiveness with the 2CMM during standard IVGTT in healthy subjects. However, the study did not test the model reliability under other conditions such as insulin-modified FSIVGTT and in subjects with type 2 diabetes (T2D).

Based on data published earlier (106), this investigation evaluated 2CMM in type 2 diabetic subjects during insulin-modified FSIVGTT and compared its performance with

1CMM. The glucose clamp technique is considered the gold standard reference method for measuring insulin sensitivity, so it is used here to assess the validity of the 1CMM and the 2CMM measurements. The Bayesian analysis was applied to estimate parameters of the 2CMM. However the model failed to give physiologically feasible estimate for S_G in 7 out of 12 subjects. The iterative two-stage population analysis was adopted, which was successful in providing feasible parameter estimates in all subjects.

6.2. Methods

6.2.1. *Subjects*

Male subjects with type 2 diabetes participated in the study (N = 12; age 59 ± 3 year, BMI 28.3 ± 0.9 kg/m^2; mean ± SE). Duration of T2D was 6.3 ± 0.6 year, see Table 6-1. No subject had any medical condition other than diabetes or received drugs except for sulfonylureas. All subjects were screened for fitness to participate in the study by a full medical history and medical examinations. Participants maintained their normal isocaloric diets and sulfonylurea therapy was omitted on the study days. The South Glamorgan Local Research Ethics Committee, Cardiff, UK, approved the study protocol, and all participants gave written informed consent.

Table 6-1. Demographic data for the 12 men with T2D participated in the study.

Sub. No.	Age (year)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Duration of diabetes (year)
1	47	92	176	30	4.1
2	57	94	172	32	9.2
3	70	72	171	25	4.2
4	43	99	182	30	7.0
5	67	62	157	25	6.7
6	53	95	168	34	6.2
7	66	85	176	28	9.3
8	70	71	172	24	6.8
9	55	75	165	28	8.0
10	61	96	176	31	7.0
11	50	77	163	29	1.7
12	69	65	165	24	5.1
Mean	59	82	170	28	6.3
SE	3	4	2	1	0.6

6.2.2. *Experimental design*

Each subject underwent a frequently sampled insulin-modified intravenous glucose tolerance test (FSIVGTT) and an isoglycaemic hyperinsulinaemic clamp in random order with 2-4 weeks between tests while participants maintained their normal isocaloric diets.

6.2.2.1. Insulin modified FSIVGTT

Subjects were studied after 12 hours overnight fast. Antecubital veins were cannulated in both arms, one for sampling and the other for administration of glucose and insulin. The insulin-modified FSIVGTT consisted of 0.3g/kg glucose bolus per body weight at 0 min over 2 min and 0.05mU/kg insulin bolus at 20 min. Overall, 30 samples were taken over 180 minutes to measure plasma glucose and insulin in addition to two pre-test samples. The 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.

6.2.2.2. Isoglycaemic hyperinsulinaemic clamp

This study was conducted after a 12 hours overnight fast. An antecubital vein was cannulated and used for glucose and insulin infusions. A second cannula was inserted retrogradely into a contralateral hand vein and the hand was warmed in a heated box to allow sampling of arterialised blood. After three basal samples for plasma glucose, an infusion of human Acrapid (NovoNordisk, Bagsavaerd, Denmark) at a rate of 160mU/min/m^2 commenced for 4 min as a priming dose and then was reduced to 40 mU/min/m^2, which was maintained for the duration of the study. The plasma glucose concentration was clamped at the basal (mean of the three basal plasma glucose values) by means of variable infusion rate of 20% D-glucose, changed on the biases of plasma glucose concentration obtained every 10 min. In addition to samples for plasma glucose, blood was sampled for the measurement of insulin at the basal period and at regular intervals during the procedure.

6.2.2.3. Essay methods

Plasma glucose was essayed using glucose oxidase method on a YSI Y2300 glucose analyser (Yellow Spring, OH; intra-assay CV <2%). Immunoreactive insulin (IRI) was assayed by using conventional radioimmunoassay (intra-assay CV <6%)

6.2.3. *Data analysis*

6.2.3.1. One compartment minimal model

The 1CMM analysis of FSIVGTT data gave insulin sensitivity S_I^1 and glucose effectiveness S_G^1, see Figure 6-1. The non-linear regression analysis was used to estimate model parameters during 1CMM. The model equations and a full description of the model structure are in chapter 2. The equations of the 1CMM model are:

$$\dot{Q}(t) = -[p_1 + X(t)]Q(t) + p_1 Q_b \qquad Q(0) = Q_b + D \tag{6-1}$$

$$\dot{X}(t) = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \tag{6-2}$$

$$G(t) = Q(t)/V \tag{6-3}$$

where Q is glucose mass (mg/kg), with Q_b denoting its basal (end-test) steady state value; D is the glucose dose (mg/kg), $p_1 = k_1 + k_5$, where k_i are rate parameters (min^{-1}); X is a variable related to insulin concentration in the insulin remote compartment, and $X(t) = (k_4 + k_6)\, I(t)$; I(t) is plasma insulin concentration (μU/ml); I_b and G_b denote basal plasma insulin and glucose concentrations respectively, G is the plasma glucose concentration; V is the distribution volume per unit body weight (ml/kg), $p_2 = k_3$, and $p_3 = k_2(k_4+k_6)$ are rate parameters expressed in min^{-1} and $min^{-2}/\mu U^{-1} \times ml$, respectively.

The 1CMM derived parameters S_I^1 and S_G^1 were calculated as follows:

$$S_G^1 = p_1 V \quad \text{(ml/min/kg)} \tag{6-4}$$

$$S_I^1 = \frac{p_3}{p_2} V \quad \text{(ml/min/kg per μU/ml)} \tag{6-5}$$

S_I^1 and S_G^1 at variance with the fractional indices S_I and S_G commonly expressed elsewhere, and have the same units as the analogous glucose clamp indices allowing for direct comparison.

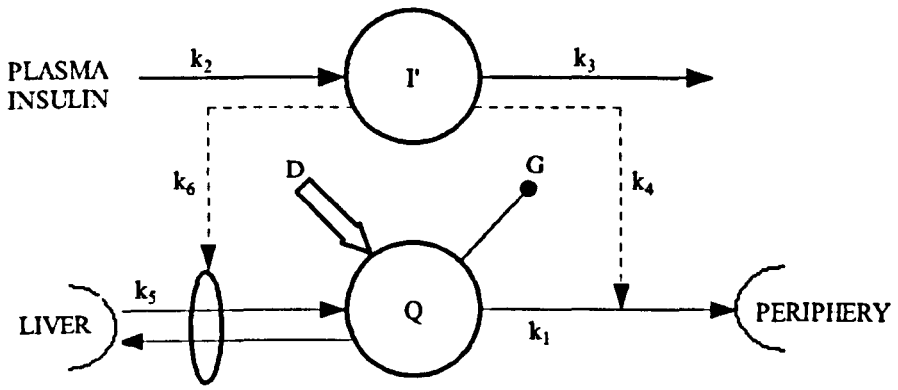

Figure 6-1. One compartment minimal model (1CMM) (115).

6.2.3.2. Two compartment minimal model

The 2CMM appends a second non-accessible compartment to the 1CMM . The 2CMM was validated in normal subjects during standard IVGTT by applying Bayesian approach to incorporate prior knowledge on k_{12} and k_{21} parameters (115). The glucose exchange kinetic parameters k_{12} and k_{21} were assumed to be normally distributed with mean and standard deviation of 0.070 ± 0.018 and 0.050 ± 0.013 min^{-1}, respectively, and with a correlation of 0.90. The model equations are as follows:

$$Q_1(t) = -[p_1 + k_{21} + X(t)]Q_1(t) + k_{12}Q_2(t) + p_1Q_{1b} \qquad Q_1(0) = Q_{1b} + D \qquad (6\text{-}6)$$

$$Q_2(t) = k_{21}Q_1(t) - k_{12}Q_2(t) \qquad Q_2(0) = Q_{2b} \qquad (6\text{-}7)$$

$$X(t) = -p_2X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \qquad (6\text{-}8)$$

$$G(t) = Q_1(t)/V_1 \qquad (6\text{-}9)$$

where Q_1 and Q_2 (mg/kg) denote the glucose masses in the accessible and non-accessible compartments, respectively, with subscript b denoting their basal (end-test) steady-state values; V_1 is the volume of the accessible compartment (mg/kg); k_{12} and k_{21} are rate parameters describing glucose exchange kinetics (1/min); D, G, I, X, p_1, p_2, and

p_3 are variables and parameters already defined for the 1CMM. The 2CMM parameters S_I^2 and S_G^2 were calculated as follows:

$$S_G^2 = p_1 V_1 \quad \text{(ml/min/kg)} \tag{6-10}$$

$$S_I^2 = \frac{p_3}{p_2} V_1 \quad \text{(ml/min/l per μU/ml)} \tag{6-11}$$

The 2CMM differs from 1CMM only in allowing an exchange of glucose between the accessible and the non-accessible compartment, see Figure 6-2. This added complexity brings a priori identifiably problems. Theoretical or a priori identifiably addresses the ability of getting unique solutions for the unknown parameters on the basis of the experiment generated data. To solve these problems and reach the unique identifiably a priori information on the glucose exchange kinetics parameters k_{12} and k_{21} was used applying Bayesian estimation (115).

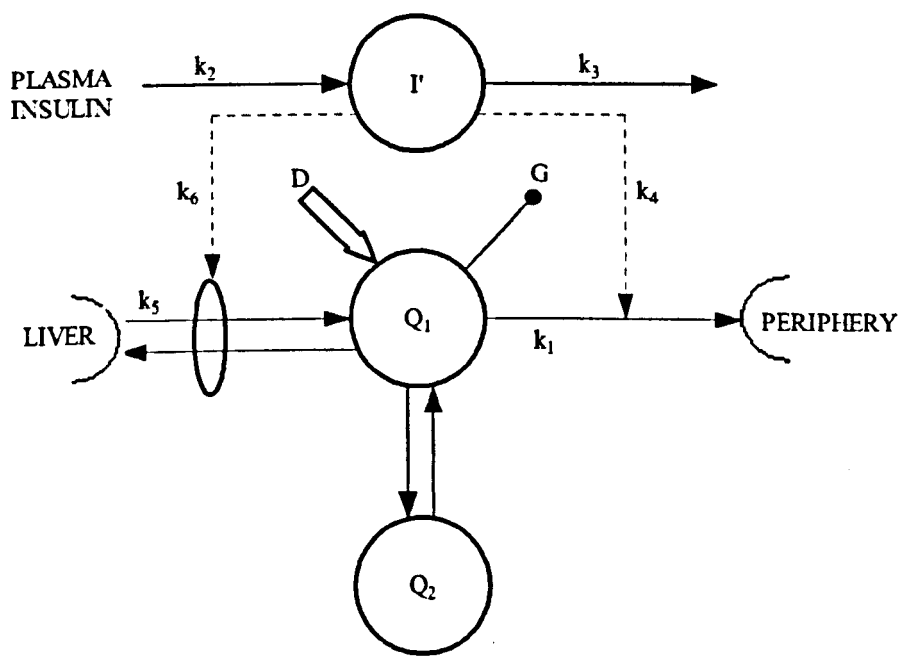

Figure 6-2. Two compartment minimal model (2CMM) (115).

6.2.3.2.1. *Bayesian prior on k_{12} and k_{21}*

Bayesian analysis was applied to estimate 2CMM parameters (insulin sensitivity S_I^2 and glucose effectiveness S_G^2) using the available prior knowledge on k_{12} and k_{21} from healthy subjects because of unavailability of prior knowledge from T2D subjects. The prior knowledge was adopted from Cobelli et al (115) who reanalysed published tracer bolus injection data obtained in the basal state in healthy subjects with a 2CMM corresponding to the one used in the current study and with no irreversible loos from the non-accessible pool. The glucose exchange kinetic parameters k_{12} and k_{21} were assumed to be normally distributed with mean and standard deviation of 0.070 ± 0.018 and 0.050 ± 0.013 min^{-1}, respectively, and with a correlation of 0.90 (115).

6.2.3.2.2. *Iterative two stage population approach*

The iterative two-stage population analysis was applied to estimate 2CMM parameters. This approach is based on calculation of the population mean and the standard deviation at each iteration and then uses them as prior information for the individual analysis. The use of a prior in the population analysis should improve the precision of the individual estimates. All parameters were log transformed before analysis to assure nonnegativity of parameter estimates. The model gives insulin sensitivity $S_I^{2\text{-POP}}$ and glucose effectiveness $S_G^{2\text{-POP}}$.

6.2.3.3. Parameter estimation

The minimal model analysis of the insulin-modified FSIVGTT data during 1CMM and 2CMM was completed using SAAM II © (software applications for kinetics analysis) version 1.2 (SAAM Institute, University of Washington, Seattle, USA) (187; 188). The precision of parameter estimates was obtained from the inverse of the Fisher information matrix and calculated as the fractional standard deviation (FSD).

6.2.3.2.3. *Model evaluation*

The following assessment procedure was applied to the 2CMM with Bayesian analysis and the 2CMM with the iterative two-stage population analysis whose parameters have been estimated successfully (a priori identifiably). The 1CMM will not undergo this assessment since it has been validated in previous studies (94).

To assess the adequacy of the 2CMM during insulin-modified FSIVGTT in T2D subjects, we evaluated practical (a posteriori) identifiably, and goodness of fit (143). The following criteria were used to assess the model validity:

- Coefficient of variation (CV) measured as the fractional standard deviation (FSD) for the assessment of precision of parameter estimates
- Distribution of normalised residual for the assessment of model's ability to fit data (goodness of fit) considering the measurement errors, and to detect any systematic deviation between the data and the model prediction
- Runs test to assess the distribution of the residuals and check for presence of model misfit.

6.2.3.4. Clamp

Isoglycaemic glucose clamp was used as the gold standard reference when comparing the insulin sensitivity indices derived by 1CMM and 2CMM. S_{ICLAMP} was derived from the steady-state glucose infusion rate [M value (min/mg/kg)] during the 3rd hour of the clamp corrected for the ambient insulin and glucose concentrations

$$S_{IClamp} = \frac{M}{\Delta I \times G} \quad \text{(ml/min/kg per μU/ml)} \qquad (6\text{-}12)$$

where ΔI (pmol/l) is the increment in insulin concentration from basal, and G (mmol/l) is the clamped glucose concentration.

6.2.3.5. Statistical analysis

Pearson's correlation coefficient was used to assess relationships between variables. Significant differences between insulin sensitivity indices among different models were determined by two-way analysis of variance, and pairwise significance was further tested by the Games-Howell multiple comparison method. Paired sample t-test was applied to assess the difference between S_G^1 and $S_G^{2\text{-POP}}$. Significance was declared at $P < 0.05$.

6.3. Results

6.3.1. *Plasma glucose and insulin*

Table 6-2 shows the basal insulin (FPI) and glucose (FPG) values during FSIVGTT. Table 6-3 shows data from isoglycaemic hyperinsulinaemic clamp and includes basal and clamped values for glucose and insulin, and the M value. FPG and FPI concentrations were not different between the two study days (P = 0.13 and P = 0.65, paired t-test, respectively). Figure 6-3 shows the profile of glucose and insulin concentrations during the FSIVGTT.

Table 6-2. Basal plasma insulin and glucose values during FSIVGTT in subjects with type 2 diabetes (N = 12).

Subject No.	Basal insulin (pmol/l)	Basal glucose (mmol/l)
1	64	9.3
2	132	7.9
3	48	6.0
4	80	11.5
5	132	8.8
6	152	11.0
7	60	7.6
8	78	12.6
9	132	8.0
10	42	8.1
11	116	7.2
12	182	9.3
Mean	102	8.9
SE	13	0.6

Table 6-3. Isoglycaemic clamp data in subjects with type 2 diabetes (N = 12).

Subject No.	Basal insulin (pmol/l)	Clamped insulin (pmol/l)	Basal glucose (mmol/l)	Clamped glucose (mmol/l)	M (min/mg/kg)
1	72	384	8.5	8.4	3.26
2	141	564	9.0	9.0	3.87
3	48	438	5.1	5.1	4.11
4	87	630	9.3	9.2	2.66
5	90	756	7.7	7.6	3.84
6	156	528	12.7	12.6	1.71
7	72	462	6.3	6.2	4.10
8	75	654	11.6	11.3	6.26
9	138	492	7.6	7.5	3.09
10	60	480	7.1	7.0	6.29
11	162	846	5.7	5.7	3.09
12	153	630	10.1	10.0	1.92
Mean	105	572	8.4	8.3	3.68
SE	12	39	0.7	0.7	0.40

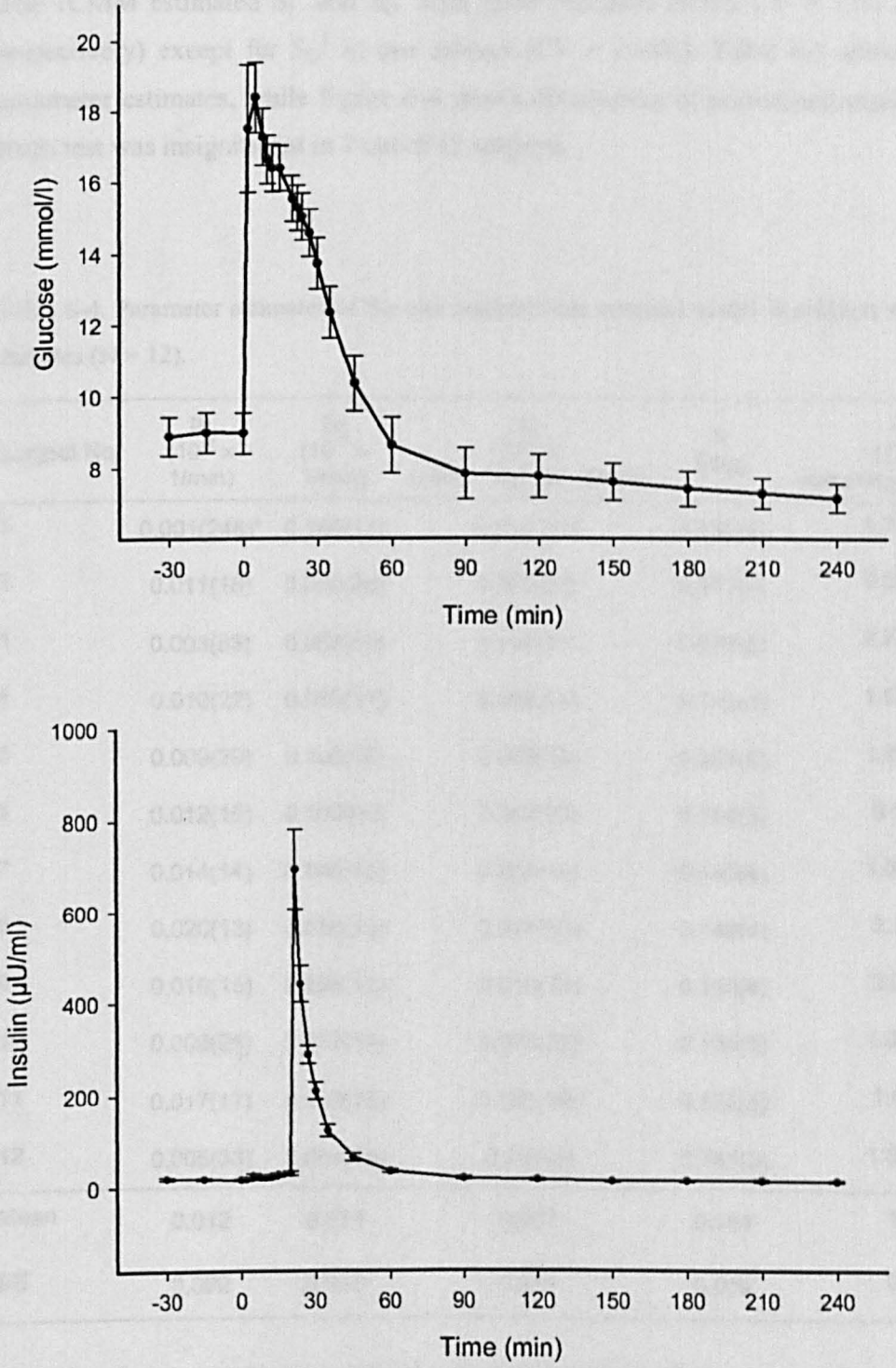

Figure 6-3. Plasma glucose and insulin profiles during FSIVGTT in subjects with T2D (N=12, mean ± SE).

6.3.2. *One compartment minimal model*

The 1CMM estimated S_I^1 and S_G^1 with good precision (mean CV = 13% and 21%, respectively) except for S_G^1 in one subject (CV = 246%). Table 6-3 shows 1CMM parameter estimates, while Figure 6-4 shows distribution of normalised residuals. The Runs test was insignificant in 7 out of 12 subjects.

Table 6-4. Parameter estimates of the one compartment minimal model in subjects with type 2 diabetes (N = 12).

Subject No.	p_1 (10^{-2} × 1/min)	p_2 (10^{-2} × 1/min)	p_3 (10^{-5} × ml/min^{-1}/kg per μU/ml)	V (l/kg)	S_I^1 (10^{-2} × ml/min/kg per μU/ml)
1	0.001(246)*	0.166(11)	0.016 (13)	0.183(4)	1.75(11)
2	0.011(18)	0.090(36)	0.003(31)	0.187(4)	0.57(30)
3	0.003(83)	0.069(10)	0.008(11)	0.233(5)	2.79(13)
4	0.010(22)	0.069(11)	0.008(16)	0.146(4)	1.62(11)
5	0.009(29)	0.100(16)	0.009(18)	0.201(5)	1.86(17)
6	0.012(15)	0.100(40)	0.002(33)	0.154(3)	0.14(2)
7	0.014(14)	0.045(14)	0.003(19)	0.140(4)	1.02(21)
8	0.020(13)	0.050(18)	0.011(13)	0.148(4)	3.35(3)
9	0.016(15)	0.056(12)	0.010(13)	0.139(4)	2.39(8)
10	0.009(25)	0.037(14)	0.003(22)	0.134(5)	1.03(17)
11	0.017(17)	0.058(15)	0.005(18)	0.124(5)	1.05(8)
12	0.005(33)	0.054(10)	0.007(9)	0.141(3)	1.82(12)
Mean	0.012	0.074	0.007	0.161	1.62
SE	0.002	0.010	0.001	0.009	0.27

*Precision of parameter estimates expressed as fractional standard deviation

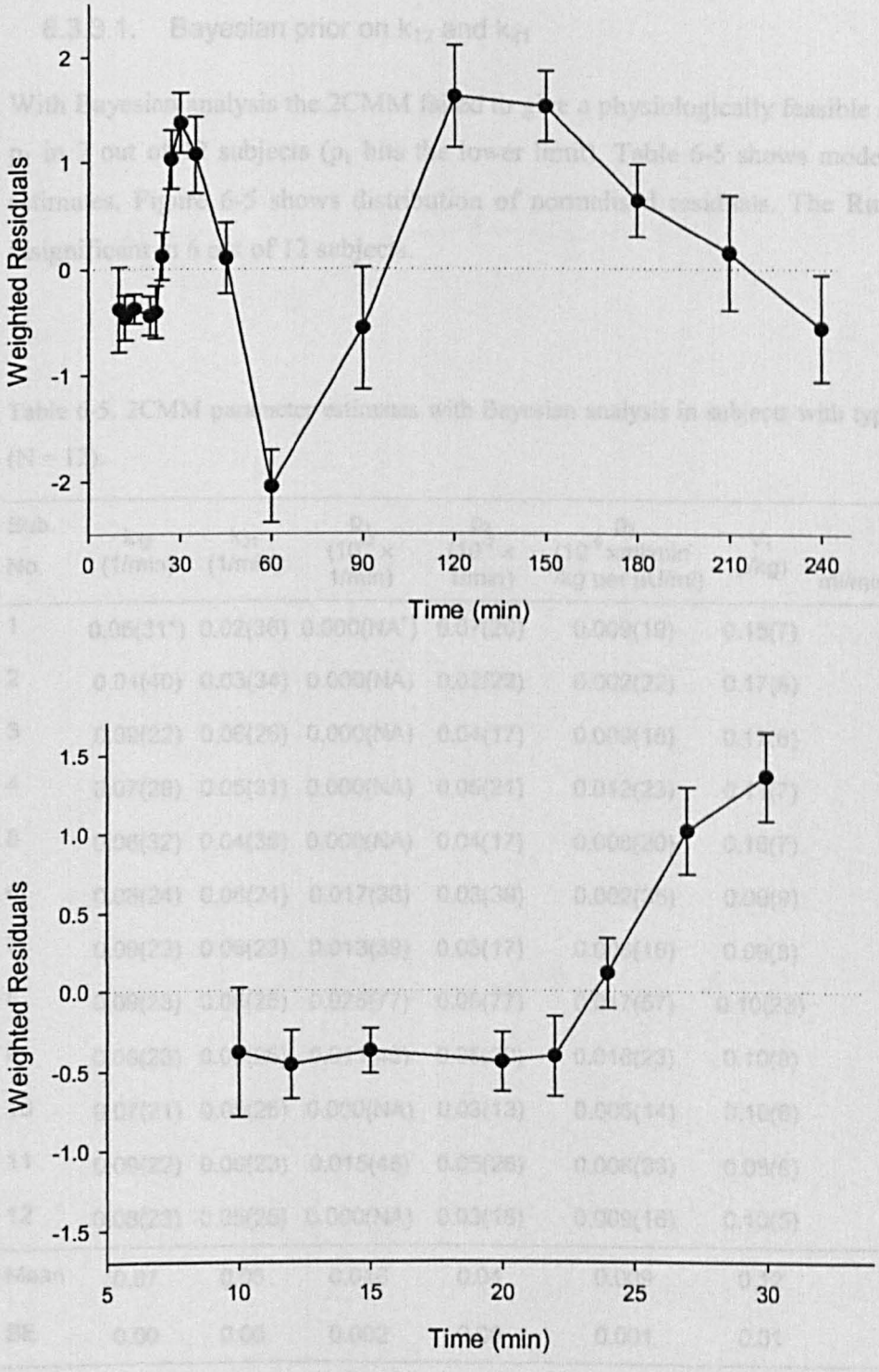

Figure 6-4. Weighted residuals during 1CMM for the full test (top panel) and the first 30 min (bottom panel), (mean ± SE).

6.3.3. *Two compartment minimal model*

6.3.3.1. Bayesian prior on k_{12} and k_{21}

With Bayesian analysis the 2CMM failed to give a physiologically feasible estimate for p_1 in 7 out of 12 subjects (p_1 hits the lower limit). Table 6-5 shows model parameter estimates. Figure 6-5 shows distribution of normalised residuals. The Runs test was insignificant in 6 out of 12 subjects.

Table 6-5. 2CMM parameter estimates with Bayesian analysis in subjects with type 2 diabetes (N = 12).

Sub. No.	k_{12} (1/min)	k_{21} (1/min)	p_1 (10^{-2} × 1/min)	p_2 (10^{-2} × 1/min)	p_3 (10^{-5} ×ml/min^{-1}/kg per μU/ml)	V_1 (l/kg)	S_I^2 (10^{-2} × ml/min/kg per μU/ml)
1	0.05(31*)	0.02(36)	0.000(NA+)	0.07(20)	0.009(19)	0.15(7)	2.00(3)
2	0.04(40)	0.03(34)	0.000(NA)	0.02(22)	0.002(22)	0.17(6)	2.06(5)
3	0.09(22)	0.06(26)	0.000(NA)	0.04(17)	0.009(18)	0.17(6)	3.51(3)
4	0.07(29)	0.05(31)	0.000(NA)	0.05(21)	0.012(23)	0.11(7)	2.70(4)
5	0.06(32)	0.04(35)	0.000(NA)	0.04(17)	0.008(20)	0.16(7)	3.78(3)
6	0.08(24)	0.06(24)	0.017(33)	0.03(39)	0.002(35)	0.09(9)	0.57(46)
7	0.09(23)	0.06(23)	0.013(39)	0.03(17)	0.006(18)	0.09(8)	1.89(25)
8	0.09(23)	0.06(25)	0.025(77)	0.05(77)	0.017(57)	0.10(23)	3.59(14)
9	0.08(23)	0.06(25)	0.011(48)	0.05(20)	0.016(23)	0.10(8)	3.32(12)
10	0.07(21)	0.05(26)	0.000(NA)	0.03(13)	0.005(14)	0.10(6)	1.93(3)
11	0.09(22)	0.06(23)	0.015(46)	0.05(26)	0.008(30)	0.08(8)	1.38(14)
12	0.08(23)	0.05(25)	0.000(NA)	0.03(15)	0.009(16)	0.10(5)	2.75(3)
Mean	0.07	0.05	0.016	0.04	0.009	0.12	2.46
SE	0.00	0.00	0.002	0.00	0.001	0.01	0.28

*Precision of parameter estimates expressed as fractional of standard deviation

+Indicates estimation failure

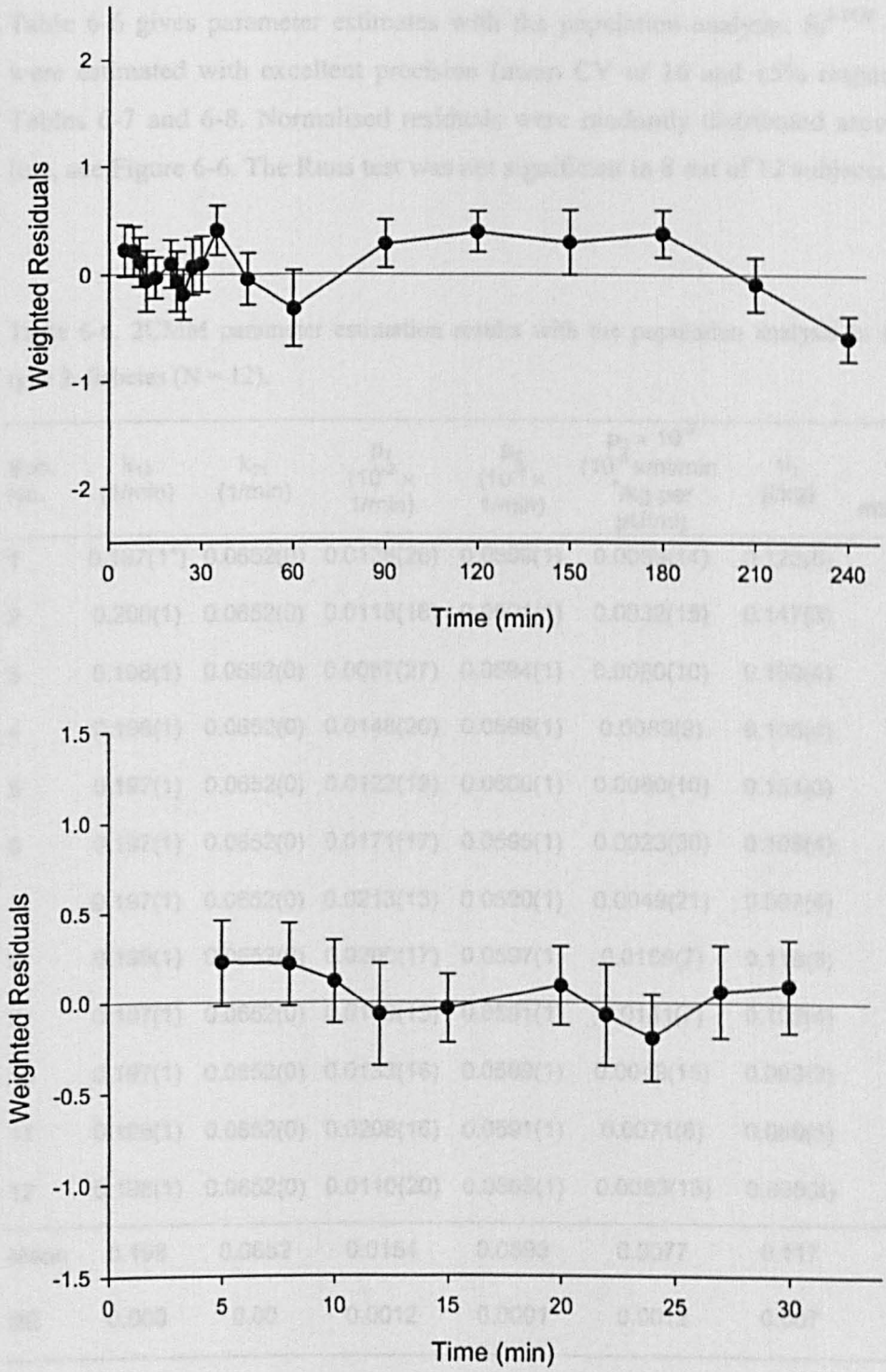

Figure 6-5. Weighted residuals during 2CMM with Bayesian analysis for the full test (top panel) and the first 30 minutes (bottom panel), (mean ± SE).

6.3.3.2. Iterative two-stage population approach

Table 6-6 gives parameter estimates with the population analysis. $S_I^{2\text{-POP}}$ and $S_G^{2\text{-POP}}$ were estimated with excellent precision (mean CV of 16 and 15% respectively), see Tables 6-7 and 6-8. Normalised residuals were randomly distributed around the zero line, see Figure 6-6. The Runs test was not significant in 8 out of 12 subjects.

Table 6-6. 2CMM parameter estimation results with the population analysis in subjects with type 2 diabetes (N = 12).

Sub. No.	k_{12} (1/min)	k_{21} (1/min)	p_1 (10^{-2} × 1/min)	p_2 (10^{-2} × 1/min)	$p_3 \times 10^{-3}$ (10^{-5} ×ml/min^{-1}/kg per µU/ml)	V_1 (l/kg)	$S_I^{2\text{-POP}}$ (10^{-2} × ml/min/kg per µU/ml)
1	0.197(1*)	0.0652(0)	0.0138(26)	0.0599(1)	0.0059(14)	0.123(6)	1.22(17)
2	0.200(1)	0.0652(0)	0.0118(16)	0.0591(1)	0.0032(15)	0.147(3)	0.80(16)
3	0.198(1)	0.0652(0)	0.0087(27)	0.0594(1)	0.0080(10)	0.169(4)	2.28(12)
4	0.198(1)	0.0652(0)	0.0148(20)	0.0596(1)	0.0089(9)	0.105(4)	1.58(12)
5	0.197(1)	0.0652(0)	0.0122(19)	0.0600(1)	0.0080(10)	0.151(3)	2.01(13)
6	0.197(1)	0.0652(0)	0.0171(17)	0.0595(1)	0.0023(30)	0.108(4)	0.41(32)
7	0.197(1)	0.0652(0)	0.0213(13)	0.0590(1)	0.0049(21)	0.097(4)	0.80(24)
8	0.199(1)	0.0652(0)	0.0200(17)	0.0597(1)	0.0169(7)	0.119(8)	3.39(8)
9	0.197(1)	0.0652(0)	0.0199(15)	0.0591(1)	0.0141(7)	0.102(4)	2.43(9)
10	0.197(1)	0.0652(0)	0.0133(16)	0.0589(1)	0.0049(15)	0.093(3)	0.77(17)
11	0.196(1)	0.0652(0)	0.0208(16)	0.0591(1)	0.0071(8)	0.089(5)	1.08(11)
12	0.198(1)	0.0652(0)	0.0110(20)	0.0585(1)	0.0083(13)	0.098(3)	1.39(14)
Mean	0.198	0.0652	0.0154	0.0593	0.0077	0.117	1.51
SE	0.000	0.00	0.0012	0.0001	0.0012	0.007	0.25

*Precision of parameter estimates expressed as fractional standard deviation

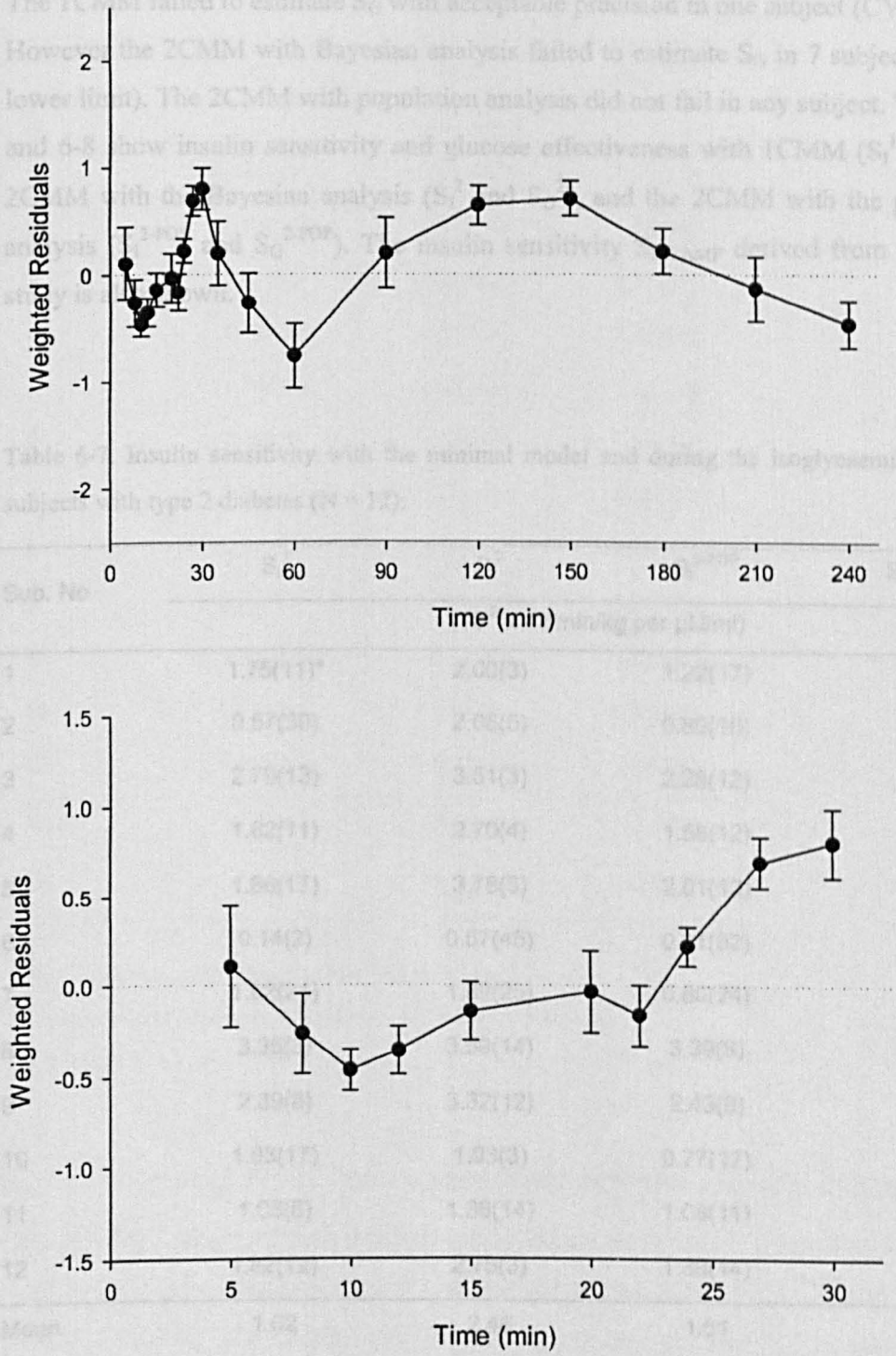

Figure 6-6. Weighted residuals during 2CMM with population analysis for the full test (top panel) and the first 30 minutes (bottom panel), (mean ± SE).

6.3.4. *Insulin sensitivity and glucose effectiveness*

The 1CMM failed to estimate S_G with acceptable precision in one subject (CV > 100%). However the 2CMM with Bayesian analysis failed to estimate S_G in 7 subjects (p_1 hits lower limit). The 2CMM with population analysis did not fail in any subject. Tables 6-7 and 6-8 show insulin sensitivity and glucose effectiveness with 1CMM (S_I^1 and S_G^1), 2CMM with the Bayesian analysis (S_I^2 and S_G^2), and the 2CMM with the population analysis ($S_I^{2\text{-POP}}$ and $S_G^{2\text{-POP}}$). The insulin sensitivity S_{ICLAMP} derived from the clamp study is also shown.

Table 6-7. Insulin sensitivity with the minimal model and during the isoglycaemic clamp in subjects with type 2 diabetes (N = 12).

Sub. No	S_I^1	S_I^2	$S_I^{2\text{-POP}}$	S_{ICLAMP}
	(10^{-2} × ml/min/kg per μU/ml)			
1	1.75(11)*	2.00(3)	1.22(17)	2.12
2	0.57(30)	2.06(5)	0.80(16)	5.64
3	2.79(13)	3.51(3)	2.28(12)	9.58
4	1.62(11)	2.70(4)	1.58(12)	6.67
5	1.86(17)	3.78(3)	2.01(13)	8.12
6	0.14(2)	0.57(46)	0.41(32)	2.00
7	1.02(21)	1.89(25)	0.80(24)	5.00
8	3.35(3)	3.59(14)	3.39(8)	12.46
9	2.39(8)	3.32(12)	2.43(9)	8.99
10	1.03(17)	1.93(3)	0.77(17)	3.73
11	1.05(8)	1.38(14)	1.08(11)	4.42
12	1.82(12)	2.75(3)	1.39(14)	3.80
Mean	1.62	2.46	1.51	6.04
SE	0.27	0.28	0.25	0.93

*Precision of parameter estimates expressed as fractional standard deviation

Table 6-8. Glucose effectiveness with the 1CMM and the 2CMM in subjects with type 2 diabetes (N = 12).

Sub. ID.	S_G^1	S_G^2	$S_G^{2\text{-POP}}$
		(ml/min/kg)	
1	0.18(246)*	0.00(NA+)	1.70(22)
2	2.15(14)	0.00(NA)	1.74(14)
3	0.67(79)	0.00(NA)	1.47(25)
4	1.48(19)	0.00(NA)	1.56(16)
5	1.74(24)	0.00(NA)	1.83(16)
6	2.64(3)	1.53(26)	1.84(14)
7	1.97(10)	1.17(33)	2.06(10)
8	3.00(10)	2.37(58)	2.39(13)
9	2.25(11)	1.12(43)	2.03(11)
10	1.24(21)	0.00(NA)	1.23(14)
11	2.06(12)	1.21(40)	1.85(12)
12	0.69(30)	0.00(NA)	1.07(18)
Mean	1.81	1.48**	1.73
SE	0.21	0.23	0.10

* Value not included in the calculation of the mean

** Mean reflects 5 subjects and does not include zero estimates

+ Indicates estimation failure

6.3.5. *Comparability of insulin sensitivity and glucose effectiveness*

The 2CMM with population analysis and 1CMM were measuring the same S_I and S_G (P = 0.99 and 0.47 respectively), with a strong correlation between $S_I^{2\text{-POP}}$ and S_{ICLAMP} (r_S = 0.91, P<0.001). The 2CMM with Bayesian approach estimated S_I 35% higher than the 1CMM but with no significant difference (P = NS), and strongly correlated with S_{ICLAMP} (r_S = 0.83, P<0.01). However it failed to estimate S_G in 7 out of 12 of cases (S_G = 0). Figure 6-7 summarises insulin sensitivity and glucose effectiveness results. Correlation results are shown in Table 6-9. Two way analysis of variance was significant for the

between parameters variability (p = 0.000). Table 6-10 shows the Games-Howell multi-comparison results. Figures 6-8 to 6-10 show the graphical representation of relationships between S_{ICLAMP}, S_I^1, S_I^2 and $S_I^{2\text{-}POP}$. Figure 6-11 shows the relationship between S_G^1 and $S_G^{2\text{-}POP}$ ($r_S = 0.76$, $P<0.01$). Due to the high failure rate in the estimate of S_G during 2CMM with Bayesian analysis it was difficult to relate S_G^2 to S_G^1 and $S_G^{2\text{-}POP}$.

Table 6-9. Pearson correlation (with the Bonferroni correction) between insulin sensitivity indices during 1CMM, 2CMM, and the clamp method.

	S_I^1	S_I^2	$S_I^{2\text{-}POP}$	S_{ICLAMP}
S_I^1	1	0.85*	0.96**	0.81*
S_I^2		1	0.86*	0.83*
$S_I^{2\text{-}POP}$			1	0.91**
S_{ICLAMP}				1

* $p < 0.01$

** $p < 0.001$

Table 6-10. The results of Games-Howell multiple comparison between insulin sensitivity indices during 1CMM, 2CMM, and the clamp method.

	S_I^1	S_I^2	$S_I^{2\text{-}POP}$	S_{ICLAMP}
S_I^1	1	NS	NS	0.003*
S_I^2		1	NS	0.012*
$S_I^{2\text{-}POP}$			1	0.002*
S_{ICLAMP}				1

* The mean difference is significant at the 0.05 level

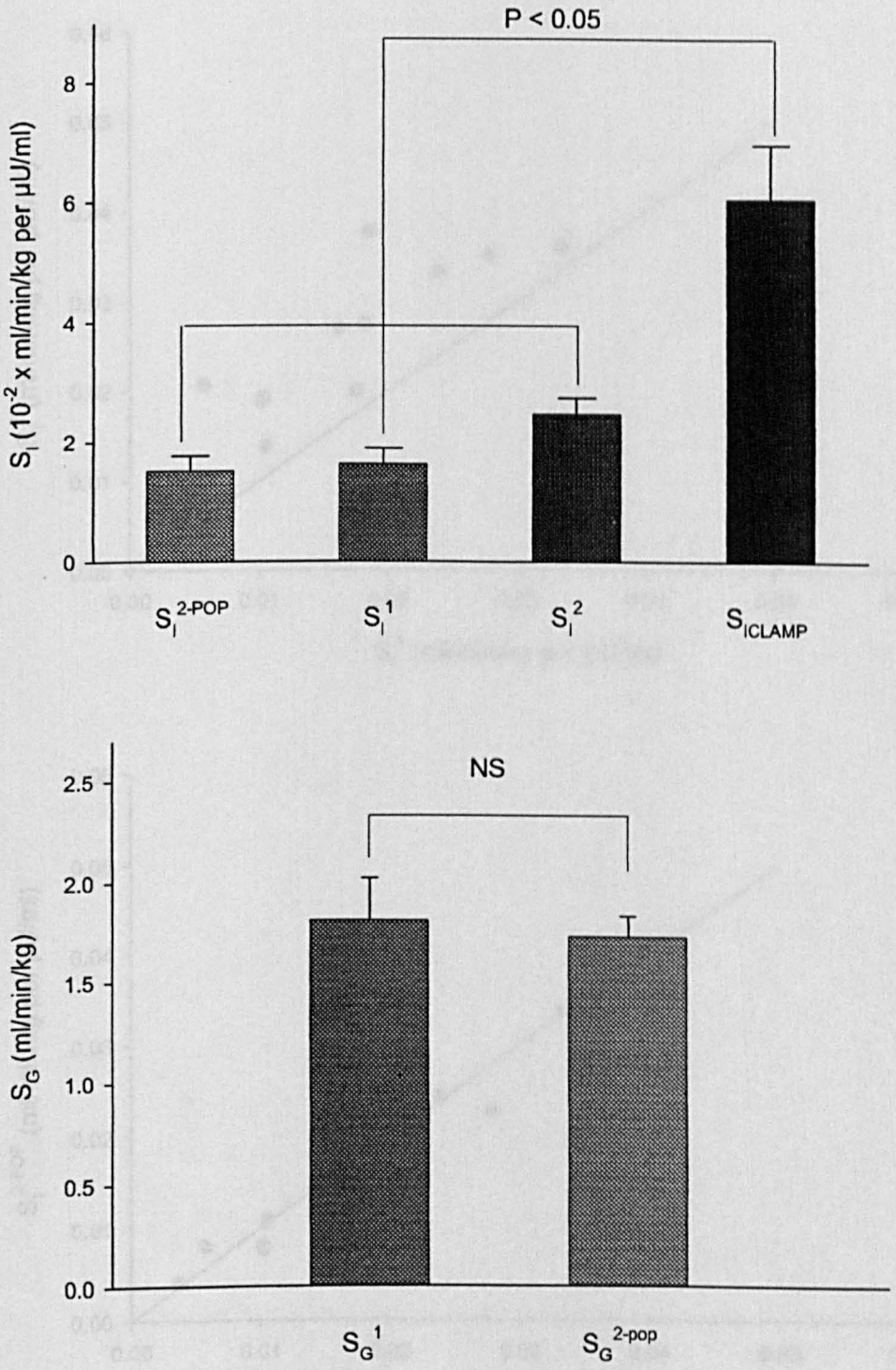

Figure 6-7. Insulin sensitivity (top panel) and glucose effectiveness (bottom panel). Results are expressed as mean ± SE, (N=12).

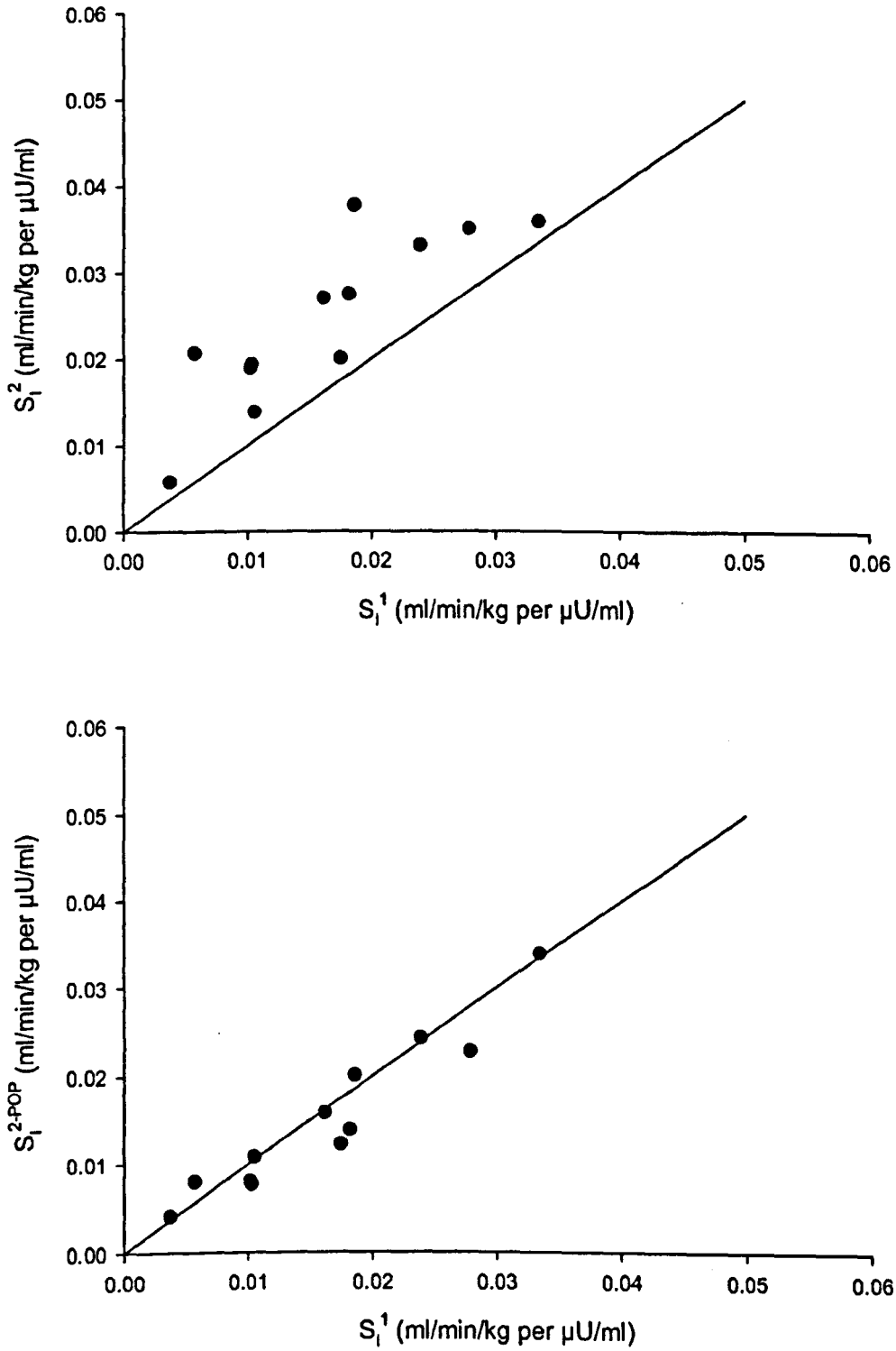

Figure 6-8. Relationship between individual estimates of S_I^1 and S_I^2 (top panel), and between S_I^1 and $S_I^{2\text{-POP}}$ (bottom panel). A unity line is shown.

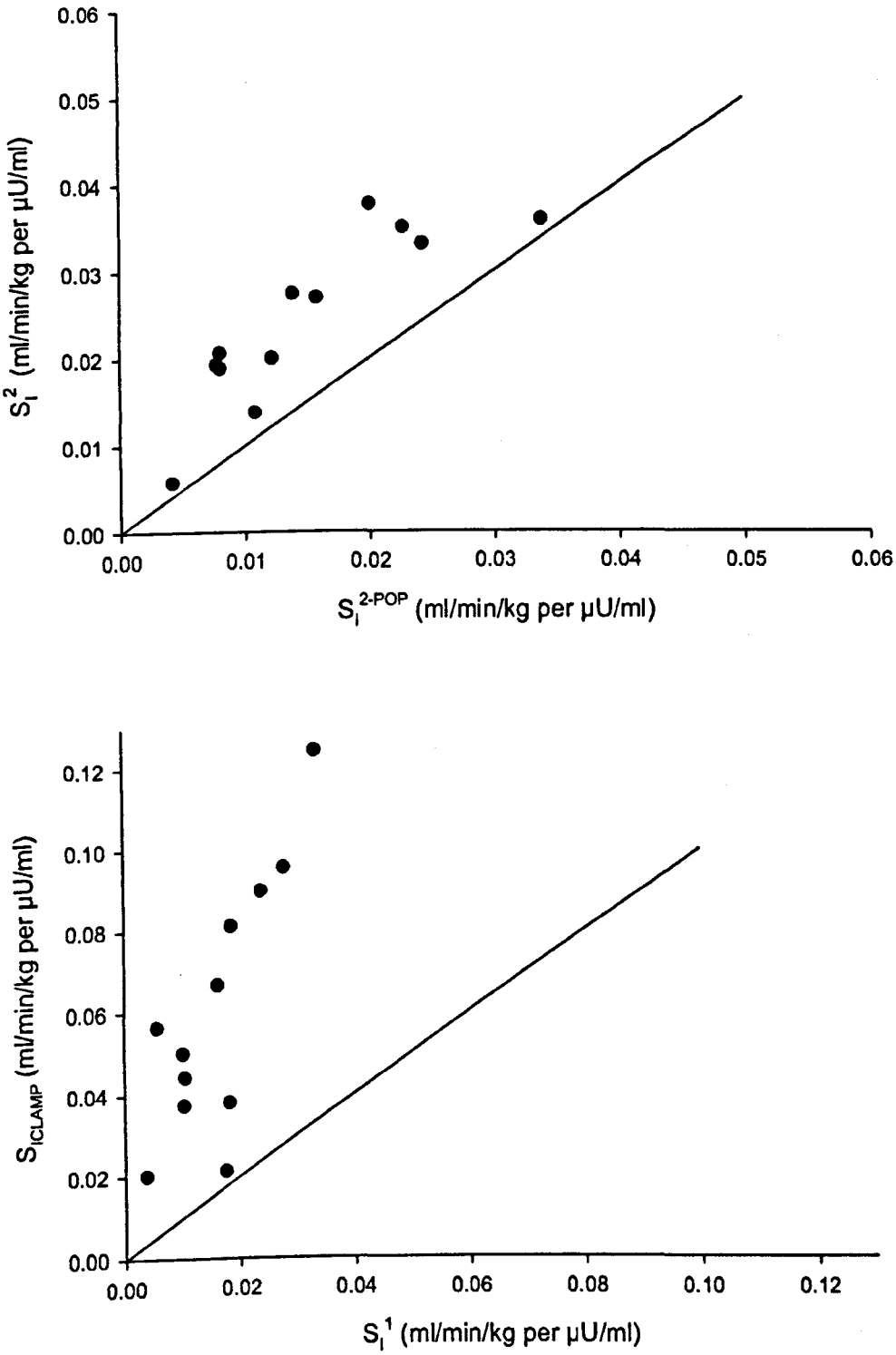

Figure 6-9. Relationship between individual estimates of S_I^1 and S_{ICLAMP} (top panel), and between S_I^2 and $S_I^{2\text{-POP}}$ (bottom panel). A unity line is shown.

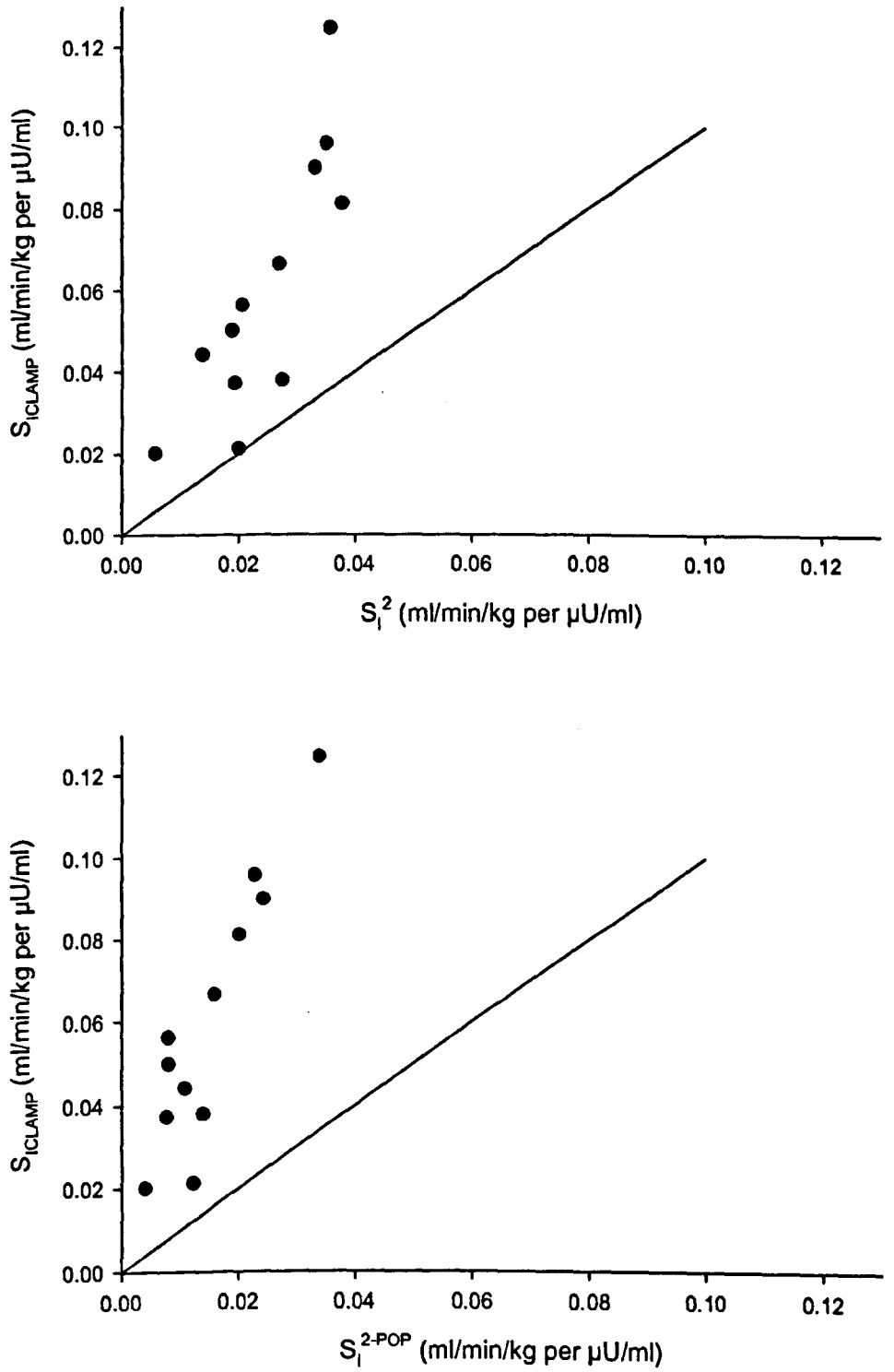

Figure 6-10. Relationship between individual estimates of S_{ICLAMP} and S_I^2 (top panel), and between S_{ICLAMP} and $S_I^{2\text{-POP}}$ (bottom panel). A unity line is shown.

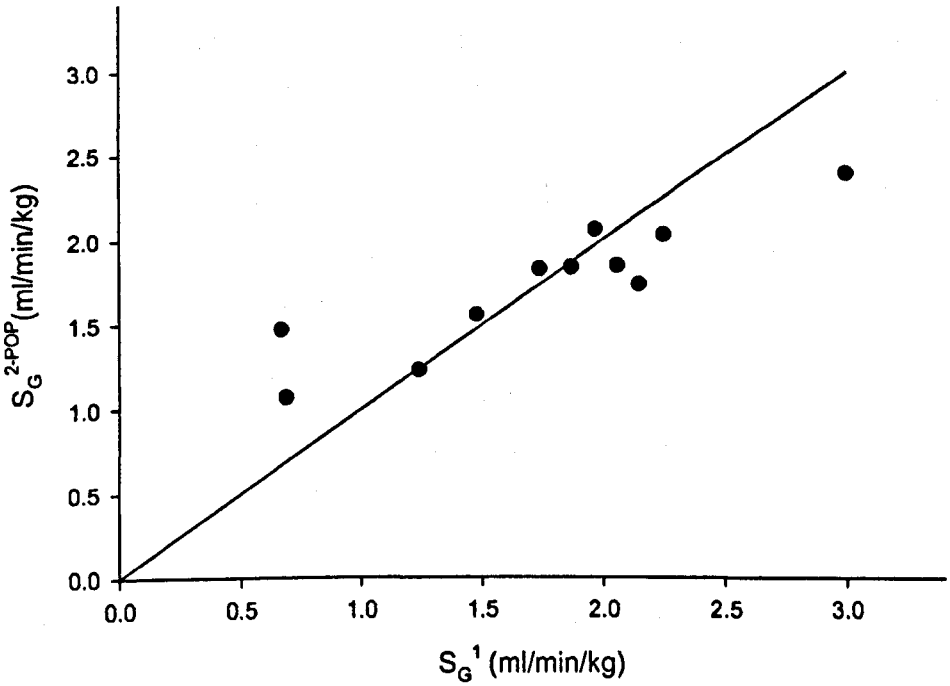

Figure 6-11. Relationship between individual estimates of S_G^1 and $S_G^{2\text{-POP}}$. A unity line is shown.

6.4. Discussion

During modified FSIVGTT in subjects with T2D, the insulin sensitivity index derived with the 2CMM applying population analysis was comparable to that measured with the 1CMM and was strongly correlated with the clamp insulin sensitivity index. Validation of the 2CMM in T2D subjects using population analysis was successful. S_I and S_G were estimated with excellent precision (mean CV for $S_I^{2\text{-POP}}$ and $S_G^{2\text{-POP}}$ was $\leq$ 13%). Normalised residuals had satisfactory behaviour in terms of both pattern and amplitude and were randomly distributed around the zero line indicating a good model fit.

In the current study, $S_I^{2\text{-POP}}$ was correlated with S_I^1, S_I^2, and S_{ICLAMP} ($r_S > 0.81$), with no significant difference from S_I^1 (1.51 ± 0.25 vs. $1.62 \pm 0.27 \times 10^{-2} \times$ ml/min/kg per μU/ml, P=NS). In healthy subjects during insulin-modified IVGTT, Omenetto et al (189) also found no significant difference between insulin sensitivity measured by 1CMM and 2CMM (12.90 ± 1.31 and $13.24 \pm 1.40 \times 10^{-2} \times$ ml/min/kg per μU/ml, S_I^1 vs S_I^2 respectively).

In a previous study by Saad et al (16), S_{ICLAMP} was correlated with S_I^1 ($r_S = 0.41$, $P<0.01$) during insulin modified FSIVGTT in T2D subjects. However it was four fold higher (0.040 ± 0.006 vs. 0.011 ± 0.003 dl/min per μU/ml). In healthy subjects, Vicini et al (107) reported a strong correlation between S_I^{1*} (* denotes an index derived from the labeled FSIVGTT) and S_I^{2*} ($r_S = 0.95$, $P<0.001$) with no significant difference between the two indices (12.98 ± 2.21 vs. $13.83 \pm 2.54 \times 10^{-2}$ ml/min/kg per μU/ml) and suggested using 1CMM instead of 2CMM for its remarkable precision and near-perfect correlation with 2CMM (it should be noted that S_I^{2*} and S_I^2 are derived from two models with two and one outfluxes, respectively, and the two indices my present different properties). Nagasaka et al (190) also found strong correlations between S_I^1, S_I^{1*} and S_I^{2*} in healthy and T2D subjects. During labelled IVGTT in healthy subjects, Hoffman et al (182) reported more reliable estimates with 1CMM than 2CMM with no difference between insulin sensitivity. In addition the insulin sensitivity indices were positively correlated (182).

In the present study, $S_G^{2\text{-POP}}$ was correlated with S_G^1 ($r_S = 0.76$, $P<0.01$) and the indices were not significantly different (1.73 ± 0.10 vs. 1.81 ± 0.21 ml/min, P=NS). Nagasaka et al (190) found in healthy and T2D subjects a strong correlation between S_G^{1*} and S_G^{2*}

with no significant difference. Vicini et al (107) also observed a weak correlation between S_G^{1*} and S_G^{2*} in healthy subjects.

Our results during modified FSIVGTT are in accordance with previous studies during both labelled and unlabelled FSIVGTT and demonstrate insignificant difference between the 1CMM and 2CMM indices of insulin sensitivity. This insignificant difference applies also on the glucose effectiveness indices during 1CMM and 2CMM.

The values for the glucose exchange kinetic parameters k_{12} and k_{21} during population analysis of the 2CMM had a negligible intersubject variability [0.198 ± 0.000 and 0.065 ± 0.000 min^{-1}, respectively (k_{21} was identical for all subjects)] suggesting that the individual data set is not rich enough to obtain individual estimate of these parameters. Parameter estimates of k_{12} values were three fold higher than k_{21} ($P < 0.001$). These results suggest that the 2CMM with population analysis tends to treat the two glucose pools as one, which could explain the comparability of 1CMM and 2CMM parameter estimates.

The 2CMM with Bayesian approach estimated insulin sensitivity with good precision, 35% higher than 1CMM with no significant difference, and a strong correlation with S_{ICLAMP} ($r_S = 0.79$, $P<0.01$). However it failed to estimate S_G in 7 out of 12 T2D subjects (more than 50% of the cases). Although Bonadonna et al (191) demonstrated no significant difference in the fractional outward transport of 3-O-methyl-D-glucose in skeletal muscle between healthy and T2D subjects during insulin clamp, the use of informative prior information on k_{21} and k_{12} obtained in healthy but not T2D subjects may have had a negative impact on the estimation results.

Applying Bayesian analysis, Cobelli et al (115) also experienced difficulties when resolving 2CMM exchange kinetics parameters especially k_{12} with acceptable precision in healthy subjects. S_I^2 and/or S_G^2 precision was unsatisfactory (CV > 100%) in 10 out of 22 subjects. Vicini et al (107) reported unsatisfactory precision for S_I estimates during labelled IVGTT with 2CMM (S_I^{2*} and/or S_G^{2*} precision was unsatisfactory in 5 out of 14 healthy subjects). Hoffman et al (182) reported difficulties in estimating S_I^{2*} and S_G^{2*} during labelled IVGTT with reliable precision and suggested using 1CMM as a robust approach in population-based studies which provides more reliable estimates

than 2CMM. Due to the high failure rate in the estimate of S_G during 2CMM with Bayesian analysis it was difficult to carry out any comparison of S_G^2 with S_G^1 and $S_G^{2\text{-POP}}$.

The difficulty of estimating 2CMM parameters with satisfactory precision in previous studies indicates that the model is too complex for the experimental data. In addition the failure of the model to estimate glucose effectiveness in the current study with the Bayesian analysis indicates the unsuitability of the 2CMM model to fit the modified FSIVGTT data in T2D subjects. The higher estimate of insulin sensitivity during 2CMM with Bayesian analysis could be explained as an over estimation attempt to compensate for zero valued glucose effectiveness.

The insulin modification appears to reduce the insulin sensitivity with the 2CMM making it similar to the 1CMM estimate. This was demonstrated by Omenetto et al (189), who studied healthy subjects with insulin-modified IVGTT and observed identical estimates of insulin sensitivity with the 1CMM and the 2CMM, while glucose effectiveness was 35% higher with 1CMM than with the 2CMM. On the other hand, result on standard (unmodified) IVGTT in healthy subjects demonstrated that the 2CMM glucose effectiveness and insulin sensitivity were respectively 60% lower and 35% higher than the corresponding 1CMM indices (115).

The main objective of the 2CMM was to solve the under-modelling effect on glucose effectiveness and insulin sensitivity. Although the model was successfully validated in T2D subjects during insulin-modified FSIVGTT, it is still measuring the same glucose effectiveness and insulin sensitivity as the 1CMM. For its simplicity and popularity and to benefit from the data available in the literature it is recommended to use the 1CMM.

6.5. Summary

The 2CMM was validated successfully using population analysis in T2D subjects during insulin-modified FSIVGTT, giving precise estimates of S_I and S_G with strong correlation with 1CMM estimates and S_{ICLAMP}. Insulin sensitivity and glucose effectiveness estimated by 2CMM with population analysis are not different from 1CMM estimates.

7. Application of ISM and 1CMM to assess glucose metabolism in newly presenting type 2 diabetes

7.1. Introduction

The pathogenesis of Type 2 diabetes is complex and has yet to be fully understood (65; 192), however, it has been established that both insulin resistance and deficient insulin secretion play decisive roles in the development of Type 2 diabetes (8; 47; 193).

The minimal model analysis (78) using the standard or insulin-modified intravenous glucose tolerance test (IVGTT) measures insulin sensitivity (S_I) and glucose effectiveness (S_G). It also provides a measure of the first phase insulin secretion (AIR_G). The minimal model has been widely used to assess insulin resistance in various pathophysiological states (78; 194) and has become invaluable especially in population studies due to its simpler experimental design compared to the glucose clamp technique (77).

In the previous chapter the one compartment minimal model (1CMM) correlated significantly with the clamp index of insulin sensitivity and was superior to the two compartment minimal model with respect to simplicity and popularity and applicability to use in subjects with T2D. The 1CMM was shown to be able to provide reproducible estimates of glucose effectiveness and insulin sensitivity (32; 195).

The insulin secretion model (ISM) is a recently developed approach to measure fasting (M_0) and postprandial (M_1) pancreatic β-cell responsiveness during a meal tolerance test (MTT) (32). The MTT is a standardised physiological test and has the benefit of a typical postprandial exposure of the pancreas to glucose, other nutrients (fat, protein), gut and vagal hormones. The insulin secretion model has been shown to discriminate across a wide spectrum of pancreatic β-cell responsiveness (32) and to provide reproducible measurements in subjects with T2D as demonstrated in Chapter 5.

The generally accepted but as yet not confirmed hypothesis is that the IVGTT and/or MTT facilitate the estimation of essential indices of the whole-body carbohydrate metabolism. The aim of this study was to investigate whether these indices 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. In this study subjects with newly diagnosed Type 2 diabetes were studied as they present the end-point of the natural development of the disease prior to therapeutic intervention.

7.2. Research Design and Methods

7.2.1. *Subjects*

Subjects with newly presenting type 2 diabetes participated in the study (N = 65; 53 males, 12 females; age 54 ± 1 year; BMI 30.5 ± 0.7kg/m^2; mean ± SE), see Table 7-1. The study was approved by Bro Taf Local Research Ethics Committee, Cardiff, UK.

Table 7-1. Demographic data for subjects with T2D participating in the study (N = 65).

	Sex (M/F)	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	53/12	54	1.72	90.0	30.5
SE		1	0.01	2.5	1.5

7.2.2. *Experimental Design*

The subjects were admitted on two consecutive study days to the Diabetes Research Unit, Llandough Hospital (Penarth, UK) following an overnight 12 hour fast. Each subject underwent two procedures in random order to assess the parameters of carbohydrates metabolism.

7.2.2.1. Intravenous glucose tolerance test

The insulin-modified intravenous glucose tolerance test (IVGTT) consisted of a 0.3g/kg glucose bolus per body weight given at 0 minute over two minutes, followed by 0.05mU/kg insulin (Actrapid, Novo Nordisk, Denmark) at 20 minutes (49). Blood 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 minutes for measurement of plasma glucose, insulin, and C-peptide.

7.2.2.2. Meal tolerance test

A standard 500 kcal meal was consumed at 0 minute (75g carbohydrates; calorie contribution: 58% carbohydrate, 23% fat and 19% protein) (49). The meal consisted of digestion of 15g Weetabix, 10g skimmed milk, 250mL pineapple juice, 50g white meat chicken, 60g wholemeal bread, and 10g polyunsaturated margarine. Subjects were required to consume the whole meal within 10 minutes. Blood samples were taken at -30, 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210 and 240 minutes to measure plasma glucose, insulin, and C-peptide.

In both tests 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. At each sample time the infusion is stopped and the first 2-ml blood withdrawn and discarded, prior to obtaining the sample for assay.

7.2.2.3. Assay method

Glucose was assayed using the glucose oxidase method (Yellow Springs Analyser, YSI 23000, USA; intra-assay CV < 2%). Insulin and C-peptide were assayed using immunoassays utilising monoclonal antibodies (Dako Dioagnostics, Ely, Cambs, UK; intra-assay CV < 5% and < 6% respectively).

7.3. Data Analysis

7.3.1. Glucose and insulin levels

Fasting plasma glucose (FPG) and fasting plasma insulin (FPI) were obtained as mean values of pre-test IVGTT and MTT measurements. $C_{max,G}$ and $C_{max,I}$ were the maximum incremental plasma glucose and insulin concentrations during the MTT. AUC_G and AUC_I were the incremental area under the curve of plasma glucose and insulin, respectively during MTT from 0 to 180 minutes.

7.3.2. *Minimal model analysis*

The minimal model analysis of IVGTT data gave 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) (19). Both S_I and S_G are measures of insulin sensitivity; the former measures insulin sensitivity at an incremental insulin concentration, the latter at the basal insulin concentration (100).

The first phase insulin response (AIR_G; measure of pancreatic β-cell responsiveness) was calculated as the incremental area under the curve from 2 to 8 minutes during the IVGTT (122). The disposition index (D_I; composite measure of insulin sensitivity and pancreatic β-cell responsiveness) was calculated as $D_I = S_I \times AIR_G$ (122).

The package used for the calculation of the minimal model parameters was IS_Ciba (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) (102).

7.3.3. *Insulin secretion model*

The insulin secretion model was used to quantify pancreatic β-cell responsiveness from MTT data, providing fasting β-cell responsiveness (M_0; ability of fasting glucose to stimulate C-peptide secretion) and postprandial β-cell responsiveness (M_I; ability of postprandial glucose to stimulate C-peptide secretion) (32).

M_0 represents fasting prehepatic insulin secretion divided by the fasting plasma glucose. M_I represents the increase in prehepatic insulin secretion given an increment in postprandial glucose (32).

The package used to calculate M_0 and M_I was version 1.0 of CPR (Calculating Pancreatic Responsiveness; written by R. Hovorka and H.C. Subasinghe, MIM Centre, City University, UK, 1997). The model is comprehensively described in Chapter 2.

Table 7-2. Glossary for dependent and independent variables

Variable	Description
	DEPENDENT VARIABLES
FPG	Fasting plasma glucose (mmol/L)
FPI	Fasting plasma insulin (pmol/L)
HbA_{1C}	Glycated haemoglobin (%)
$C_{max,G}$	Maximum (above fasting) plasma glucose during MTT (mmol/L)
$C_{max,I}$	Maximum (above fasting) plasma insulin during MTT (pmol/L)
AUC_G	Integrated (above fasting) plasma glucose during MTT (mmol/L per 180 min)
AUC_I	Integrated (above fasting) plasma insulin during MTT (mmol/L per 180 min)
	INDEPENDENT VARIABLES
S_I	Insulin sensitivity (IVGTT-derived) (1/min per pmol/L)
S_G	Glucose effectiveness (IVGTT-derived) (1/min)
M_I	Post-prandial β-cell sensitivity (MTT-derived) (1min)
M_0	Fasting β-cell sensitivity (MTT-derived) (1/min)
AIR_G	First phase insulin response during IVGTT (pmol/L per 6 min)
D_I	Disposition index ($D_I = S_I * AIR_G$) (IVGTT-derived) (1/min per 6 min)

7.4. Statistical analysis

A Spearman correlation analysis with a Boniferroni correction was carried out to assess relationships between indices classified as independent variables for the purposes of the study (measures of insulin sensitivity and pancreatic β-cell responsiveness: S_I, S_G, AIR_G, D_I, M_0, and M_I), and dependent variables (clinical measures of glucose control: HbA_{1C}, FPG, FPI, AUC_G, $C_{max,G}$, AUC_I and $C_{max,I}$). The step-wise multi-linear regression analysis was used to relate the measures of insulin sensitivity and pancreatic β-cell responsiveness to the clinical measures of glucose control. The amount of explained inter-individual variability was calculated by the analysis of variance (ANOVA). The dependent and independent variables were tested for normal distribution and where as appropriate logarithmically transformed. The results are expressed as mean ± SE unless stated otherwise. SPSS for Windows V9.0 (SPSS Inc., Chicago, IL, USA) was used to carry out statistical calculations.

7.5. Results

7.5.1. *Plasma glucose, insulin and C-peptide*

Elevated fasting plasma glucose and HbA_{1C} shown in Table 7-3 document the lack of control in the newly diagnosed subjects who also presented elevated BMI (see *Subjects*). However, fasting plasma insulin was comparable to that measured in healthy subjects indicating a gross reduction in insulin secretion when corrected to the glucose stimulus. The AIR_G mean value was quite low and close to the FPI values because of the weak pancreatic response in newly diagnosed Type 2 diabetes.

The profiles of plasma glucose, insulin, and C-peptide during IVGTT and MTT are shown in Figure 7-1 and 7-2. During IVGTT, the effect of exogenous insulin at 20 minutes on glucose lowering is clearly visible. At the start of the experiment, the glucose bolus failed to stimulate an immediate insulin response as documented by an early drop in C-peptide and resulted in a paradoxical temporary suppression of insulin secretion. During MTT, the glucose and insulin levels remained elevated for longer than in non-diabetic subjects with peak values reached at 60–90 minutes.

Table 7-3. Summary statistics of variables representing glucose control: fasting plasma glucose (FPG), fasting plasma insulin (FPI), incremental area under the curve of glucose and insulin during MTT (AUC_G, AUC_I), incremental glucose and insulin concentration during MTT ($C_{max,G}$, $C_{max,I}$), and glycated haemoglobin (HbA_{1C}); and variables representing characteristics of glucose metabolism: insulin sensitivity (S_I), glucose effectiveness (S_G), first phase insulin secretion (AIR_G), disposition index (D_I), and fasting (M_0) and postprandial (M_I) pancreatic β-cell responsiveness.

Variable	Mean	SE (Interquartile range)
FPG (mmol/L)	11.0	0.4
FPI (pmol/L)	60	(31-81)
AUC_G (mmol/L per 180 min)	607	41
$C_{max,G}$ (mmol/L)	5.2	0.2
AUC_I (mmol/L per 180 min)	28.7	(13.6-36.6)
$C_{max,I}$ (pmol/L)	233	(115-320)
HbA_{1C} (%)	7.9	(6.1-9.3)
$S_I \times 10^{-5}$ (1/min per pmol/L)	1.07	(0.40-1.51)
$S_G \times 10^{-2}$ (1/min)	1.5	0.1
AIR_G (pmol/L per 6 min)	313	(143-393)
D_I (1/min per 6 min)	488	(202-732)
$M_I \times 10^{-9}$ (1/min)	20.1	(11.3-27.4)
$M_0 \times 10^{-9}$ (1/min)	5.7	(3.4-7.8)

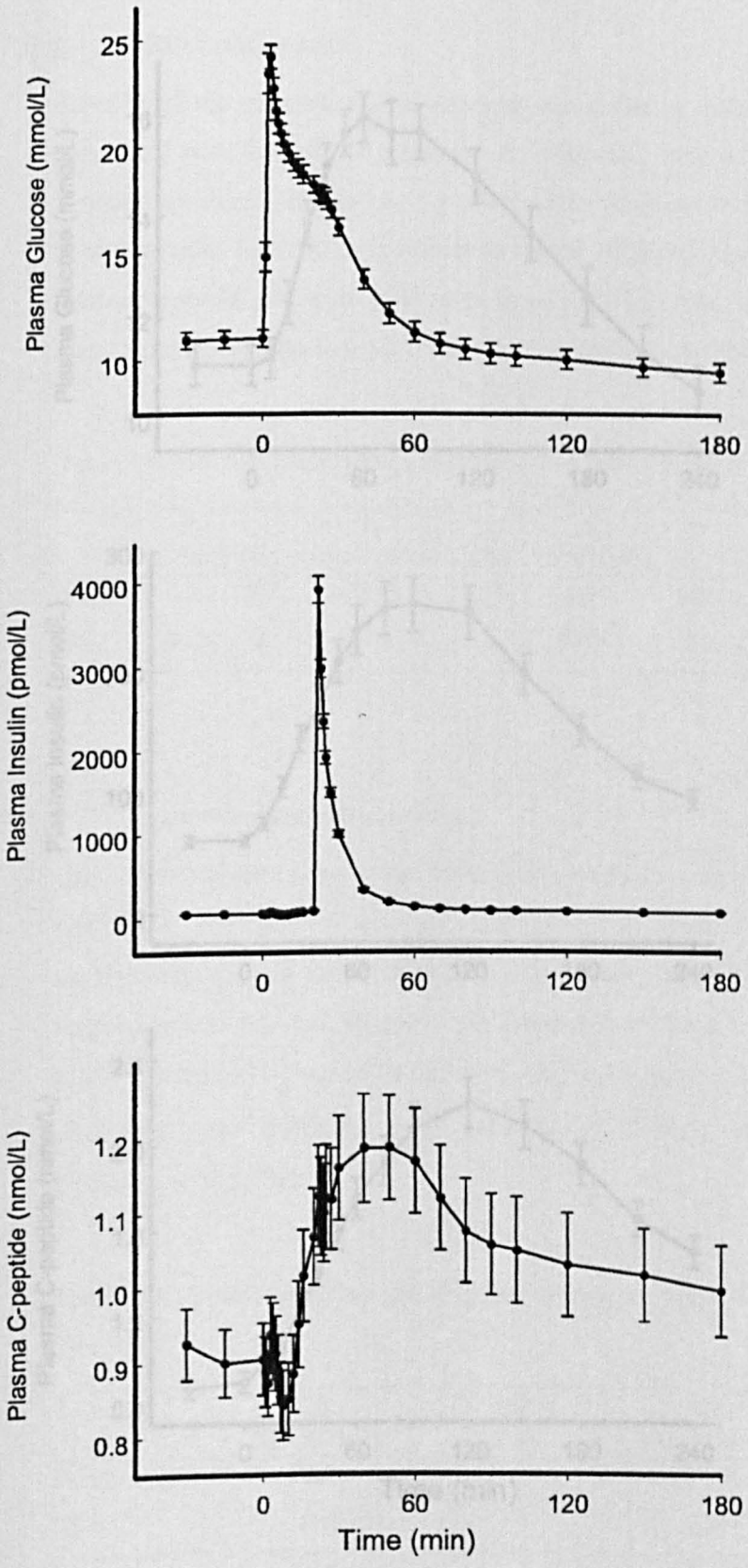

Figure 7-1. Plasma glucose, insulin, and C-peptide profiles during IVGTT (mean ± SE; N=65) in newly presenting subjects with T2D.

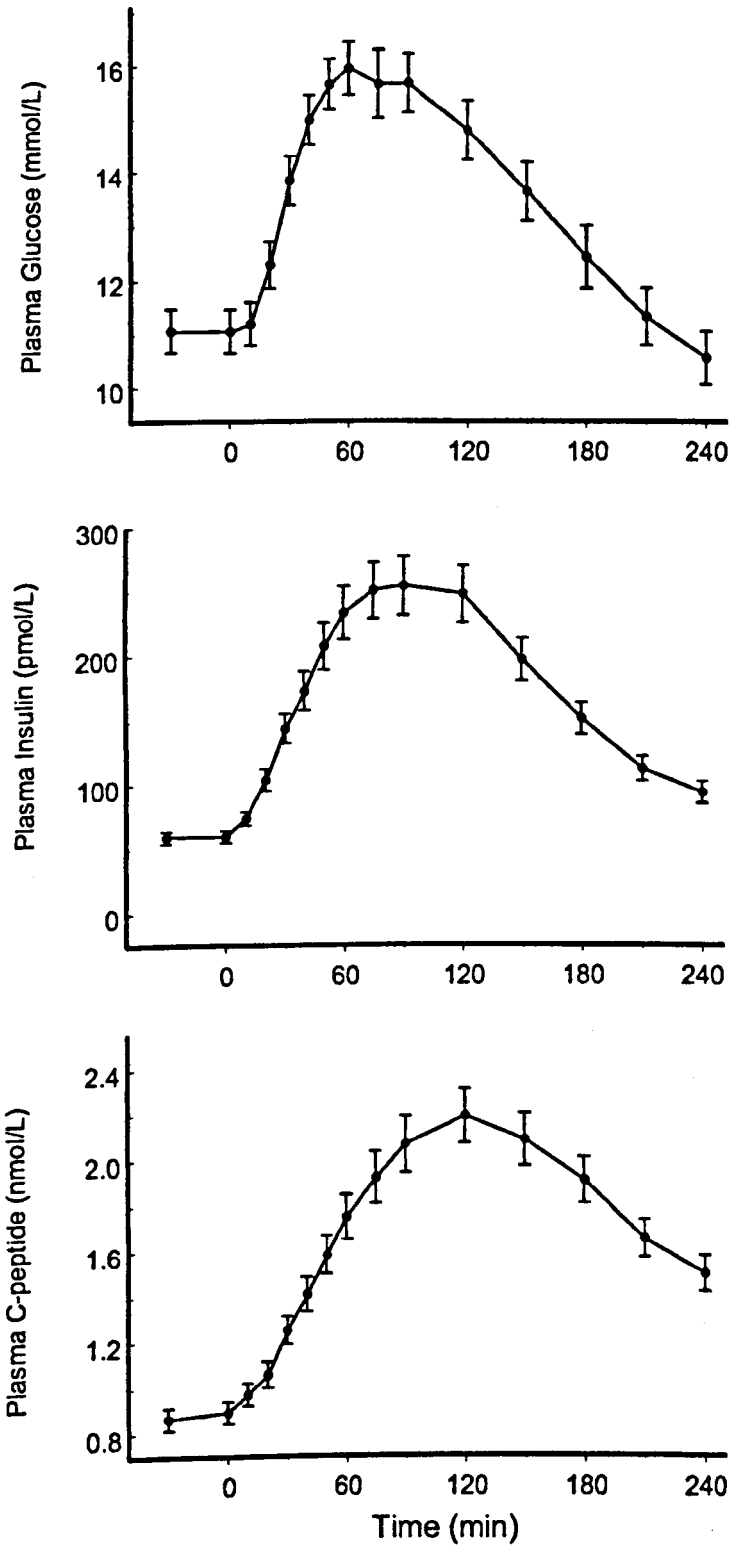

Figure 7-2. Plasma glucose, insulin, and C-peptide profiles during MTT (mean ± SE; N=65) in newly presenting subjects with T2D.

7.5.2. *Minimal model*

Results of the minimal model analysis are given in Table 7-4. All parameters were estimated with acceptable accuracy. As expected, insulin sensitivity S_I was markedly reduced by about 70% and S_G by about 20% compared to healthy subjects (197). Table 1 in appendix I shows the parameters for all subjects. The model failed to estimate S_I with acceptable precision (CV more than 150%) in four subjects. No common reason was found among the four subjects, which might explain the failure.

Table 7-4. Minimal model results in subjects with newly presenting T2D (N = 65).

	P_2 (1/min)	CV (%)	P_3 ($1/min^2 \times pmol/L$)	CV (%)	S_G ($10^{-2} \times 1/min$)	CV (%)	S_I ($10^{-5} \times 1/min$ per pmol/L)	CV (%)
Mean	0.24	23	0.70	27	1.54	20	1.8	18
SE	0.03	3	0.09	4	0.07	2	1.3	3

7.5.3. *Insulin secretion model*

The individual estimates of the pancreatic responsiveness indices during MTT together with their precision of estimates are given in Table 2 in appendix I. Table 7-5 summarises the results of ISM analysis. Fasting and postprandial pancreatic β-cell responsiveness M_0 and M_I were low compared to those measured in healthy subjects (197) (reduction by about 50 and 80% respectively; N = 16, age: 50 ± 10 years, BMI: 29.2 ±3.6 kg/m^2, FPG: 5.1 ± 0.5 mmol/L) (197). They were estimated with excellent precision (CV ≤ 7%).

Table 7-5. Insulin secretion model (ISM) results in subjects with newly presenting T2D (N = 65).

	M_I ($10^{-9} \times 1/min$)	CV (%)	M_0 ($10^{-9} \times 1/min$)	CV (%)
Mean	20.15	7	5.72	3
SE	1.66	1	0.36	0

7.5.4. *Correlation analysis*

The results of the Spearman correlation analysis are given in Table 7-6. FPG was negatively correlated with all measures of pancreatic β-cell responsiveness (most strongly with M_I) and the composite index D_I (Figure 7-3). HbA_{1C} followed a similar pattern with an even stronger correlation with M_I but correlation with D_I failing to reach significance after the Boniferroni (conservative) correction, see Figure 7-4. The two MTT-related glucose variables $C_{max,G}$, and AUC_G were also negatively correlated with M_I.

All insulin variables (FPI, $C_{max,I}$ and AUC_I) were positively correlated with measures of pancreatic β-cell responsiveness. In addition, FPI was strongly negatively and AUC_I was weakly negatively correlated with S_I (Figure 7-5). These were the only correlations demonstrated by the two insulin sensitivity indices S_I and S_G.

Table 7-6. Spearman correlation with the Bonferroni correction between measures of glucose control (FPG, FPI, AUC_G, $C_{max,G}$, HbA_{1C}, AUC_I and $C_{max,I}$) and indices of insulin sensitivity and pancreatic β-cell responsiveness (S_I, S_G, AIR_G, D_I, M_0, and M_I).

	S_I	S_G	AIR_G	D_I	M_I	M_0
FPG	-0.16	0.10	-0.49**	-0.58***	-0.73***	-0.61***
FPI	-0.70***	0.23	0.74***	-0.06	0.40*	0.76***
HbA_{1C}	-0.12	-0.03	-0.37	-0.43	-0.81***	-0.52**
$C_{max,G}$	0.16	-0.01	-0.26	-0.08	-0.49**	-0.36
$C_{max,I}$	-0.38	0.15	0.64***	0.21	0.78***	0.77***
AUC_G	0.01	-0.01	-0.26	-0.2	-0.65***	-0.42*
AUC_I	-0.43*	0.15	0.64***	0.19	0.75***	0.76***

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

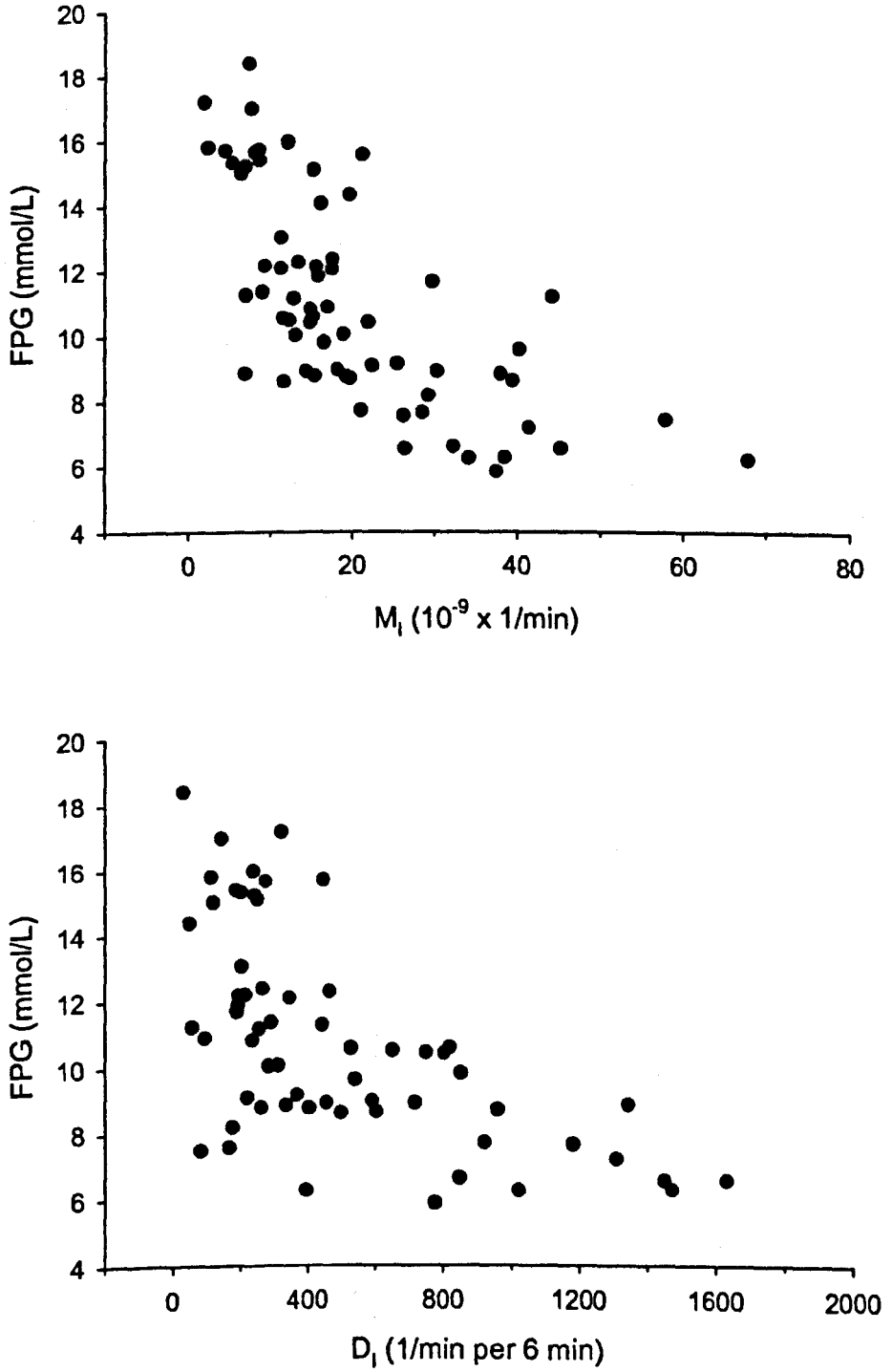

Figure 7-3. Relationship between FPG and M_I (top panel), and between FPG and D_I (bottom panel) in subjects with T2D (mean ± SE).

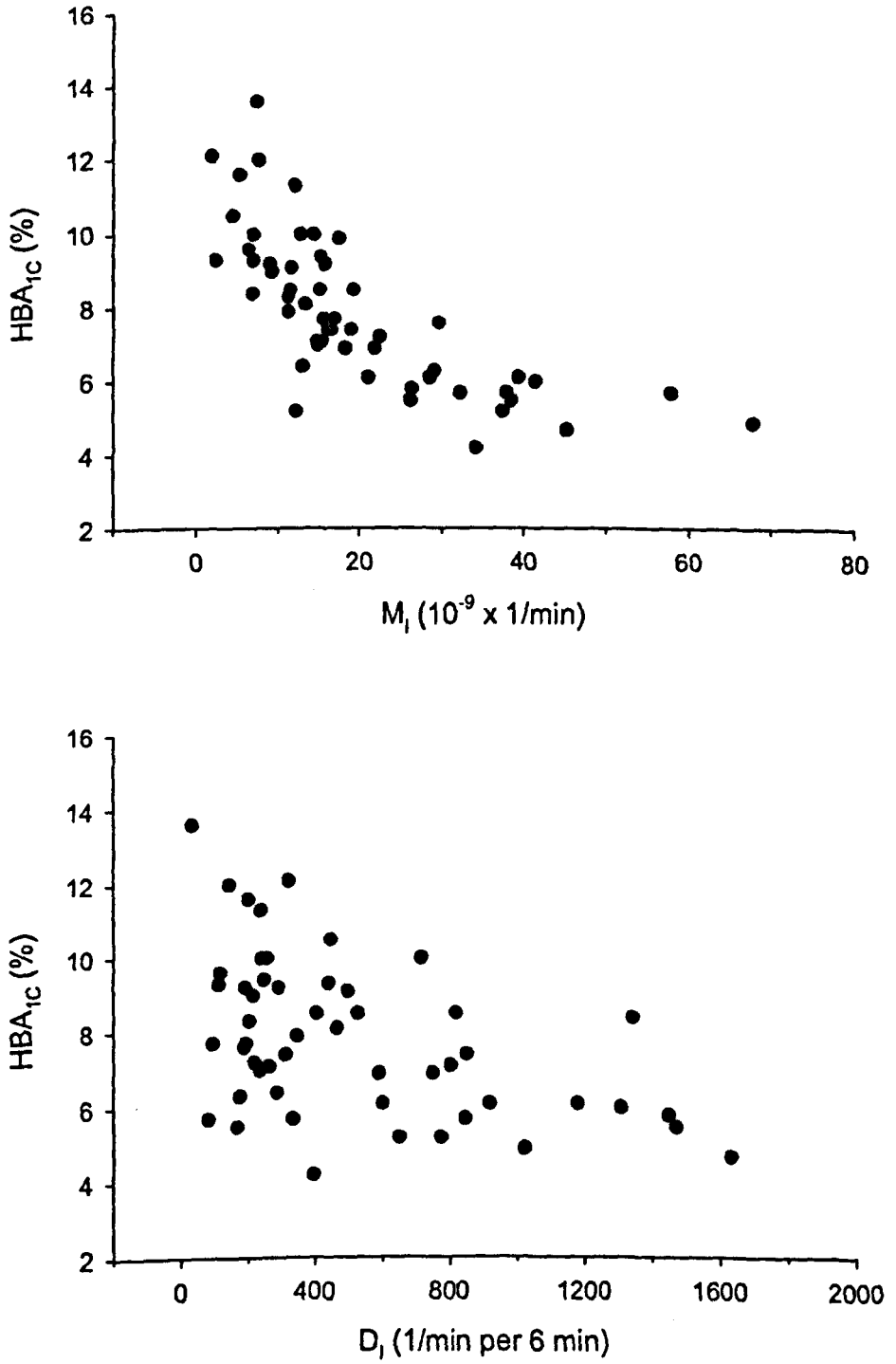

Figure 7-4. Relationship between HBA_{1C} and M_I (top panel), and between HbA_{1C} and D_I (bottom panel) in subjects with T2D (mean ± SE).

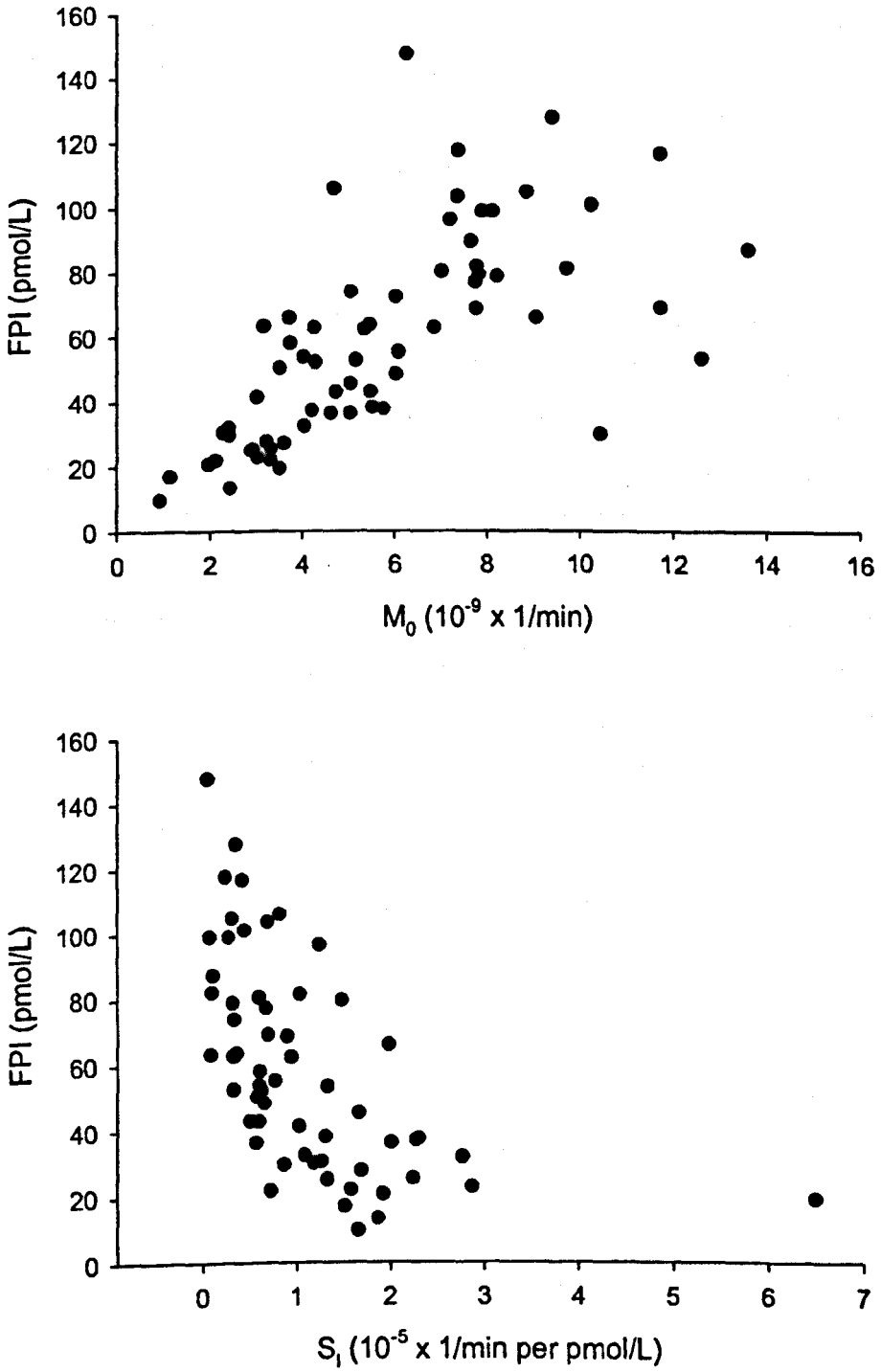

Figure 7-5. Relationship between FPI and M_0 (top panel), and between FPI and S_I (bottom panel) in subjects with T2D (mean ± SE).

7.5.5. *Regression analysis and explained inter-individual variability*

The results of the step-wise multi-linear regression analysis are shown in Table 7-6. The table lists normalised regression coefficients (z-scores; a higher absolute z-score indicates a stronger explanatory ability - this is achieved by transforming the independent variables to standardised form which makes the coefficients more comparable since they are all in the same units of measure), which entered the regression formulae.

The postprandial pancreatic β-cell responsiveness M_I entered all formulae with the exception of that associated with FPI and was the strongest predictor in these regressions. The disposition index D_I was the second strongest predictor. S_I was a strong predictor of FPI and also entered the formula associated with $C_{max,I}$. AIR_G predicted AUC_I and M_0 predicted FPI. S_G did not enter any regression.

The linear regression analysis was powerful in explaining inter-individual variability of all variables with the exception of glucose responses to MTT, see Figure 7-6. Linear regression explained 70--80% inter-individual variability of FPI, FPG, HbA_{1C} and insulin responses to MTT, and only 25--40% interindividual variability of glucose responses to MTT.

Table 7-7. Results of step-wise linear regression are shown in the form of z-scores (regression coefficients when all variables are expressed in standardised form). Dash (-) indicates that the independent variables (S_I, S_G, AIR_G, D_I, M_0, and M_I) did not enter the regression formula for the dependent variables (FPG, FPI, AUC_G, $C_{max,G}$, AUC_I and $C_{max,I}$).

	S_I*	S_G	AIR_G	D_I*	M_0*	M_I*	P value
FPG	-	-	-	- 0.41	-	- 0.66	<0.001
FPI*	- 0.83	-	-	0.44	0.35	-	<0.001
HbA_{1c}*	-	-	-	- 0.21	-	- 0.73	<0.001
$C_{max,G}$	-	-	-	-	-	- 0.50	<0.001
$C_{max,I}$*	- 0.47	-	-	0.26	-	0.69	<0.001
AUC_G	-	-	-	-	-	- 0.65	<0.001
AUC_I*	-	-	0.28	-	-	0.65	<0.001

* Variable log transformed to assure normality

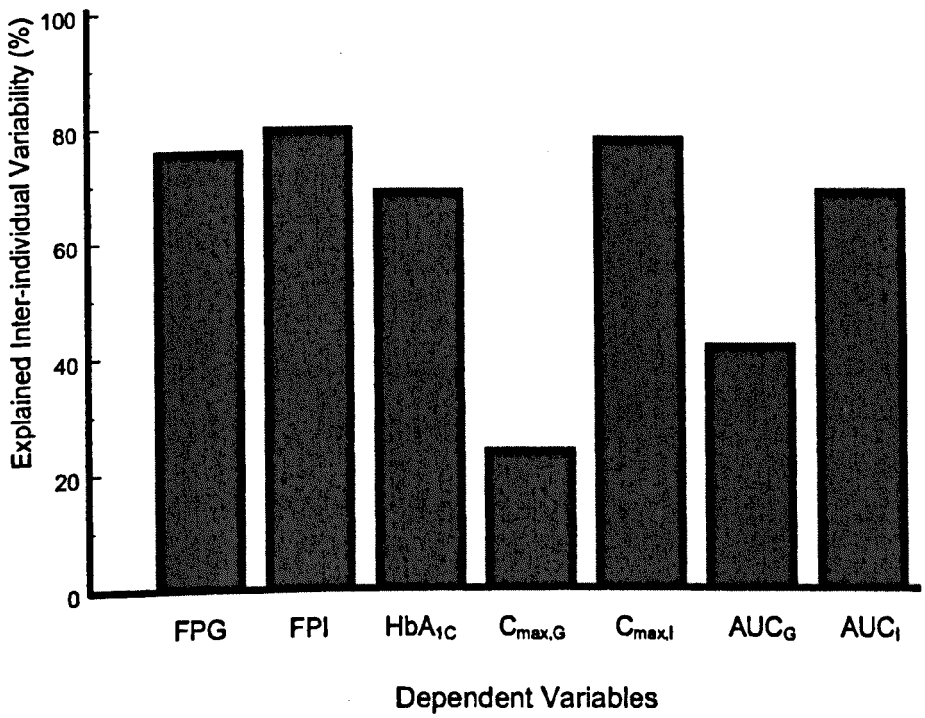

Figure 7-6. Explained inter-individual variability of clinical measures of glucose control using indices of insulin resistance and pancreatic β-cell responsiveness.

7.6. Discussion

The present study documents that at time of presentation, pancreatic β-cell deficiency plays the key role in explaining fasting glucose levels at Type 2 diabetes. This finding is consistently supported by MTT and IVGTT data.

Impaired postprandial pancreatic β-cell responsiveness is the most powerful explanatory factor of impaired glucose control. This suggests that most effective normalisation of glucose levels in Type 2 diabetes is associated with increased postprandial insulin appearance.

During MTT, fasting plasma glucose and HbA_{1C} were strongly inversely related to the ability of basal/fasting and, to a greater extent, postprandial pancreatic β-cell responsiveness. Fasting plasma glucose was also inversely correlated with the IVGTT-derived first phase insulin response and more strongly with the disposition index. This confirms that the disposition index is useful in characterising the overall state of glucose metabolism (122). However, in newly presenting Type 2 diabetes, postprandial insulin secretion is even more useful as it is more closely correlated with fasting plasma glucose.

Glucose meal responses were only correlated with pancreatic β-cell responsiveness and not with insulin resistance. Insulin sensitivity S_I failed to demonstrate any relationship with fasting plasma glucose and glucose meal responses. Reaven et al (198) also failed to find a simple relationship between insulin resistance and fasting plasma glucose in non-obese individuals (normal, impaired glucose tolerance and Type 2 diabetes subjects). However, Van Haeften et al (199) reported the effect of insulin sensitivity as assessed by hyperglycaemic clamp on fasting glucose in subjects with normal and impaired glucose tolerance. Levy et al (200) documented that the ongoing fall in β-cell function assessed by HOMA modelling closely followed a rise in fasting plasma glucose in a 10-year prospective study of newly presenting Type 2 diabetes but also failed to find any effect of insulin sensitivity.

The insulin dependent glucose disposal (production) is the product of two factors, the ambient insulin levels and the ability of insulin to stimulate (suppress) glucose disposal (production). The former factor is influenced by pancreatic β-cell responsiveness and

the latter corresponds to the insulin sensitivity index. It is a paradox that only one factor, the pancreatic deficiency, is related to glucose control (primarily fasting plasma glucose) in the studied subjects. It is unclear why there is a lack of relationship between glucose control and insulin resistance and whether methodological issues associated with measuring insulin resistance or unaccounted physiological mechanism(s) are responsible.

Our interpretation is that when fasting plasma glucose exceeds say 7mmol/l, insulin sensitivity is greatly reduced with little or no further reduction with increasing fasting hyperglycaemia (subjects in the present study had already achieved their maximum insulin resistance). In highly insulin resistant state, insulin dependent glucose disposal during fasting becomes negligible and fasting plasma glucose is regulated primary via the insulin independent pathways such as the mass effect of glucose on its disposal (glucose effectiveness). Thus at fasting, insulin resistance is so high that insulin fails to exercise any detectable effect on glucose disposal and production, and in turn on glucose concentration.

This interpretation is not, however, fully consistent with another study finding. Insulin sensitivity S_I and fasting plasma insulin have been found tightly (negatively) correlated in the present study. This correlation is well documented by others and is normally interpreted by a causal chain reasoning that includes plasma glucose. The argumentation is that insulin resistance results in elevated plasma glucose, which in turn stimulates insulin secretion. Thus insulin resistance is the cause of increased fasting plasma insulin. However, this argumentation does not hold in the present study due to the lack of correlation between S_I and fasting plasma glucose and we must seek alternative explanations. Two candidate theories emerge. Either chronic elevation of plasma insulin induces insulin resistance possibly due to the down regulation of insulin receptors or some other metabolic variable acts as the "control" messenger between insulin resistance and insulin secretion.

Note that tight correlation between S_I and FPI in the diabetes group supports methodological validity of S_I estimates and suggests that insulin modification of IVGTT enabled insulin sensitivity to be successfully estimated.

Subjects with Type 2 diabetes demonstrate both insulin resistance and reduced pancreatic β-cell responsiveness (45; 193). A previous study on newly diagnosed T2D showed that both insulin sensitivity (S_I) and postprandial β-cell responsiveness (M_I) are reduced by about 80% compared to BMI-matched healthy subjects, whereas glucose effectiveness (S_G) and fasting β-cell responsiveness (M_0) are reduced by approximately 25% and 50%, respectively (197).

Subjects were referred directly after diagnosis by their GPs. The subjects had no treatment and did not have any dietary advice. It is possible that they may have made their own dietary adjustments, for example they may have given up sugar in their tea once they knew they were diabetic, but to all extents and purposes they had had absolutely no treatment for diabetes (treatment naive) before undergoing MTT and IVGTT.

Subjects presented a wide range of fasting plasma glucose and fasting plasma insulin (5.9--18.4mmol/L and 20--150pmol/L respectively) probably due to the duration of undetected diabetes and/or individual differences in diet and life style. The mean value of fasting plasma insulin was close to that observed in healthy subjects, while the mean value of fasting plasma glucose was considerably elevated. This observation supports the hypothesis that overt diabetes does not appear until the pancreas is not able to meet the body's demand for insulin in the face of increasing insulin resistance (193; 201; 202). This process is then accelerated as glucose is then toxic (glucose toxicity) to the β-cell and peripheral tissues (202; 203).

It has been shown that during the natural development of Type 2 diabetes, fasting plasma insulin increases and then decreases as insulin resistance develops (Starling's curve of the pancreas). The increase in fasting plasma insulin is generally regarded as a compensation mechanism aiming to reverse the effect of insulin resistance and the subsequent decrease as a decompensation mechanism reflecting β-cell 'exhaustion'. However, such analysis fails to take into account the level of the stimuli, i.e. the fasting glucose level. When insulin secretion is normalised to fasting glucose (such as when calculating fasting responsiveness M_0), no increase in insulin secretion, i.e. compensation, is observed and there is a consistent pattern of continuously deteriorating

fasting pancreatic β-cell responsiveness accompanying elevated fasting glucose. This suggests that no compensation mechanism *per se* exists and that the apex on the Starling curve represents a point when the stimuli are not high enough to overcome deteriorating pancreatic β-cell responsiveness. It is also known that the early insulin release (parameter comparable to M_1) during an oral load decreases progressively as the 2-h plasma glucose increases. There is no Starling curve for this parameter of insulin secretion, which is compatible with our data.

Fasting plasma insulin correlates negatively with insulin sensitivity (-0.70, $p < 0.001$). This correlation can be explained by the effect of insulin resistance on the stimulation of insulin secretion (46; 204) and suggests that elevated fasting plasma insulin is a reliable index of insulin resistance. Bonora et al (159) also found a negative correlation between insulin sensitivity and fasting plasma insulin in mild glucose intolerance and suggested that overproduction of insulin is due to insulin resistance. Olefsky et al (46) found a similar correlation in normal subjects, subjects with impaired glucose tolerance and Type 2 diabetes, and explained fasting plasma insulin elevation as a result of an attempt to overcome insulin resistance.

The explained inter-individual variability of fasting plasma glucose and HbA_{1C} were excellent (> 75%) if we consider intra-individual (day-to-day) variability, which could account for 10--20% of unexplained variability (153). A similarly excellent explanation was found for fasting plasma insulin and insulin responses to meal.

Glucose responses after a meal were poorly explained (< 45%). It appears that other variables not included in the study such as gut absorption and endogenous glucose production are responsible for the residual amount of unexplained variability. Thus the standard indices of insulin sensitivity and pancreatic β-cell responsiveness do not enable reliable predictions of postprandial glucose to be made.

7.7. Summary

In conclusion, pancreatic β-cell responsiveness indices from IVGTT and MTT are better explanatory factors of fasting plasma glucose, HbA_{1C}, and insulin and glucose responses to meal than insulin resistance indices in newly presenting Type 2 diabetes. Postprandial

insulin deficiency is the most powerful explanatory factor of elevated fasting plasma glucose, glycated haemoglobin and glucose responses to meal. Indices of insulin sensitivity and pancreatic β-cell responsiveness are able to explain glucose control well with the exception of glucose response to a meal.

8. Final discussion

The primary aim of this thesis was to use modelling techniques employing data collected during MTT and IVGTT to progress our understanding of the pathology of type 2 diabetes. The secondary aim was to evaluate the domain of validity of the insulin secretion model (ISM) and, in part, the minimal model.

Various methods with varying degrees of complexity have been established and developed for the measurement of insulin sensitivity and β-cell function. A review of these methods was given in Chapter 2. However, for methodological considerations and to eliminate or reduce the effect of the measurement error and the inter-subject variability in insulin and C-peptide kinetic, model-based approaches are preferred over a simple one or two concentration-point assessment.

The insulin secretion model (ISM) with MTT is a simple relatively non-invasive tool to investigate pancreatic responsiveness (32). It measures the prehepatic insulin secretion and assesses pancreatic β-cell responsiveness providing postprandial pancreatic β-cell responsiveness (M_1) and fasting pancreatic β-cell responsiveness (M_0).

A reduced sampling scheme consisting of nine samples has been used and was planned to be used in the course of studies in this thesis (49) but its performance with the model was still unknown. In Chapter 3, ISM indices of pancreatic β-cell responsiveness were successfully estimated and evaluated during MTT with nine-sample and five-sample schemes. The five-sample scheme will further reduce the time, labour and cost, facilitating a wide use of the model.

The insulin secretion model was successfully validated during OGTT in healthy subjects, and subjects with T2D. The OGTT is the most widely used test because of its simplicity. The OGTT is the standard diagnostic test of type 2 diabetes and impaired glucose tolerance IGT approved by the World Health Organisation (1).

In the same chapter (Chapter 4), a comparison between model performance and indices during MTT and OGTT was performed. In healthy subjects, as a result of higher

C-peptide secretion during MTT with respect to the glucose concentration, MTT resulted in 1.5 fold higher postprandial pancreatic β-cell responsiveness than OGTT. In subjects with T2D, glucose response during OGTT was higher than during MTT ($P = 0.001$), whereas the insulin response was slightly higher during MTT (P = NS). As a result M_I was two fold higher during MTT than OGTT ($P = 0.001$). The two indices were also correlated. The meal tolerance test provides a more physiological challenge to the β-cell than OGTT. Therefore, MTT is expected to facilitate a more comprehensive assessment of β-cell function.

In Chapter five, the reproducibility of pancreatic β-cell responsiveness and the reproducibility of glucose, insulin, and C-peptide responses to MTT and OGTT were investigated. In healthy subjects the indices of pancreatic β-cell responsiveness failed to show sufficient reproducibility due to lack of reproducibility of glucose and C-peptide to MTT and OGTT. However insulin responses to MTT and OGTT were reproducible. In subjects with T2D, M_I and M_0 were sufficiently reproducible in line with reproducible glucose, insulin, and C-peptide responses to MTT. Due to the data limitation in subjects with T2D, the reproducibility of these parameters was studied during MTT but not OGTT.

The one compartment minimal model (1CMM) of glucose kinetics is a powerful relatively non-invasive tool to investigate glucose metabolism. However it has been criticised by several studies reporting that S_G is overestimated (109-111; 113; 182-184) and S_I is underestimated (109-113; 184) due to the under modelling effect of using one compartment to represent the glucose pool (113; 182; 185; 186). The new two compartment minimal model (2CMM) was suggested and used as an alternative to the 1CMM, but the 2CMM performance in subjects with type 2 diabetes is still unknown.

In Chapter 6, the one and two compartmental minimal model performance was evaluated and compared to the clamp in subjects with type 2 diabetes during the modified IVGTT.

The iterative two-stage population approach was the successful one among several other approaches applied during the evaluation and validation of 2CMM. Although the model was successfully validated in subjects with T2D during insulin-modified FSIVGTT,

insulin sensitivity and glucose effectiveness estimated by 2CMM with population analysis were not different from 1CMM estimates. For its simplicity and popularity and to benefit from the data available in the literature it is recommended to use the 1CMM.

In Chapter seven the one compartment minimal model and the insulin secretion model with MTT were then applied to study newly presenting type 2 diabetes in order to gain more understanding of the disease pathology and to investigate the ability of the IVGTT and MTT derived indices to explain the 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.

Two sets of indices were defined and analysed. One set contained model-based indices assessing and presenting the level of insulin sensitivity and pancreatic β-cell responsiveness. A second set included several indices of clinical measures describing the blood glucose and insulin control ranging from fasting to postprandial measures.

The impaired postprandial pancreatic β-cell responsiveness was the most powerful explanatory factor of impaired glucose control. The explained inter-individual variability of fasting plasma glucose and HbA_{1C} were excellent (> 75%) if we consider intra-individual (day-to-day) variability, which could account for 10--20% of unexplained variability (153). A similarly excellent explanation was found for fasting plasma insulin and insulin responses to a meal.

Glucose meal responses were only correlated with pancreatic β-cell responsiveness. In addition insulin sensitivity S_I failed to demonstrate any relationship with fasting plasma glucose and glucose meal responses. Fasting plasma glucose and HbA_{1C} were strongly inversely related to the ability of fasting and, to a greater extent, postprandial pancreatic β-cell responsiveness during MTT.

Fasting plasma insulin correlates strongly and negatively with insulin sensitivity (-0.70, $p < 0.001$). This correlation was explained by the effect of insulin resistance on the stimulation of insulin secretion and suggesting that elevated fasting plasma insulin is a reliable index of insulin resistance.

Subjects with Type 2 diabetes demonstrated both insulin resistance and reduced pancreatic β-cell responsiveness. When insulin secretion was normalised to fasting glucose (such as when calculating fasting responsiveness M_0), no increase in insulin secretion, i.e. compensation, was observed and there was a consistent pattern of continuously deteriorating fasting pancreatic β-cell responsiveness accompanying elevated fasting glucose.

This observation supports the hypothesis that overt diabetes does not appear until the pancreas is not able to meet the body's demand for insulin in the face of increasing insulin resistance (193; 201; 202). This process is then accelerated as glucose is then toxic (glucose toxicity) to the β-cell and peripheral tissues (202; 203).

In conclusion, the model-based approaches used in this thesis, provided simple and reproducible relatively non-invasive measures to assess the pancreatic β-cell responsiveness and insulin sensitivity. These methods enhanced and raised our understanding of type 2 diabetes pathology.

8.1. Achievement of objectives

An outline of the achieved objectives is given below. The achievements are divided as the objectives into methodological and clinical. The methodological achievements are:

- Pancreatic β-cell responsiveness was successfully estimated and evaluated during MTT with nine-sample and five-sample schemes

- Insulin secretion model was validated during OGTT in healthy subjects, and subjects with T2D. The model is able to assess pancreatic β-cell responsiveness from MTT as well as OGTT data

- In healthy subjects the indices of pancreatic β-cell responsiveness failed to show sufficient reproducibility due to lack of reproducibility of glucose and C-peptide to MTT and OGTT

- In subjects with T2D, M_1 and M_0 were sufficiently reproducible in line with reproducible glucose, insulin, and C-peptide responses to MTT

- The 2CMM was validated successfully using population analysis in T2D subjects during insulin-modified FSIVGTT, giving precise estimates of S_I and S_G with strong correlation with 1CMM estimates and S_{ICLAMP}.

- Insulin sensitivity and glucose effectiveness estimated by 2CMM with population analysis were not different from 1CMM estimates. For its simplicity and popularity and to benefit from the data available in the literature it is recommended to use the 1CMM.

The clinical achievements are:

- Pancreatic β-cell responsiveness indices from IVGTT and MTT were better explanatory factors of fasting plasma glucose, HbA_{1C}, and insulin and glucose responses to meal than insulin resistance indices in newly presenting Type 2 diabetes

- Postprandial insulin deficiency was the most powerful explanatory factor of elevated fasting plasma glucose, glycated haemoglobin, and glucose responses to meal

- Indices of insulin sensitivity and pancreatic β-cell responsiveness were able to explain glucose control well with the exception of glucose response to a meal in subjects with T2D.

8.2. Future work

Several research questions were raised during the course of study. Recommendations for future research encompass clinical and methodological aspects:

- It is recommended to follow up subjects with newly diagnosed T2D to gain more understanding of the disease progression and to detect the effects of therapeutic interventions

- Future work is needed to understand the variability of glucose meal responses and its main explanatory factors

- It is recommended to study the reproducibility of the pancreatic β-cell responsiveness during OGTT in subjects with T2D

- It is recommended to compare the performance of the one compartment minimal model with both iterative two-stage population analysis and standard two-stage analysis in different pathogenesis states including T2D.

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Appendices

Appendix I has three tables including data from 65 subjects with newly presenting type 2 diabetes who participated in the study described in Chapter 7. Table 1 shows individual estimates of minimal model parameters. Table 2 shows individual estimates of insulin secretion model. Individual fasting values of glucose, insulin, and C-peptide together with HbA_{1C} are given in Table 3.

Appendix II contains the published papers and a list of publications derived from the work included in this thesis.

Appendix I

Table 1. Individual estimates of minimal model analysis in subjects with newly presenting T2D.

Sub. No.	P_2 1/min	CV %	P_3 ($1/min^2 \times pmol/L$)	CV %	S_G ($10^{-2} \times 1/min$)	CV %	S_I ($10^{-5} \times 1/min$ per pmol/L)	CV %
1	0.01	54	0.06	45	1.40	11	1.01	24
2	0.05	25	0.22	32	1.18	17	0.71	23
3	0.13	15	2.39	17	0.93	36	3.12	5
4	5.59	n/a*	n/a	n/a	1.57	17	0.16	127
5	0.00	n/a	0.00	122	1.20	44	n/a	n/a
6	5.60	0	n/a	n/a	3.28	12	n/a	n/a
7	0.03	35	0.35	28	3.51	8	2.22	14
8	0.05	18	0.41	23	1.52	15	1.28	16
9	0.02	56	0.04	56	2.04	9	0.47	31
10	0.19	211	0.09	206	1.86	6	0.08	102
11	0.10	10	1.25	13	0.22	104	2.08	6
12	0.04	8	0.37	10	0.92	16	1.59	8
13	0.06	18	0.92	22	1.38	19	2.48	7
14	0.07	14	0.74	16	1.19	20	1.72	10
15	0.05	27	0.87	28	1.38	38	2.82	6
16	0.06	10	0.96	12	0.68	29	2.78	9
17	0.21	126	0.17	122	1.77	7	0.14	57
18	0.07	49	4.85	39	2.72	38	10.83	13
19	0.31	164	0.35	161	1.73	7	0.19	58
20	0.04	16	0.25	19	1.67	10	0.94	15
21	0.05	13	0.33	16	1.60	11	1.00	9
22	0.05	15	0.32	19	1.64	11	1.01	12
23	0.06	7	0.73	10	1.07	15	2.19	5
24	0.06	12	1.07	14	1.34	16	2.77	4
25	0.03	21	0.63	25	1.11	33	3.32	8
26	0.05	9	0.58	11	1.16	13	1.82	5
27	0.07	19	0.24	20	1.73	8	0.57	15
28	0.08	21	1.65	24	1.37	31	3.35	6
29	0.07	21	0.23	22	1.38	12	0.57	16
30	0.08	8	0.99	10	0.88	19	2.12	5
31	0.00	n/a	n/a	n/a	2.03	13	n/a	n/a
32	0.04	21	0.46	23	0.96	33	1.98	26
33	0.02	30	0.07	34	1.90	8	0.55	19
34	0.03	14	0.21	18	1.31	13	1.03	10
35	0.02	25	0.10	32	1.17	14	0.86	22
36	0.05	9	0.69	12	1.01	20	2.53	7
37	0.04	12	0.23	15	1.18	11	0.96	12
38	0.04	23	0.60	23	1.49	26	2.65	6
39	0.06	35	1.76	27	1.92	35	4.62	10
40	0.02	18	0.06	21	1.58	6	0.53	12
41	0.09	18	1.17	22	1.15	31	2.22	10
42	0.08	26	1.73	28	1.42	38	3.78	7
43	0.04	29	0.14	35	1.62	13	0.55	26
44	0.00	n/a	n/a	n/a	1.61	15	n/a	n/a
45	0.05	30	0.22	28	1.18	19	0.74	28
46	0.05	13	0.34	15	1.46	10	1.11	13
47	0.07	20	0.46	21	1.32	15	1.11	16
48	0.08	15	0.58	18	1.48	18	1.17	9
49	0.07	9	1.44	12	3.77	59	3.22	5
50	0.09	30	0.66	36	2.01	21	1.22	15
51	0.08	18	0.48	22	1.57	17	1.00	12
52	0.09	27	0.80	29	1.19	43	1.45	6
53	0.09	17	1.98	19	1.17	28	3.83	5
54	0.04	17	0.49	19	1.64	18	2.22	6
55	0.06	18	0.65	23	1.34	26	1.74	9
56	0.05	12	0.43	15	1.35	12	1.50	12
57	0.09	16	0.32	18	1.81	8	0.61	11
58	0.07	35	1.62	35	1.56	44	3.73	7
59	0.05	35	1.38	27	1.61	38	4.79	9
60	0.23	38	0.56	40	1.70	7	0.42	17
61	0.03	19	0.24	21	1.53	11	1.39	18
62	0.06	33	0.20	33	1.59	11	0.54	29
63	0.03	12	0.20	18	1.52	8	1.17	12
64	0.12	78	0.11	73	2.04	5	0.15	47
65	0.05	14	0.18	18	1.75	8	0.60	14
Mean	0.24	23	0.70	27	1.54	20	1.79	18
SE	0.03	3	0.09	4	0.07	2	0.21	3

* The estimation did not return any value

Table 2. Individual estimates of ISM analysis in subjects with newly presenting T2D.

Sub. No.	M_I (10^{-9} × 1/min)	CV %	M_0 (10^{-9} × 1/min)	CV %
1	40.22	5	7.02	4
2	30.23	8	11.72	3
3	8.10	6	2.44	3
4	44.21	4	7.78	3
5	21.17	8	7.65	3
6	8.53	7	3.72	3
7	8.63	8	2.93	3
8	25.47	6	6.09	3
9	17.53	7	8.12	3
10	19.62	8	6.25	3
11	19.72	6	7.20	3
12	11.54	5	5.35	3
13	26.38	6	7.83	3
14	1.94	28	3.02	3
15	18.21	4	3.22	3
16	11.64	4	5.05	3
17	16.94	9	7.90	3
18	28.50	4	3.51	3
19	57.91	4	13.62	4
20	22.40	4	5.04	3
21	15.77	7	4.73	3
22	14.84	5	3.75	3
23	32.26	4	5.52	4
24	2.41	8	.92	3
25	45.24	5	9.06	3
26	11.30	7	4.05	3
27	6.46	21	4.26	3
28	14.78	6	4.62	3
29	12.84	7	5.07	3
30	13.06	5	2.29	4
31	17.45	6	6.04	3
32	37.44	5	10.44	3
33	29.14	4	5.17	4
34	29.63	4	4.29	3
35	5.37	20	5.48	3
36	7.01	4	1.15	3
37	12.12	7	3.51	3
38	34.14	3	3.30	4
39	6.93	5	2.42	4
40	26.20	6	8.21	3
41	11.26	6	2.89	3
42	21.84	6	4.21	3
43	15.56	8	6.85	3
44	16.09	6	3.60	3
45	15.40	9	10.25	3
46	37.94	6	7.75	3
47	9.28	11	6.03	3
48	16.51	7	7.35	3
49	7.04	5	1.97	3
50	7.70	5	2.12	3
51	15.28	11	4.03	3
52	4.47	6	2.42	3
53	14.38	8	5.75	3
54	38.48	6	12.62	4
55	41.40	6	9.72	3
56	39.39	4	7.77	4
57	9.02	7	5.46	3
58	13.36	5	3.32	4
59	15.15	4	3.03	3
60	18.91	7	7.36	3
61	12.23	5	4.69	3
62	19.27	6	8.85	3
63	67.77	5	11.75	3
64	7.41	15	3.16	4
65	21.07	6	9.41	3
Mean	20.15	7	5.72	3
SE	1.66	1	0.36	0

Table 3. Fasting plasma glucose (FPG), insulin (FPI), C-peptide (FPC), and glycated haemoglobin (HbA_{1C}) in subjects with newly presenting T2D.

Sub. No.	FPI (pmol/L)	FPG (mmol/L)	FPC (nmol/L)	HBA1C (%)
1	81	9.6	0.994	n/a*
2	117	8.9	1.598	n/a
3	13	15.7	0.560	n/a
4	82	11.2	1.385	n/a
5	90	15.6	1.793	n/a
6	66	15.8	0.941	n/a
7	25	15.4	0.628	n/a
8	55	9.2	0.764	n/a
9	99	12.4	1.701	n/a
10	148	14.4	1.418	n/a
11	97	8.7	0.921	n/a
12	63	10.6	0.907	8.5
13	80	6.6	0.685	5.8
14	42	17.2	0.813	12.1
15	28	9.0	0.491	6.9
16	46	8.6	0.711	9.1
17	99	10.9	1.479	7.7
18	19	7.7	0.435	6.1
19	87	7.5	1.403	5.7
20	36	9.1	0.678	7.2
21	43	11.9	0.793	9.2
22	58	10.8	0.646	7.0
23	38	6.6	0.644	5.7
24	10	15.8	0.255	9.3
25	66	6.6	1.299	4.7
26	32	13.1	0.898	8.3
27	63	15.0	n/a	9.6
28	36	10.4	0.766	7.1
29	74	11.2	1.206	10.0
30	31	10.1	0.366	6.4
31	72	12.1	1.074	9.9
32	30	5.9	0.922	5.2
33	53	8.2	0.797	6.3
34	52	11.7	0.839	7.6
35	43	15.4	0.910	11.6
36	17	15.3	0.276	10.0
37	51	16.0	0.862	11.3
38	22	6.3	0.306	4.2
39	32	8.9	0.411	8.4
40	79	7.6	1.219	5.5
41	25	12.1	0.512	7.9
42	37	10.4	0.662	6.9
43	63	12.2	1.217	7.7
44	27	14.1	0.691	7.4
45	101	8.8	1.390	7.1
46	77	8.9	0.993	5.7
47	49	12.2	1.046	9.0
48	104	9.8	1.218	7.4
49	21	11.3	0.436	9.3
50	22	17.0	0.531	12.0
51	54	15.1	0.868	9.4
52	30	15.7	0.585	10.5
53	38	8.9	0.923	10.0
54	54	6.3	1.339	5.5
55	81	7.2	1.345	6.0
56	69	8.7	0.848	6.1
57	152	11.0	n/a	9.2
58	26	12.3	0.573	8.1
59	23	10.6	0.625	8.5
60	118	10.1	1.153	7.4
61	106	10.5	0.744	5.2
62	105	8.8	1.194	8.5
63	69	6.3	0.990	4.9
64	63	18.4	0.973	13.6
65	128	7.7	1.107	6.1
Mean	60	11.0	0.901	7.9
SE	4	0.4	0.045	0.3

* The estimation did not return any value

Appendix II

List of publications

1. **Albarrak, A. I. S., Luzio, S. D., Chassin, L., Playle, R., Owens, D. R., and Hovorka, R.** Explaining inter-individual variability of clinical measures of diabetes control in newly presenting type 2 diabetes. Diabetic Medicine 16 (Suppl 1), 46. 1999.

2. **Owens DR, Albarrak AIS, Luzio SD, Chassin LJ, Playle R and Hovorka R.** Relationships between clinical measures of diabetes control, and insulin sensitivity and pancreatic reponsiveness in newly presenting type 2 diabetes. *Diabetes* 48: 1298, 1999.

3. **Hovorka, R., Albarrak, AIS, Chassin, L., Luzio, S. D., Playle, R., and Owens, D. R.** Close association between -cell responsiveness and fasting glucose in subjects with newly presenting Type 2 Diabetes. Diabet.Med. 17(1), 136. 2000.

4. **Hovorka R, Albarrak A, Chassin L, Luzio SD, Playle R and Owens DR.** Relationship between beta-cell responsiveness and fasting plasma glucose in Caucasian subjects with newly presenting type 2 diabetes. *Diabet Med* 18: 797-802, 2001.

5. **Albarrak, AIS, Luzio, S. D., Hovorka, R., Playle, R., and Owens, D. R.** Reproducibility and Comparability of Pancreatic beta-cell Responsiveness during MTT and OGTT in Healthy Subjects. Diabet.Med. 18(1), 136. 2001.

6. **Albarrak AIS, Luzio SD, Playle R, Hovorka R and Owens DR.** Pancreatic beta-cell responsiveness is reproducible in type 2 diabetes during MTT. *Diabetes* 50: A508-A509, 2001.

7. **Agbaje S, Playle R, Luzio SD, Albarrak AIS, Lunn DJ, Owens DR and Hovorka R.** Population approach to estimate insulin sensitivity and glucose effectiveness by minimal model. *Diabetologia* 44: 211, 2001.

8. **Albarrak AIS, Luzio SD, Playle R, Hovorka R and Owens DR.** Reproducibility of pancreatic beta-cell responsiveness during MTT and OGTT in healthy subjects. *Diabetologia* 44: 524, 2001.

9. **Albarrak AI, Luzio SD, Chassin LJ, Playle RA, Owens DR and Hovorka R.** Associations of glucose control with insulin sensitivity and pancreatic beta-cell

responsiveness in newly presenting type 2 diabetes. *J Clin Endocrinol Metab* 87: 198-203, 2002.

10. **Albarrak, A. I. S., Luzio, S. D., Hovorka, R., and Owens, D. R.** Pancreatic β-cell responsiveness during mtt and ogtt in type 2 diabetes subjects. Diabet.Med. 19(2), 16. 2002.

11. **Albarrak A, Luzio SD, Hovorka R and Owens DR.** Full phase insulin response is more closely associated with deteriorated glucose control than first phase insulin response in newly presenting Type 2 diabetes. *Diabetologia* 45: 439, 2002.

12. **Albarrak AI, Luzio SD, Owens DR and Hovorka R.** Evaluation of cold two compartment minimal model in type 2 diabetes. Will be published in *IFAC* 2003.