PHARMACOKINETICS AND PHARMACODYNAMICS OF GLIBENCLAMIDE IN TYPE 2 DIABETICS

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Pharmacokinetics and Pharmacodynamics of glibenclamide in type 2 diabetics

by

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Dedication

Shanta and Euvir for making a dream that Mum and Dad inspired, a reality

Declaration

This document describes the original work by the author and has not been submitted in any form to any other University. Where use was made of the work of others it has been duly acknowledged in the text.

The study was supervised by Dr G Pillai (B Pharm M Pharm Phd), Modeling and Simulation, Novartis, Switzerland, Professor B Maharaj (MBChB MD PhD FCP FRCP), Department of Therapeutics, University of KwaZulu-Natal, South Africa and Dr LM Robertson [MBChB MMed (Prac)], Dot Shuttleworth Centre for Diabetes, South Africa.

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List of abbreviations

Item	*Definition*
ADA	America Diabetes Association
AUC	Area under the curve
BMI	Body Mass Index
Cl	Clearance
Cmax	Maximum concentration
DAMT	Dose amount
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
FBG	Fasting blood glucose
HbA_{1c}	Glycated haemoglobin
HLA	Human leukocyte antigen
HOMA-IR	Homeostatic model assessment of insulin resistance
IDDM	Insulin depenent diabetes mellitus
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
II'	Modified insulinogenic index
IR	Insulin resisitance
IRF	Impaired renal function
Ka	Absorption rate constant
NIDDM	Non-insulin-depenent diabetes mellitus
NRF	Normal renal function
OGTT	Oral glucose tolerance test
PD	Pharmacodynamics
PK	Pharmacokinetics
PPG	Postprandial plasma glucose
QUICKI	Quantitative Insulin Sensitivity Check Index
SAMJ	South African Medical Journal
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa
SU	Sulphonylurea
$T_{1/2}$	Half life
Tmax	Time to maximum concentration
TZD	Thiazolidinedione
Vd	Volume of distribution
FBI	Fasting Blood Insulin

Abstract

The dose of a number of pharmacological agents in clinical use differ from that initially recommended when the compound was first introduced into clinical use. For some time now, anecdotal reports have suggested that glibenclamide, a widely used oral hypoglycaemic in type 2 diabetic patients, is being used at doses that exceed those likely to produce clinical control of elevated blood glucose. This is also reflected by the discrepancy in the maximum recommended dose from different manufacturers and in different countries – some recommend 15 mg as the maximum dose and others recommend 20mg.

A survey of 6 state institutions in the greater eThekwini/Durban area revealed that up to 25% (range 7-45%) of patients were prescribed 20 mg of glibenclamide per day, confirming the use of high doses of glibenclamide. Even more disconcerting was the observation that elderly patients, who are potentially more susceptible to the adverse effects of glibenclamide, are given this high dose.

A clinical study was therefore conducted to determine whether patients benefit from the use of these high doses of glibenclamide. Twenty two type 2 diabetics who attended an outpatient diabetes clinic were recruited into a within-subject dose escalation study. In order to evaluate the dose-response relationship of glibenclamide, blood glucose, blood insulin and blood glibenclamide concentrations were measured in these subjects. After an initial washout period, a zero dose study was conducted followed by dose escalation through 2.5, 5, 10, 15 and 20mg daily doses at 14 day intervals. Dose escalation was guided by careful clinical examination, monitoring of blood glucose concentrations and checks for symptoms of hypoglycaemia. The relationship between dose and selected metrics of pharmacokinetic and pharmacodynamic (PKPD) response on glucose and insulin were investigated. Data analysis procedures included graphical exploration, use of conventional statistical methods and mathematical modeling using the non-linear mixed effects models as implemented in the software package, NONMEM.

Clinical evaluation of glycaemic control based on the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) conservative criteria indicated that only 57% of subjects benefited clinically even when their dose was escalated to the maximum dose of 20 mg per day. Exploratory graphical analysis suggested that doses beyond 5 - 10 mg per day were unlikely to provide any additional reduction in blood glucose concentrations. Subsequent PKPD modeling revealed that the maximum mean reduction in blood glucose concentrations (E_{max}) was only ~ 34% from a baseline of ~15 mMol/L. The glibenclamide dose producing 50% inhibition of glucose concentration (ED_{50}) was estimated from the models to be in the region of 2.5 to 5mg per day. These 2 parameters in combination confirm that on average, escalating doses of glibenclamide in these subjects was unlikely to produce any substantial clinical benefit.

The subjects included in this study were considered to be typical diabetic patients that attended this clinic. Factors that were identified for the poor response to dose-escalation were the duration of diabetes and insulin resistance. Diabetic patients who share characteristics of the diabetic population and who are insulin resistant and on glibenclamide therapy, are more likely to benefit from combination therapy i.e., the addition of insulin sensitizers and/or insulin. This approach to their therapeutic control should be used rather than escalation of doses beyond 5 – 10mg per day since the use of high doses of glibenclamide is not without risk. High doses can mask the severity of a myocardial infarction, increase cardiovascular mortality with the added disadvantage that many patients are not likely to benefit from this expensive dose escalation.

STRUCTURE OF THE DISSERTATION

Introduction

The motivation for the study based on the observations of effectiveness of low dose glibenclamide and a survey on high dose glibenclamide use are presented.

Literature review

In this section a clinical overview of diabetes is presented followed by the pharmacology and treatment of the disease. The pharmacokinetics and pharmacodynamics of glibenclamide and modeling are also reviewed.

Patients and Methods

The demographics of the study population, the study design and the methods of analyses are presented

Results and Discussion

Results and discussion are presented separately for demographics, insulin resistance, dose-exposure-response relationship, clinical benefit, NCA, Population-PK and PKPD modeling.

Limitations

Limitations identified in the study are presented.

Recommendations

Recommendations based on the findings with respect to optimal dose, high dose, PK and PKPD models are presented.

Summary of Findings

A global summary of the research concludes the thesis.

Introduction

A prerequisite for optimal drug therapy is an appropriate dose. A review of the literature by Heerdink et al. (2002) suggests that doses of a wide variety of pharmacological agents currently used in clinical practice differ from the doses initially recommended at the time of drug registration. Some of the classes of drugs for which doses have been changed include antibiotics, cardiovascular, respiratory, neurological agents and others. Oral antidiabetic agents, in particular sulphonylureas, thus far lack, this degree of post-approval evaluation.

1 Diabetes - an epidemic

Diabetes mellitus (DM) is a global problem which is expected to reach epidemic proportions. DM is classified into type 1 (insulin-dependent DM or IDDM) and type 2 (non-insulin-dependent DM or NIDDM). Approximately 70-80% of diabetic patients have type 2 diabetes (Alberti and Zimmet, 1998). The World Health Organisation (WHO) estimates that the global number of persons with diabetes will rise from 151 million in the year 2000 to 221 million by the year 2010, and to 300 million by 2025 (Zimmet, 1999). Most of this increase will occur in the developing countries and will be due to population ageing, unhealthy diets, obesity, sedentary lifestyle and rural-urban migration.

Diabetes is approaching epidemic proportions in South Africa (Naicker, 2002). The present prevalence in South Africa is estimated to be between 4% and 5% and is expected to increase to 8% by the year 2010 i.e., from 1.6 million to 3.5 million and possibly 10 million. In South Africa, the magnitude of the diabetes epidemic is reflected in its prevalence in the different population groups. It is estimated that 8-10 % of coloureds, 13-18% South Africans of Indian origin, the majority of whom reside in the province of Kwazulu-Natal and 3.5-4% of whites are diabetic (Naicker, 2002; Trutter, 1998). The incidence in South African blacks is 5-8% and rising due to urbanisation e.g., the prevalence of DM in blacks in rural QwaQwa (Free State province) is 4.8% whilst in urbanised Mangaung (Free State Province) it is 6% (Mollentze et al., 1995). It may thus be postulated that lifestyle and dietary changes have contributed to this trend.

Motala et al. (2003) in their community-based study on a cohort of South Africans of Indian origin, aimed to determine the incidence of type 2 DM and the risk factors associated with its development, over a 10 year period. The crude cumulative incidence of DM in this population was 9.5% and significant predictors were body mass index (BMI) and a high baseline blood glucose.

2 Glibenclamide – which dose?

Oral hypoglycaemic agents (OHA's) are the mainstay of pharmacological management of type 2 diabetes mellitus. Glibenclamide, a second generation high potency sulphonylurea (SU) (Feldman, 1985), is one of the most widely used oral hypoglycaemic agents (OHA's) in public health institutions. It has been used in clinical practice for the past two decades both worldwide and in South Africa. The low cost and ready availability to the state makes it a popular agent in the management of type 2 diabetes mellitus. Glibenclamide is available as a generic preparation at a fraction of the cost of the original product. As at October 2003, Glycomin® was 50% the price of Daonil® in the private sector and only 14% of the cost of Daonil® in the public sector. Glycomin® is much cheaper in provincial institutions (R16 for 500 tablets) because of the highly competitive tender system.

The package insert for DiaBeta® (glyburide USP, 2003) states that the usual maintenance dose is in the range 1.25 to 20 mg daily. The maximum dose, it cautions, should not exceed 20 mg per day. The package insert for Daonil® (glibenclamide) in South Africa limits the maximum daily dose to 15 mg and for Glycomin® the maximum dose is 20 mg.. Therefore, manufacturers are inconsistent and even in conflict in their recommendations on the maximum dose of glibenclamide.

This discrepancy in dose recommendation amongst the manufacturers, has translated to inappropriate doses of glibenclamide being prescribed.

In South Africa, the case against the use of high dose glibenclamide came from Robertson and Jackson (1989). They showed that a reduction in the dose of glibenclamide from 15 mg/day to 2.5 mg/day in 15 type 2 DM patients resulted in 12 (80 %) patients achieving a reduction in fasting blood glucose. As there were no reported hypoglycaemic episodes, they suggested that their patients were often eating to 'keep up with their glibenclamide.' However, despite this study and the manufacturers' recommendations, glibenclamide continues to be used in high doses in clinical practice in South Africa.

A long term study comparing glibenclamide and glipizide showed little or no improvement in blood glucose control at doses greater than 10 mg/day (Groop et al., 1987). In the case of glipizide, dosage increases from 15 to 25 mg/day resulted in increased rather than decreased blood glucose levels (Wahlin-Boll et al., 1982). The authors therefore concluded that there may be a narrow range of plasma concentration below which sulphonylureas (SU's) are ineffective and above which there is little additional pharmacological benefit.

High dose glibenclamide is not without risk. Firstly, because of its high potency and long duration of action, it carries the risk of prolonged hypoglycaemia. This is particularly evident in the elderly and patients with irregular eating habits, and can result in fatalities. Secondly, the danger of high dose glibenclamide (e.g., 20 mg/day usually given as 10 mg in the morning and 10 mg at night) is potentially a hypoglycaemic risk, especially in patients who consume small meals. To overcome the symptoms of hypoglycaemia, the patient increases his glucose intake which increases his postprandial blood glucose level, which in turn elicits an unnecessary further increase in the dose of glibenclamide. A vicious cycle is thus set in motion. Thirdly, Huizar et al. (2003) found that diabetic patients treated with sulphonylureas did not display the level of ST-segment changes on electrocardiogram that would make them candidates for thrombolytic therapy. In essence, SUs can mask the severity of a myocardial infarction. If this finding is confirmed by larger studies, then guidelines for the use of thrombolytics may need to be reconsidered in this group of patients. Indeed, the warning on the Daonil® package insert merely states "increased cardiovascular mortality", without further elaboration.

Other studies reviewing dose-response effects of glibenclamide are presented in the literature review.

3 Survey of the prescribing of glibenclamide at selected provincial institutions in KwaZulu-Natal

3.1 Introduction

The majority of South Africans (up to 80%) utilize the health services offered by the province. These institutions serve the population of the greater eThekwini/Durban area (approximately 3 064 624, for 2002, projected from 1996 census, www.durban.gov.za). Hence, the medical systems at provincial institutions, with their captive diabetic population, offer a simple and effective way to evaluate prescription and usage patterns. These institutions purchase medications in bulk packs and prepack these according to the doses prescribed by the attending clinicians. For chronic care, patients usually return to the institution on a monthly basis for their medication. In the case of glibenclamide (5 mg dose unit) pack sizes of 14, 28, 42, 56, 70, 84 and 112 would correspond to daily doses of 2.5 to 20 mg/day based on a 28-day month.

The objective of this survey was to evaluate the prescribing patterns and the extent of high dose (20mg/day) glibenclamide usage in state and provincial institutions.

3.2 Methods

Pharmacy managers of the 10 major institutions in the greater eThekwini area were contacted telephonically and asked to provide their records of the number of units per pack size of glibenclamide prepacked per annum for the years 2000, 2001 and 2002/3. Data (Appendix 1) from the individual institutions is anonymised.

3.3 Results

This evaluation will only focus on the extent of usage of high dose (20mg/day) glibenclamide at the institutions surveyed. Six of the 10 state institutions responded with the requested data. Only data for the year 2002/3 was complete and is presented in table 1 and figure 1. Twenty five percent of all dose unit packs dispensed, comprised the 20mg dose of glibenclamide. Although not presented here, a similar picture was noted for the years 2000 and 2001 from those centres that did supply data.

At Centre A, which supplies chronic diabetic medication to geriatric homes and clinics, the usage was 25 %.

At a major provincial hospital (Centre B), the usage was 44% for 2002 and for the years 2000 and 2001 the figures were 47% and 46%, respectively.

In the case of a community clinic (Centre C), the figure was 7% for the year 2002.

At a provincial hospital (Centre D), the figure was 24% for 2002 and 17% and 8 % for 2000 and 2001, respectively.

Centre E, a provincial hospital situated in central eThekwini, the figures are 45% for the year June 2002 - June 2003.

At center F, a provincial hospital also situated in central eThekwini, 7% of all glibenclamide packed comprised the 20mg/day dosage.

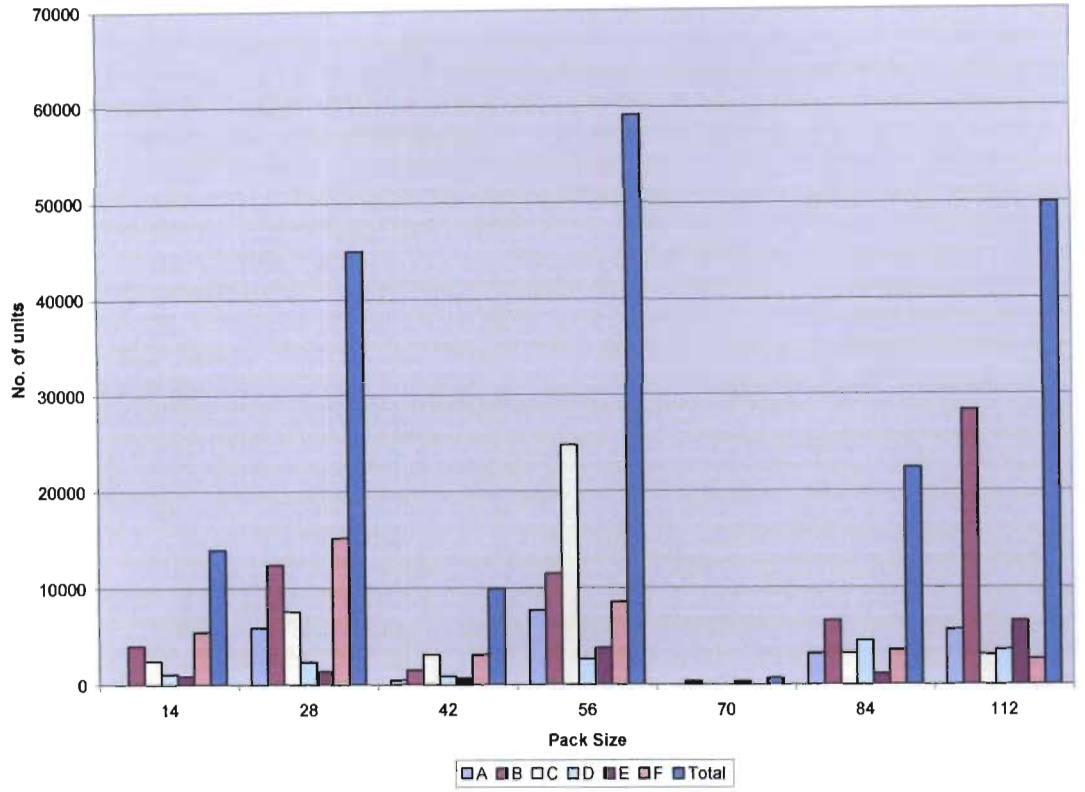

Figure 1: **Number of units of various pack sizes of glibenclamide 5mg tablets prepared as prepacks for out-patient dispensary use at 6 provincial health care institutions (A- F) in the greater eThekwini area. One-month pack sizes of 14 – 112 correspond to dosage regimens of 2.5mg/day to 20mg/day**

Table 1: **Usage of 20mg dose as inferred from number of pre-packed units of 112 glibenclamide tablets dispensed by provincial institutions in the greater eThekwini area in 2002/3**

Centre	Number of 20mg/day dose packs prepared in 2002	Total of all packs prepared in 2002	Percentage
A	5694	23116	25
B	28334	64743	44
C	3083	44415	7
D	3594	15230	24
E	6590	14758	45
F	2621	38750	7
Total	49916	201012	25

Discussion

This survey of glibenclamide supply in the public sector in the greater eThekwini/Durban area confirms that the drug is used in excess of the maximum recommended dose of 15 mg per day. This high dose usage is consistent over the previous 2 years for those centres that were able to supply this data. From this survey, it is not possible to determine whether these doses are associated with efficacy or safety issues. These prescribing patterns might reflect the status of the different dosage requirements in stabilised patients or the trends of titration of individual patients to their final dosage. It is particularly disconcerting that the high dosage appears to have been used by centres supplying medication to mainly geriatric patients.

The short term and long term adverse effects of hypoglycaemia and cardiovascular events associated with glibenclamide use will be highlighted in this dissertation. Although the cost of glibenclamide to the province is low due to the nature of the present tender system of purchase, drug wastage is, nevertheless, an important consideration. It can become significant if the purchasing system for the province is changed (as has been legislated for the private sector) due to legislation advocating a single exit price for medicines from manufacturers. In such an event, the province may then be forced to pay higher prices or the same price as the private sector for all medications. Drug wastage might be due to use of doses that do not provide (additional) clinical benefit, or due to poor compliance as a result of adverse effects.

This survey however, is not without its limitations e.g., not all the major institutions in the greater eThekwini area were surveyed. This is not a serious limitation since the areas serviced by the institutions that did provide data, are representative in terms of geography and population. A more relevant limitation however, is that it is not known whether the tendency towards use of high doses noted in this public sector survey are also applicable in the private sector due to the known discrepancy in the nature and quality of care. This extrapolation cannot be made and would require a similar survey in the private sector.

Pack sizes are an indirect measure of dosage and by implication, usage. However, while compliance is not measured in this survey and is assumed to be total, this limitation must be borne in mind in extrapolations of dose usage.

Pack size does not always mean one month supply. Patients could be making more than one visit per month for various reasons (dose adjustment, loss of medications).

The number of diabetics served by the institutions surveyed is not known, but a crude approximation can be inferred from the number of units of medication packs dispensed.

Prescribing patterns are best determined by examining individual prescriptions, reviewing data from private medical aid societies or health maintenance organizations, and reviewing pharmacoeconomic databases. These sources are reluctant to divulge this type of information often due to valid patient confidentiality concerns. Where this information is available it requires considerable time and effort to extract the data. Therefore, this public sector survey uses a simple method to provide macro trends on glibenclamide use and prescribing patterns.

In spite of these reservations, this survey serves a useful purpose not only as motivation for this study, but also to concientise the relevant institutions on high dose glibenclamide use.

In conclusion, this survey of glibenclamide usage in the greater eThekwini/Durban area confirms that the maximum recommended dose of 15 mg per day is being exceeded in public institutions. An evaluation of the dose-exposure response relationship to identify the clinical benefit of these higher doses is therefore warranted.

4 Aims and objectives

To date, there is no clinical study relating glibenclamide dosage to its blood glucose lowering effect in a South African diabetic population. A study on the dose-exposure-response relationship of glibenclamide in type 2 diabetic patients might clarify the issue. The aim was therefore to investigate the effect of increasing doses of glibenclamide on blood glucose levels and blood insulin levels in type 2 diabetic patients, with the view to determining which dosage regimen best controlled blood glucose levels.

This study was therefore designed to:

- characterise the within-subject blood glucose response and insulin response to increasing doses of glibenclamide
- characterize the pharmacokinetics of glibenclamide in type 2 diabetic patients
- develop a pharmacokinetic/pharmacodynamic (PKPD) model for the relationship between glibenclamide and blood glucose levels
- examine the relative contribution of pharmacokinetics to the overall variability in pharmacodynamic response to glibenclamide
- establish the dose at which glibenclamide optimally controls blood glucose in type 2 diabetics
- make dosage recommendations based on PKPD principles to diabetes caregivers

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Literature Review

1 Diabetes mellitus

Diabetes mellitus (DM) is a term describing a metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Beers, 1999).

DM spans a very broad clinical spectrum ranging from asymptomatic individuals (in whom the diagnosis is proven by biochemical investigation) at one extreme, to severely symptomatic, ill patients at the other extreme. The single defining feature common to this broad clinical spectrum is the diagnostic demonstration of hyperglycaemia. Symptoms of marked hyperglycaemia include polyuria, polydipsia, and unexplained weight loss. Other symptoms include pruritis, polyphagia, impaired vision and susceptibility to infections (Harris and Zimmet, 1992).

The vast majority of cases of DM fall into 2 broad categories.

Type 1 Diabetes Mellitus

In this category, the cause is an absolute deficiency of insulin secretion which leads to a variety of metabolic consequences. The clinical picture is of a severe hyperosmolar state due to severe hyperglycaemia, the symptoms of which are drastic, insatiable thirst, polyuria and rapid, severe weight loss. Poorly regulated lipolysis results in elevated concentrations of ketone bodies which, if high enough in the blood, lead to metabolic acidosis which leads to coma and death if left untreated. Insulin treatment is essential to sustain life in patients with type 1 diabetes (Harris and Zimmet, 1992).

Type 2 Diabetes Mellitus

In this more prevalent category, the cause is a combination of resistance to insulin action and an inadequate insulin secretory response. In such subjects, hyperglycaemia without clinical symptoms may be present for a long period before DM is detected. The result is that complications of DM such as retinopathy, nephropathy, atherosclerotic coronary disease, stroke or neuropathy may be the first clinical indications of the disease (DECODE study, 1999). Despite the presence of hyperglycaemia, ketone bodies in the blood and urine are absent and insulin treatment is not necessary to sustain life, although insulin may be necessary to achieve and maintain glycaemic control.

There are a few cases of diabetes, about 5%, that are secondary to identifiable causes. These include malnutrition-related diabetes, pancreatic disease, endocrine disease, gestational diabetes, drug-induced and toxin-mediated and some rare conditions.

1.1 Clinical characteristics

The World Health Organisation (WHO) classification of DM provides a framework within which to identify and differentiate the various types and stages of DM. Various other classifications **[*National Diabetes Data Group, American Diabetes Association Classification (ADA), South African Medical Journal (SAMJ) Classification*]** are based in essence on the WHO classification. Table 2 tabulates the various components of the WHO classification of DM

Table 2: WHO aetiological classification of DM (Alberti and Zimmet; 1998)

1. Type 1 diabetes *(β-cell destruction usually leading to absolute insulin deficiency)
   - A. immune-mediated
   - B. idiopathic

ii. Type 2 diabetes* (ranges from predominant insulin resistance with relative insulin deficiency to a predominant secretory defect with insulin resistance)

iii. Other specific types

- A. Genetic defects of β-cell function
  - 1. Chromosome 12, HNF-1 α (MODY 3)
  - 2. Chromosome 7, glucokinase (MODY 2)
  - 3. Chromosome 20, HNF ± α (MODY 1)
  - 4. Mitochondrial DNA
  - 5. Others
- B. Genetic defects in insulin action
  - 1. Type A insulin resistance
  - 2. Leprechaunism
  - 3. Rabson-Mendenhall syndrome
  - 4. Lipoatropic diabetes
  - 5. Others
- C. Diseases of the exocrine pancreas
  - 1. Pancreatitis
  - 2. Trauma/pancreatectomy
  - 3. Neoplasia
  - 4. Cystic fibrosis
  - 5. Haemochromatosis
  - 6. Fibrocalculous pancreatopathy
  - 7. Others
- D. Endocrinopathies
  - 1. Acromegaly
  - 2. Cushing's Syndrome
  - 3. Glucagonoma
  - 4. Aldosteronoma
  - 5. Hyperthyroidism
  - 6. Somatostatinoma
  - 7. Phaeochromocytoma
  - 8. Others
- E. Drug-or-chemical-induced
  - 1. Glucocorticoids
  - 2. Thiazides
  - 3. Diazoxide
  - 4. Vacor
  - 5. Thyroid hormone
  - 6. Pentamidine
  - 7. β-adrenergic agonists
  - 8. Nicotinic acid
  - 9. Phenytoin
  - 10. α-interferon
  - 11. Others
- F. Infections
  - 1. Congenital rubella
  - 2. Cytomegalovirus
  - 3. Others
- G. Uncommon forms of immune-mediated diabetes
  - 1. Stiff-man syndrome
  - 2. Anti-insulin receptor antibodies
  - 3. Others
- H. Other genetic syndromes sometimes associated with diabetes
  - 1. Down's syndrome
  - 2. Klinefelter's syndrome
  - 3. Turner's syndrome
  - 4. Wolfram's syndrome
  - 5. Friedreich's ataxia
  - 6. Huntington's chorea
  - 7. Laurence-Moon-Biedl syndrome
  - 8. Myotonic dystrophy
  - 9. Poryphyria
  - 10. Prader-Wils syndrome
  - 11. Others

IV. Gestational diabetes mellitus (GDM)

Patients with any form of diabetes may require insulin at some stage of their diseases. Such use of insulin does not in itself, classify the patient.

Table 3 and 4 outline the differences between type 1 and type 2 diabetes according to the SAMJ classification [Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) Guidelines, (2002) and Edwards et al., (1995)].

Table 3: Characteristics of type 1 and type 2 diabetes based on SEMDSA Guidelines (SEMDSA, 2002)

Characteristic	Type 1 DM	Type 2 DM
Onset	<40 years	>40 years
BMI	Thin	Fat
Insulin	Dependent	Requiring
DKA	Very prone	Less prone
Symptoms	Acute	Gradual

Key: BMI body mass index
DKA diabetic ketoacidosis

Table 4: Differences between type 1 and type 2 DM (Edwards et al., 1995)

	Type 1 or IDDM	Type 2 or NIDDM
Metabolic features		
Insulin deficiency	Severe (C-peptide negative)	Moderate, variable (C-peptide positive)
Spontaneous ketosis	Yes	No
Need insulin to survive	Yes	No
Insulin insensitivity	Mild, variable	Severe, variable
Aetiology		
Genetic susceptibility	Moderate	Very strong
HLA markers	Yes	None known
Autoimmune features	Yes	No
Environmental factors	? Viruses	? Overeating
Clinical features at presentation		
Age (not very useful)	Most <40 years (peak at 13 years)	Most >40 years (peak at 70 years)
Body weight	BMI mostly <25; recent loss common	BMI mostly >25; recent loss sometimes
Microvascular complications	Rare	Sometimes present
Signs and Symptoms	Abrupt onset, weight loss, ketosis, polydypsia, polyuria, fatigue	Polydypsia, polyuria. Weight gain, fatigue, blurred vision, susceptability to infection, impotence in men, pruritis, unusual sensations in the periphery, polyphagia
Prevalence	10-15 % of all diabetics	85-90% of all diabetics
Risk factors	Age < 30, childhood, adolescence, genetic susceptability	Obesity Age >30 Geographical and ethnic factors History of gestational diabetes Hypertension Hyperlipidaemia Familial

Key: HLA human leucocyte antigen
BMI body mass index

Diagnosis

The revised criteria [American Diabetes Association (ADA) report, 2003] avoids the discrepancy between FBG and 2-hour postload glucose (PG) and facilitates and encourages the use of a simpler and equally accurate test viz., fasting blood glucose, for diagnosing diabetes. The cut off point for the 2-hour PG is justified because it is at approximately at this point that the prevalence of microvascular complications considered specific for diabetes viz., retinopathy and nephropathy, increases dramatically. The relationship between FBG, the 2-hour PG and the development of retinopathy was confirmed in the Pima Indians and Egyptians and in the NHANES III studies (Harris et al., 1998). The relationship between macrovascular disease and FBG and 2-hour PG has been examined. In the Paris Prospective Study (Balkau et al., 1999), coronary artery disease and all cause mortality were related to these two parameters. Therefore, both FBG and 2-hour PG provide important information regarding the risk for both microvascular and macrovascular disease.

Diagnostic Criteria for Diabetes Mellitus

The ADA Expert Committee (2003) has revised and set the following criteria for the diagnosis of diabetes. Diabetes can be diagnosed in three different ways and each must be confirmed on a subsequent day by one of the three recommended methods.

The revised criteria for diagnosis of DM are still based on measures of hyperglycaemia. The metabolic defects underlying the hyperglycaemia are referred to independently i.e., in the classification of the disease.

Blood glucose levels are distributed over a continuum but there is an approximate threshold separating subjects who are at increased risk for some complications of DM from those who are not. Based partly on estimates of the thresholds for microvascular disease, the previous WHO criteria defined DM by fasting blood glucose (FBG) 7.0 mmol/L, 2 hour blood glucose (PG) 11.1 mmol/L in the OGTT, or both (Alberti and Zimmet; 1998).

However, almost all subjects with a raised FBG had an elevated 2 hour PG by OGTT, but only one-fourth with a raised 2-hour PG by OGTT had a raised FPG. It is this discrepancy, and the necessity to devise a simpler diagnostic test (the FBG) than the OGTT, that led to the revision of the criteria for the diagnosis of DM (Alberti and Zimmet; 1998).

The revised criteria are for diagnosis and not for treatment or goals of therapy. The new diagnostic cut-off point (FPG 6.1 mmol/L) is based on the observation that this degree of hyperglycaemia reflects a metabolic abnormality that has been shown to be associated with serious complications (Alberti and Zimmet; 1998).

Diabetes mellitus is diagnosed according to various criteria. The revised criteria for the diagnosis of type 2 diabetes according to the ADA, SEMDSA, WHO, and SAMJ are listed in table 5 below.

Table 5: Criteria for diagnosis of diabetes (SEMDSA, 2002; Alberti and Zimmet, 1998; DECODE, 1999; ADA, 2004)

Characteristic	SEMDSA	WHO	ADA	SAMJ
FBG	>7	>7	>7.0	>7.8
2hr pp	>11.1	>11.1	>11.1	---
RBG	>11.1	>11.1	---	>11.1
IGT	---	>7.8-<11.1*	---	---
IFG	---	---	>5.6-<6.9*	---

Key:
FBG Fasting blood glucose confirmed on the following day
2hr pp 2 hour postprandial blood glucose
RBG Random blood glucose
IFG Impaired fasting glucose
OGTT Oral glucose tolerance test (75 g glucose)
IGT Impaired glucose tolerance
All values in mmol/L
*- Value based on OGTT

The ADA Expert Committee (2003) also recognises an intermediate group of subjects whose glucose levels are too high to be considered altogether normal. The criteria for defining this group of subjects are as shown in Table 6.

Table 6: Catergories for defining DM

	Normal	Intermediate	Provisional diagnosis of DM
Fasting	<6.1	6.1 <7.0*	7.0
2-hour post-	<7.8	7.8 <11.1**	11.1
load (OGTT)			

Key:

*IGT Impaired glucose tolerance
**IFG Impaired fasting glucose
OGTT Oral glucose tolerance test
All units in mmol/L

Diagnostic Tests for Type 2 Diabetes

When symptoms suggest diabetes the diagnosis may be confirmed by the presence of glycosuria with or without ketonuria, and a random blood glucose concentration greater than 11.1 mmol/L.

It is now recommended that persons over the age of 45 years be tested regularly for the presence of DM (Alberti and Zimmet, 1998). In addition, younger adults presenting with the following, should also be tested:

- A weight that is 20% more than ideal body weight
- High blood pressure
- Low HDL cholesterol levels (0.91 mmol/L) and high triglyceride levels (2.82 mmol/L)
- A close relative with diabetes
- A high-risk ethnic background
- Delivered a baby weighing over 4.08kg
- A history of gestational diabetes

Some experts recommend that any asymptomatic child over age 10 years should be tested for type 2 diabetes if they are overweight and have at least two of the above mentioned risk factors. Children who have symptoms of diabetes are usually diagnosed as type 1. This is of particular concern given the rise in childhood type 2 diabetes with some centers reporting a misdiagnosis in 25% of cases (Alberti and Zimmet, 1998).

Pathogenesis

Type 1 DM is an example of T cell-mediated autoimmune disease characterized by selective destruction of pancreatic β-cells. Genetic and environmental factors play a role in pathogenesis.

Type 2 DM is a polygenic disorder with environmental influences playing a major role in its onset and progression. It is a disorder with dual defects involving insulin resistance and β-cell dysfunction. The progression of the disease is related to deterioration in β-cell function and increased insulin resistance.

Genetic factors: The clustering of type 2 DM in families, provides evidence for a genetic basis for DM. Studies on identical twins provide further confirmation for this theory. Later studies showed that concordant rates of type 2 diabetes in this population was as high as 33% (Lebovitz, 1998). Monogenic and polygenic disorders result in maturity onset diabetes in the young (MODY) and an altered metabolic state (obesity, insulin resistance, and impaired β-cell secretory function) respectively (Lebovitz, 1998).

Environmental factors: It has been postulated that polygenic forms of type 2 diabetes are the consequence of having evolved a *"thrifty" genotype* that had survival benefits in the past but is detrimental in our modern indulgent society. This thrifty genotype is a disadvantage and leads to obesity, insulin resistance and type 2 diabetes (Neel, 1962).

An alternate view is that individuals with a low birth weight have a higher prevalence of obesity, insulin resistance and type 2 diabetes in adult life than those who had normal birth weight. This is due to the exposure to the indulgences of modern day living in the face of a relatively small pancreatic β-cell mass (Barker, 1993).

Studies have shown that rural (traditional-living) populations are experiencing a major increase in the burden of type 2 DM as they move to urban (non-traditional) situations, often with a 5- to 10-fold increase in the prevalence of type 2 DM (Harris, 1995).

Biochemical defects: There is agreement that type 2 DM is closely associated with two features namely, insulin resistance and insufficient insulin secretion. The former suppresses hepatic glucose output and promotes peripheral glucose disposal, while the later attempts to overcome the degree of insulin resistance. As long as β-cell function remains adequate to match or overcome insulin resistance in the prediabetic state, the chronic hyperglycaemia of type 2 DM does not appear (DeFronzo et al., 1992).

Immunologic factors: These also contribute to the aetiology of DM by auto-immune damage to the pancreatic β-cells, acutely as in type 1 diabetes, or slowly evolving auto-immune damage, as in latent auto-immune diabetes in an adult (LADA).

Metabolic factors:

The "glucocentric" theory of metabolic derangement in type 2 DM: The traditional thinking in the pathogenesis of type 2 DM centres around impaired β-cell function resulting in impaired secretion of insulin the consequence of which is hyperglycaemia. This is a necessary defect in all stages of abnormal glucose tolerance. This glucocentric theory forms the basis for pharmacotherapy of type 2 DM, namely OHA's and insulin (DeFronzo et al., 1992).

The "lipocentric" theory for the metabolic derangement of type 2 DM shifts the basis for the pathogenesis of type 2 DM to abnormal lipid dynamics. This theory is based on evidence that insulin resistance in tissues such as liver and muscle might, at least in part, be a consequence of a genetically programmed abnormal accumulation of fat in these sites. It is also likely that lipotoxicity plays a role in β-cell demise in type 2 DM.

Prevalence

The WHO estimates that between 120 and 140 million people suffer from DM worldwide and that this number could double by the year 2025. Most of the increase will occur in the developing countries and will be due to population aging, unhealthy diets, obesity and sedentary lifestyle (Alberti and Zimmet, 1998).

In the UK, DM affects about 2% of the population, and 7.8% in the USA. Diabetes mellitus is approaching epidemic proportions in South Africa, the present estimated prevalence being between 4 and 5 % and is expected to increase to 8% by 2010 i.e., from 1.6 million to 3.5 million. In South Africa, it is estimated that 5-8% of blacks, 8-10 % of coloureds, 13-18% South Africans of Indian origin and 3.5-4% of whites are diabetic (Naicker, 2002; Trutter, 1998).

Diabetes is universal with widely varying prevalence rates between and within different populations. There is great variation in the frequency of type 2 diabetes in different countries, the highest being noted in the Pima Indians of America and the South Pacific islands. More than 50% of Pima Indians have type 2 diabetes. The Pima Indians once had an agricultural lifestyle, but, due to a rapid development of a westernised lifestyle, they are now overweight and inactive and thus prone to developing insulin resistance and type 2 diabetes (Unger, 1995).

The risk of developing type 2 diabetes increases with age, obesity and lack of physical activity. Type 2 diabetes is more common in individuals with a family history of diabetes and in members of certain racial or ethnic groups (Edwards et al., 1995). It occurs more frequently in individuals with hypertension or dyslipidaemia and women with prior gestational diabetes mellitus (GDM).

The correlation of risk factors with development of diabetes is never 100%. However, the greater the number of risk factors present in an individual, the greater the chance of that individual developing or having diabetes (Edwards et al., 1995).

Pathophysiology

Impaired β-cell function is a necessary defect in impaired glucose regulation (IGR). However, it manifests itself in a different manner in fasting and glucose-stimulated conditions. In the fasting state, the basal insulin secretory rate increases as a function of the progressive decline in insulin action. Thus, fasting insulin levels are taken as a marker of insulin sensitivity. A specific alteration of acute insulin release is an early and progressive defect after a glucose challenge.

To understand the impact of β-cell dysfunction in type 2 diabetes on metabolic homeostasis, the different phases of insulin secretion need to be considered separately. Insulin secretion can be divided into the basal (post absorptive) state and the stimulated (postprandial) state. The former plays a major role during the interprandial phases and the overnight fast. The latter regulates glucose metabolism when carbohydrate is abundant and needs to be disposed of.

Basal insulin secretion

Adequate basal insulin secretion is important in glucose regulation both in the liver and the peripheral tissues viz. muscle and adipose tissue. Endogenous glucose production (EGP) is modulated and modified by basal insulin levels and is very sensitive to minor changes in portal insulin concentration.

Skeletal muscle is the major peripheral site for glucose disposal while the liver is the prime site for gluconeogenesis. In the fasting state, blood glucose concentrations are maintained by a delicate balance between glucose production by the liver and glucose disposal by peripheral tissue. Glucose disposal is regulated by insulin and various non-insulin mechanisms. Hepatic glucose production is regulated by the combined effects of insulin and glucagon.

Glucose stimulates release of insulin. Insulin response to a glucose stimulus is biphasic with a rapid early insulin peak followed by a second slow-rising peak. Figure 2 describes the biphasic secretion of insulin to a glucose stimulus. The first phase peak is due to the release of insulin from the β-cells while the second phase peak represents newly synthesized insulin and is directly related to the level of glucose (Pratley and Weyer, 2001).

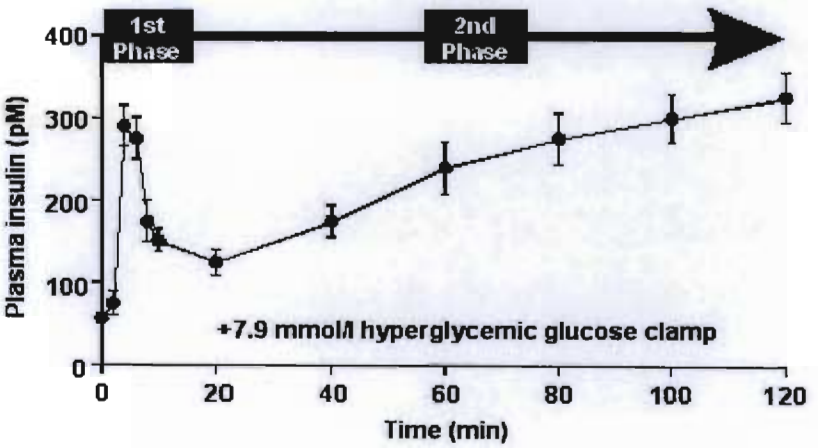

Figure 2: Biphasic insulin secretion *in vivo* (Pratley and Weyer, 2001)

Type 2 DM is characterized by a blunting or loss of the first phase of insulin secretion as illustrated in figure 3 below. This phenomenon has also been observed in the Pima Indians and is not just a marker of type 2 DM but a pathophysiological mechanism (Del Prato et al., 2002).

In type 2 diabetic patients, the combination of insulin resistance and inadequate basal insulin secretion to compensate for the defect in insulin action may well account for excessive hepatic glucose production. Basal level of insulin is important in modulating the intracellular fate of glucose uptake by peripheral tissues and a dysfunction in basal insulin secretion may contribute to the release of free fatty acids (FFAs) and lactate. The latter is a preferential substrate for gluconeogenesis and the former provides the energy required for driving the gluconeogenesis, the result of which is enhanced EGP and fasting hyperglycaemia. Correction of this relative hypoinsulinaemia in type 2 diabetics is thus a rational therapeutic approach (Del Prato, 2002).

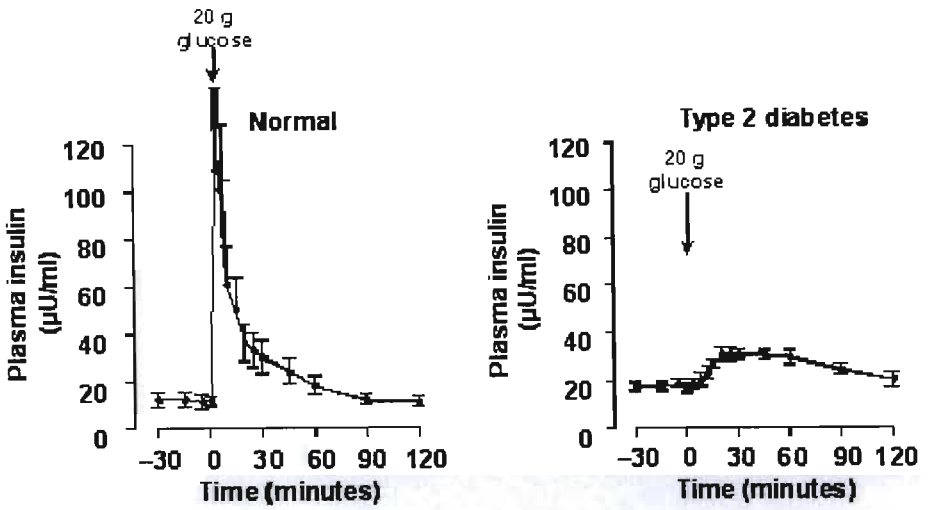

Figure 3: Loss of first-phase insulin response (Pfeifer et al., 1981)

In the early stages of type 2 DM, first-phase insulin release in invariably lost despite the enhancement of second-phase insulin secretion. The acute elevation of blood glucose concentration and the subsequent biphasic insulin release in hyperglycaemic clamp studies are associated with progressive suppression of hepatic glucose production and an increase in glucose disposal. Abolition of first-phase insulin release by somatostatin and insulin replacement is not associated with changes in glucose disposal. However, the impact on suppression of hepatic glucose production is dramatic with the liver releasing glucose at a greater rate despite the presence of hyperglycaemia and hyperinsulinaemia. These results are in agreement with the correlation between plasma concentration 30 minutes after an oral glucose load and the rate of glucose appearance in patients with IGT. Further, an inverse correlation between the rate of appearance of glucose and the insulin/glucagon ratio is found in patients with IGT. In the postprandial phase, plasma glucagon levels remain high despite normal or increased insulin levels while EGP is not suppressed (Flier et al., 1979).

A significant improvement in postprandial glucose tolerance is apparent with early administration of insulin to restore or mimic first-phase insulin release (Bruce et al., 1988). This may be interpreted as a sparing effect on β-cell function due to prevention of excessive rise in glucose concentrations. The lower plasma C-peptide values in the late phase are associated with lower glucose and insulin concentrations. This correlates directly with the 120-minute post-OGTT blood glucose and insulin levels.

Postprandial hyperglycaemia

Prevention of postprandial hyperglycaemia is important as it is implicated in the development of macro- and microvascular complications associated with diabetes (Baron, 1998). Postprandial hyperglycaemia may be defined as elevated glucose concentrations following the ingestion of a meal. It is generated by a combination of impaired insulin secretion, unsuppressed hepatic glucose production and reduced glucose uptake into the periphery (Cozma et al., 2002). In normal individuals, glucose levels rarely rise above 7.8 mmol/L during the postprandial period. There is a progressive rise in postprandial glucose levels associated with increasing degrees of impairment of glucose tolerance. Individuals with post-challenge glucose levels in excess of 11.1mmol/L have diabetes by definition. However, fasting glucose levels are often below the current glycaemic threshold of 7.0 mmol/L for the diagnosis of diabetes. It has thus been concluded that fasting glucose level is not a good predictor of postprandial levels as it often underestimates post-challenge glucose levels (Baron, 1998).

The risk of cardiovascular disease is related to the degree of postprandial hyperglycaemia rather than fasting blood glucose levels (Hanefield and Temelkova-Kurktschier, 1997). Postprandial blood glucose, triglycerides and blood pressure are risk factors for myocardial infarction, whereas fasting blood glucose is not a risk factor. Thus prevention of postprandial hyperglycaemia could be important in the prevention of diabetic complications (Baron, 1998). It is also noted that individuals with impaired glucose tolerance (IGT) have elevated postprandial glucose levels and an increased risk of atherosclerosis (Pan et al., 1993).

The National Health and Examination Survey (NHANES) in the USA reported an association with post-load hyperglycaemia and increased all-cause and cardiovascular mortality (Saydah et al., 2001). The incidence of retinopathy is higher in patients with postprandial glucose levels higher than 11.1 mmol/L (Bando et al., 2001). Macrovascular disease is a major cause of death in type 2 diabetes and conventional treatments of diabetes have had minimal effect on diabetes-related cardiovascular mortality.

A study of patients with gestational diabetes showed that monitoring and targeting postprandial hyperglycaemia rather than fasting blood glucose, resulted in greater reduction of HbA_{1c}, significantly smaller babies and a reduction in the number of babies that were large for their gestational age (de Veciana et al., 1995). A study conducted by Edward et al. (2000) showed that adding a second antihyperglycaemic agent lowers HbA_{1c} and glucose levels. However, when insulin lispro was used to focus on postprandial blood glucose, there was a greater impact on overall metabolic control. Insulin lispro and acarbose prevented postprandial blood glucose excursions in obese type 2 patients. Regulation of postprandial hyperglycaemia by agents such as acarbose has been shown to decrease Apo B and triglyceride levels. This may have a favourable effect on atherosclerosis and coronary artery disease (Reaven et al., 1990).

1.2 Insulin Resistance (IR)

A constellation of insulin resistance, reactive hyperinsulinaemia, increased triglycerides, decreased high- density lipoprotein cholesterol and hypertension was designated as 'syndrome X' by Reaven (1988). The close association of type 2 diabetes and cardiovascular disease led to the hypothesis that the two arise from a common antecedent. This concept is termed the Metabolic Syndrome (Wilson et al., 1999). Table 7 lists the criteria for characterization/classification of metabolic syndrome.

Table 7: Criteria for characterization/classification of the Metabolic Syndrome*(Ford et al., 2002)

Parameter	Value
Waist circumference	>102 cm in men >88 cm in women
Hypertriglyceridaemia	1.69 mmol/L
Low HDL cholesterol	< 1.04 mmol/L in men < 1.29 mmol/L in women
Blood pressure	130/ 85 mm Hg
Fasting blood glucose (FBG)	> 6.1 mmol/L

****The prescence of three or more of the above criteria are a pre-requisite for the classification of metabolic syndrome***

Type 2 DM represents an extreme of insulin resistance. Insulin resistance (IR) is the body's inability to correctly utilize its normal (endogenous) insulin supply, even though that insulin is present in sufficient amount. As a result of IR the pancreas produces more insulin than is required (hyperinsulinaemia).

Insulin resistance reflects defective insulin action in skeletal muscle and the liver. The major causes of skeletal muscle insulin resistance in the prediabetic state may be grouped into genetic, obesity and physical inactivity. At present there is no clear understanding of the factors that define the genetic basis of insulin resistance.

There is a close relationship between obesity and insulin resistance. While being overweight does not cause IR, losing weight reduces IR. Diabetics are obese and the risk of developing diabetes increases progressively in both men and women with the degree of obesity. This is due to increasing IR and decreasing insulin sensitivity (IS) as body mass increases. A diet high in fat, a sedentary lifestyle and a genetic predisposition all contribute to obesity. Central or truncal obesity, more typical of men, but often seen in female type 2 daibetics, offers the greatest risk for the development of diabetes. This distribution of fat is due to the deposition of adipose tissue both subcutaneously and intra-abdominally.

This visceral fat is metabolically active and releases non-esterified fatty acids (NEFAs) that cause insulin resistance among other effects, thus contributing to the hyperinsulinaemia and reduced IS that is Syndrome X. The worldwide epidemic of type 2 DM is fuelled by a parallel epidemic of obesity and reduced physical activity, clearly pointing to the prevention of obesity as the most direct route to the prevention of the metabolic syndrome and its sequelae (Reaven, 1988).

Insulin resistance and diabetes increase the atherosclerotic process. There is mounting evidence that insulin-resistant subjects have a defect in insulin-mediated nitric oxide production which parallels their defect in glucose transport (Radikova, 2003).

Measures of insulin resistance and acute insulin response

Type 2 DM represents an extreme of insulin resistance. Insulin resistance is a condition in which the response to the hormone insulin, is muted and the body must produce excess insulin to maintain normal blood glucose concentrations. This condition is also called low insulin sensitivity.

Insulin sensitivity is measured by the hyperinsulinaemic euglycaemic clamp technique, which is the gold standard, homeostatic model assessment (HOMA) and more recently, Quantitative Insulin Sensitivity Check Index (QUICKI). These measures have proven to be suitable surrogates for more complex gold standard technique. HOMA (Mathews et al., 1985) and QUICKI (Katz et al., 2000) utilise fasting blood glucose and fasting insulin levels in determining insulin sensitivity. Katz et al. (2000) concluded that fasting glucose and insulin levels contain sufficient information to accurately assess insulin sensitivity *in vivo* over a wide range in a diverse population. They observed that QUICKI is a novel, simple, accurate, and reproducible method for determining insulin sensitivity in humans.

The formula for HOMA is:

Equation 1: R_{HOMA}= Fasting Glucose*Fasting Insulin /22.5

The formula for **QUICKI is**,

Equation 2: QUICKI is: 1/[log fasting insulin + log fasting glucose]

When glucose is measured in mmol/L, the factor 22.5 is used and when glucose is measured in mg/dl, the factor is 405. HOMA is an index of insulin resistance (R_{HOMA}) which is the inverse of the corresponding index of sensitivity (S_{HOMA}) i.e., (R_{HOMA}=1/ S_{HOMA}). HOMA and QUICKI are related by the following equation:

Equation 3: QUICKI= 1/[log(HOMA) + log(22.5)]

Indices of insulin sensitivity/resistance derived from fasting glucose and insulin concentrations reflect hepatic insulin sensitivity and basal hepatic glucose production. Lichnovska et al. (2002) report a mean HOMA normal value ± SD of 1.57 ± 0.87 and a mean QUICKI of 0.366 ± 0.029 in healthy subjects of both genders.

Numerous authors have shown good correlations between HOMA and clamp-derived insulin sensitivity (Radziuk, 2000). 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. It has also been stated that hyperglycaemia and hyperinsulinaemia are necessary in the insulin resistance state, to maintain near normal peripheral glucose uptake when metabolic glucose clearance at a specific insulin concentration is decreased, as a result of insulin resistance.

In view of the direct relationship between basal hepatic glucose production and fasting blood glucose concentration, adequate overnight insulin secretion is needed to ensure euglycaemia in the post absorptive phase. In type 2 diabetic patients, the combination of insulin resistance and inadequate basal insulin secretion to compensate for the defect in insulin action may account for the excessive endogenous glucose production. It has been hypothesized that dysfunction in basal insulin secretion may contribute to an increase in free fatty acids (FFA) and lactate. Lactate is a preferential substrate for gluconeogenisis and FFAs provide the energy required for driving this gluconeogenesis, the final result is enhanced endogenous glucose production and fasting hyperglycaemia (Avogaro et al., 1998). Therefore correction of absolute or relative hypoinsulinaemia in type 2 DM is a rational therapeutic approach.

Development of diabetes occurs more frequently in individuals with low insulinogenic values (Del Prato et al., 2002). Impaired beta-cell function is a necessary defect in all conditions of impaired glucose regulation. In the fasting state, the basal insulin secretory rate increases as a function of the progressive decline in insulin action – therefore fasting blood insulin concentration is often used as a marker for insulin sensitivity. After a glucose challenge, the change in acute insulin release (AIR) is an early and progressive defect. While this may be an intrinsic defect its continuous decline is affected by glucose toxicity (Leahy et al., 1992) and lipotoxicity (Del Prato et al., 2002).

Del Prato et al. (1990) studied type 2 DM patients with secondary failure to OHAs before and after adding a preprandial subcutaneouos injection of long acting insulin while maintaining preprandial administration of a SU. There was improvement in the post-absorptive concentration of blood insulin associated with a reduction of basal hepatic glucose production, fasting blood glucose and FFAs, in keeping with the hypothesis proposed above. The improvement of basal insulin levels, was associated with a positive influence on daily blood glucose profile, as indicated by decrease in fasting blood glucose and a reduction in 24 hour glucose levels.

In summary, a high fasting insulin concentration is a surrogate marker for insulin resistance. An incremental 30 min insulin concentration is an indicator of early phase insulin response. The insulinogenic index (II) which is a measure of the ratio of the increment of insulin to that of blood glucose 30 min after glucose load, provides a parameter of insulin response and a parameter of insulin release (Del Prato et al., 2002).

Insulin secretion and sensitivity are highly interdependent phenomena. Any decrease in insulin sensitivity is immediately translated into minute increases in blood glucose concentration that will in turn produce a compensatory stimulus for insulin secretion. This prevents greater hyperglycaemia. When the β-cell is unable to compensate for the prevalent insulin resistant state by further augmenting insulin secretion, hyperglycaemia continues to increase, producing impaired fasting glucose or diabetes. Therefore assessment of insulin secretion and sensitivity has clinical relevance. Equation 4 shows the insulinogenic index (Del Prato et al., 2002) is calculated as the ratio of the increment of insulin to that of blood glucose 30 min after glucose load and provides a parameter of insulin response as represented below.

Equation 4:

$$II = \frac{Insulin(time = 30\,\text{min}) - Insulin(time = 0)}{Glu\cos e(time = 30) - Glu\cos e(time = 0)}$$

Low values of insulinogenic index occur more frequently in diabetic individuals than in normal insulin responders. In the study by Jensen et al. (2002) diabetic subjects and patients with impaired glucose tolerance had insulinogeic values of 36% and 64% that of normal subjects, respectively. Suzuki et al. (2003) in their study reported an insulinogenic index of approximately 64 for insulin measured in pmol (9.22 when insulin is measured in μU/mL) and 34 for insulin measured in pmol (4.9 when insulin measured in μU/mL) for subjects with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) respectively.

1.3 Complications

While the chronic complications of DM are probably the single greatest cause of anxiety for most diabetic patients, it must be noted that over 40% of type 1 diabetics survive for over 40 years, half of them without developing significant complications (DCCT, 1993). Furthermore, with continuing advances in diabetic care as seen by the decreasing incidence of nephropathy, the damage due to diabetic complications is likely to decrease even further.

Chronic diabetic complications can be classified as:

Microvascular (small vessel/microangiopathic) disease

The Diabetes Control and Complications Trial (DCCT, 1993) linked the severity of microvascular complications with the degree and duration of hyperglycaemia in type 1 DM while the United Kingdom Prospective Diabetes Study (UKPDS) produced analogous evidence in type 2 DM (UKPDS 33, 1998).

Basement membrane thickening and abnormal leakiness in the microcirculation are common features and are essential components of retinopathy and nephropathy, and to a lesser extent in neuropathy. The lesions are identical in both type 1 and type 2 diabetics, suggesting that hyperglycaemia or some other metabolic disturbance is responsible. These changes are very often found at diagnosis in type 2 diabetes, reflecting the long, unsuspected presence of the disease.

Microangiopathy involving the capillaries and pre-capillary arterioles may occur in any tissue, but occurs especially in the kidneys, eyes and peripheral nerve endings in type 2 diabetic patients. This association of hyperglycaemia with microvascular complications has been recognized implicitly in type 2 diabetes.

- **Retinopathy**

Diabetic retinopathy is the commonest cause of blindness, but is easily detected and now often treatable. Background changes affect almost all cases after 20 years of diabetes. Maculopathy is a common cause of blindness in type 1 DM and may be invisible on fundoscopy. Laser photocoagulation prevents blindness in 50% of cases with new vessel disease while vitreo-retinal surgery restores vision in 50% of cases with advanced eye disease (DCCT, 1993).

The prevalence of non-proliferative diabetic retinopathy rises from 5% among patients with duration of diabetes of less than 5 years to 30%, 45%, and 62% among those with duration of 5-9 years, 10-14 years and 15years, respectively (Lebovitz for the Framingham Eye Study, 1980). Klein et al. (1984) showed that the risk of retinopathy is lower in type 2 than in type 1 DM and that the level of hyperglycaemia at baseline examination has a significant impact on the development of retinal changes. In patients with initial early retinopathy, the average risk of progression was reduced by 50% in patients who received intensive therapy (DCCT, 1993).

Proliferative retinopathy is uncommon in type 2 diabetes, the cumulative incidence being less than 4% (Klein et al., 1984).

- **Diabetic nephropathy**

Hyperglycaemia is necessary for the development of diabetic renal damage, as again shown by both the DCCT and UKPDS. Diabetic nephropathy affects mainly the glomerulus, but tubular lesions may occur and is the commonest cause of premature death in type 1 DM. In the UK alone, it accounts for 25% of patients with end-stage renal failure (DCCT, 1993; UKPDS 33, 1998; UKPDS 35, 2000).

Nephropathy is rarer in type 2 DM. Hypertension is an important association with nephropathy which, if not treated, accelerates the decline in the rate of glomerular filtration (GFR). The main significance of microalbuminuria is the increased risk of developing overt proteinuria which is associated with a high mortality by way of renal failure (66% of proteinuric patients). Type 2 diabetic patients frequently have proteinuria at diagnosis which tends to be persistent with increasing age, yet the risk of progressing to renal failure is less than in patients with type 1 diabetes (Caterson et al., 1997).

Control of hypertension, cessation of smoking and improving glycaemic control significantly slow the deterioration in renal function. Hypoglycaemia is a common problem in patients with with compromised renal function. Insulin and sulphonylureas, that are excreted partly through the kidneys, may accumulate thus prolonging their duration of action. Insulin is preferred when creatinine levels rise. Metformin should not be given because of the very high risk of lactic acidosis.

- **Diabetic neuropathy**

Diabetic neuropathy involves the somato-sensory system, causing variable sensory and motor deficits. The autonomic outflow to various organs is also affected by diabetes. Epidemiological studies have associated severe nerve damage with poor diabetic control while tightening glycaemic control has produced short-term neurophysiological and clinical benefits (Harris and Zimmet, 1992). Hyperglycaemia could damage nerves through glycation of proteins and/or overactivity of the polyol pathway. Vascular damage through diffuse occlusion of capillaries supplying the nerves occurs and may occlude larger vessels causing localised infarction and demyelination.

Macrovascular (large-vessel/macroangiopathic) disease

The association between macrovascular disease and either the duration of diabetes or the level of glycaemia is weak. Factors such as insulin resistance, hyperinsulinaemia or the metabolic syndrome may be more important in the development of macrovascular disease. UKPDS 35 (2000) showed that at near normal levels of glycosylated haemoglobin (HbA_{1c}) the risk of myocardial infarction was 2-3 times the risk for a microvascular endpoint. The Kumomoto study (1995) showed a definite decrease in macrovascular disease with strict glycaemic control. This outcome is due to control of not only FBG, but also postprandial blood glucose levels. The atherosclerosis of diabetes is distinguished from that affecting non-diabetic people by its rapid and extensive development.

Atherosclerosis is very common in both types of diabetes and is a major cause of death in type 2 DM. Hyperglycaemia amplifies those predisposing factors which operate in the non-diabetic subject viz.,

- Hypertension is two to three times commoner in diabetics than in the general population and affects up to one third of diabetic patients. The association may be partly explained by sodium retention, altered vascular reactivity and insulin resistance (Caterson et al., 1997).
- Hyperlipidaemia is common, with an atherogenic pattern of increased VLDL and LDL-cholesterol and reduced HDL cholesterol, which is more pronounced in type 2 DM. Triglycerides are also increased in untreated diabetes (Lebovitz, 1998).
- Smoking is another predisposing factor and the 10-year mortality in diabetic smokers is twice as high as in non-diabetic smokers and most premature deaths are from macrovascular deaths (Lebovitz, 1998).
- The atheroma in diabetic patients is more extensive and multifocal and tends to involve the more distal arteries (Lebovitz, 1998).

- **Coronary heart disease**

Cardiovascular disease is the commonest cause of death in type 2 diabetes (Reaven, 1988; UKPDS 35, 2000). Diabetic women are notably affected since they lose the protection from cardiovascular disease enjoyed by non-diabetic women and have a relatively greater risk of developing atherosclerosis than diabetic men. IGT lends itself to an increased frequency of cardiovascular disease (Taskinen, 1995).

- **Stroke and Peripheral vascular disease**

The prevalence of stroke and peripheral vascular disease is similar to that of cardiac macrovascular complications in diabetic patients, and their clinical presentation is no different from that of the non-diabetic population (Reaven, 1988; DIGAMI study, 1995).

Management involves the treatment of risk factors. Active treatment of hypertension with drugs that do not worsen blood glucose or lipid levels, optimizing glycaemic control and treatment of hyperlipidaemia reduce the cardiovascular complications. Life-style modification, dietary control, reducing obesity and cessation of smoking are crucial in the management.

Other Complications

- **The Diabetic Foot**

Foot problems are one of the commonest causes of hospital admissions for diabetic patients and are usually the result of neuropathic and vascular changes that complicate DM. Proliferative retinopathy and neuropathy causing foot ulceration frequently co-exist (Edwards et al., 1995).

Diabetic Metabolic Emergencies

Coma in diabetic patients is a medical emergency and is commonly due to the metabolic complications of the disease and/or its treatment, as well as other factors.

- **Diabetic ketoacidosis (DKA)**

DKA is uncontrolled diabetes with hyperglycaemia and metabolic acidosis due to high circulating ketone-body levels. DKA is a life-threatening condition that occurs secondary to insulin deficiency resulting in unrestrained lipolysis. It develops mostly in type 1 diabetic patients but can occur in type 2 diabetic patients due to a precipitating factor eg., infection or myocardial infarction (Edwards et al., 1995).

Treatment of DKA consists of insulin therapy, rehydration and correction of the metabolic acidosis and electrolyte imbalance as well as treatment of the precipitating cause (Edwards et al., 1995).

- **Hyperosmolar non-ketotic coma (HONK)**

Patients with type 2 diabetes mellitus have partial insulin deficiency. The anticatabolic effect of insulin may be relatively well preserved whilst its anabolic effect is impaired. In this setting lipolysis is not markedly accelerated and ketone body concentrations remain relatively normal despite severe hyperglycaemia. Insulin therapy and fluid replacement form the basis for management of hyperosmolar non-ketotic coma (Edwards et al., 1995).

- **Hypoglycaemia**

Hypoglycaemia is defined as an arterial blood glucose level less than 2.2 mmol/L but in practice the diagnosis is made on the basis of venous blood glucose levels in association with symptoms of hypoglycaemia.

Factors predisposing to hypoglycaemia are high insulin levels, deficiency of counter-regulatory hormones, hypothyroidism, liver damage, intense exercise, and prolonged starvation. Drugs and toxins which may cause hypoglycaemia include ethanol, pentamidine and salicylate overdosage especially in children.

The management of hypoglycaemia involves an oral glucose load followed by a low glycaemic index (GI) snack for mild episodes and intramuscular glucagon or intravenous dextrose for severe episodes (SEMDSA, 2002).

2 Pharmacology of agents used for the management of Diabetes Mellitus

The pharmacological management of diabetes has undergone unprecedented expansion. The increasing number of anti-diabetic agents will ensure that a larger number of patients achieve glycaemic control, but the adverse effects, cost of therapy, ease of administration and urgency for blood glucose normalization will govern their use (Chehade and Mooradian, 2000).

Table 8 provides a list of pharmacological agents for the management of type 2 diabetes mellitus.

Table 8: Pharmacological agents used in the management of patients with type 2 diabetes mellitus (Chehade and Mooradian, 2000)

- Sulphonyureas
  - First generation
    - Acetohexamide
    - Chlorpropamide
    - Tolazamide
    - Tolbutamide
  - Second generation
    - Glimepiride
    - Glipizide
    - Glibenclamide
    - Gliclazide
    - Gliquidone
    - Glisoxepide
    - Glibornuride
- Biguanides
  - Metformin
  - Phenformin (Withdrawn)
- Thiazolidinediones
  - Troglitazone (withdrawn)
  - Pioglitazone
  - Rosiglitazone
- α-Glucosidase inhibitors
  - Acarbose
  - Miglitol
  - Voglibose
- Meglitinide analogues
  - Repaglinide
  - Nateglinide
- Bodyweight-reducing agents
  - Anorectics
  - Lipase inhibitors
- Insulin
  - Regular
  - NPH
  - Semilente
  - Lente
  - Ultralente
- Insulin analogues
  - Insulin Lispro
  - Neutral protamine lispro (NPL) insulin
  - Insulin aspart (Novorapid)
  - Insulin glargine (Lantus)
- Amylin agonists
  - Pramlintide
- Glucagon-like peptide 1 (7-36)-amide
- Glucagon antagonists (in early development phase)

Mechanism of Action of the Different Therapeutic Agents

Table 9 provides the general mechanisms of action of various anti-diabetic agents (Chehade and Mooradian, 2000).

Table 9: Mechanism of Action of the Different Therapeutic Agents

Mechanism	Drug groups
Prolongation of glucose absorption	α-Glucosidase inhibitors, glucagon-like peptide-1, amylin analogues
Reduction of hepatic glucose output and enhancement of insulin effect	Biguanides
Enhancement of insulin sensitivity	Thiazolidinediones
Stimulation of insulin release	Sulphonyureas, meglitinide analogues
Insulin replacement	Insulin formulations and analogues

2.1 Insulin and Insulin Analogues

Several studies have shown that insulin therapy improves peripheral insulin sensitivity and decreases endogenous glucose production in subjects with type 1 as well as type 2 diabetes (Yki-Jarvinen and Koivisto, 1984; Lager et al., 1983). Even short-term intensive insulin therapy for 2–3 weeks using large amounts of insulin per day to attain normoglycaemia, ameliorates both peripheral insulin resistance as well as endogenous glucose production quite substantially (Scarlet et al., 1982; Garvey et al., 1985). Interestingly, these beneficial effects were maintained after withdrawal of insulin therapy for at least 2 weeks (Andrews et al., 1984). Furthermore, the study by Garvey et al., (1985) suggests that second-phase insulin secretion is enhanced approximately 6-fold after restoration of normoglycemia using continuous subcutaneous insulin infusion, while first-phase insulin secretion was not significantly affected. These results indicate that short-term restoration of normoglycemia is able to reduce the detrimental effects of glucose toxicity on both peripheral glucose disposal and hepatic glucose production, as well as insulin-secretory capacity.

The Veterans Affairs Cooperative Study on Glycaemic Control and Complications in type 2 diabetes (VACSDM) showed that intense stepped insulin therapy is effective in maintaining near normal glycaemic control for greater than 2 years in type 2 diabetic patients whose glycaemia could not be controlled with oral hypoglycaemic agents (Abraira et al., 1995).

The Kumamoto Trial showed that intensive insulin therapy not only improved glycaemic control but also prevented the onset and progression of microvascular complications (Okhubo et al., 1995).

The UKPDS provided additional support for intensive therapy by demonstrating that macrovascular as well as microvascular complications could be minimized by such therapy (UKPDS 33, 1998). The findings of the UKPDS 33 (1998), regarding intensive therapy are:

- Diet therapy alone inadequate in two thirds of patients
- Pharmacologic therapy plus nutrition/exercise is necessary
- No threshold for HbA_{1c} reduction in reducing complications
- Insulin does not increase macrovascular disease

The DCCT (1993) demonstrated that the use of a wide range of insulin preparations can be used for intensive insulin therapy. A summary of the findings of this study are presented below:

- Insulin therapy which resulted in 62% reduction in retinopathy, 56% less progression of kidney disease and 60% less progression of neuropathy.
- The need for tight glycaemic control was emphasised.

Insulin Preparations

Table 10 below lists some of the commonly used insulin preparations and their pharmacokinetic profiles.

Table 10: The various available insulin preparations (Adapted from FDA, 2002)

Type of Insulin	Examples	Onset of Action	Peak of Action	Duration of Action
Rapid-acting	Humalog® (lispro) (Eli Lilly)	15 minutes	30-90 minutes	3-5 hours
	NovoRapid® (aspart) (Novo Nordisk)	15 minutes	40-50 minutes	3-5 hours
Short-acting (Regular)	Humulin R® (Eli Lilly) Actrapid® (Novo Nordisk)	30-60 minutes	50-120 minutes	5-8 hours
Intermediate-acting (NPH)	Humulin N® (Eli Lilly) Protophane® (Novo Nordisk)	1-3 hours	8 hours	20 hours
	Humulin L® (Eli Lilly) Monotard® (Novo Nordisk)	1-2.5 hours	7-15 hours	18-24 hours
Intermediate- and short-acting mixtures	Humulin® 70/30 Humalog Mix® 25 (Eli Lilly) Actraphane® NovoMix® 30 (Novo Nordisk)	The onset, peak, and duration of action of these mixtures would reflect a composite of the intermediate and short- or rapid-acting components.		
Long-acting	Ultralente® (Eli Lilly)	4-8 hours	8-12 hours	36 hours
	Lantus® (glargine) (Aventis)	1 hour	none	24 hours

An insulin regimen must be tailored to the individual needs of the patient. Hypoglycaemia remains the most common adverse effect of insulin therapy and is due mainly to erratic meal timing, excessive insulin dosage or unplanned exercise (Buse, 1999).

The currently available insulin preparations fail to reproduce insulin patterns that closely mimic physiological responses.

Regular insulin is not fast - or short-acting enough, and the need for it to be injected 30 or 60 minutes before a meal impacts on a patients lifestyle.

Insulin lispro was developed to overcome some of the limitations of regular insulin. It acts within 10 to 20 minutes, peaks around 1 to 2 hours, and is cleared from the system within 4 to 5 hours. It has been shown to improve postprandial hyperglycaemia and to reduce hypoglycaemic episodes (Garg et al., 1996). The overall glycaemic control, as measured by HbA_{1c} levels, was not different when patients were taking lispro insulin as compared with regular insulin (Melki et al., 1998).

Studies have shown that insulin aspart, another rapid acting insulin analogue, shows favourable effects on postprandial hyperglycaemia with the added advantage that the insulin can be administered at the beginning of the meal. This advantage, however, is offset by the 10-20% variability of action of insulin aspart (Home et al., 1998).

Insulin glargine, when injected once a day, demonstrates a constant peakless profile over a 24-hour time period (Linkeschowa, 1999).

2.2 Oral hypoglycaemic drugs

2.2.1 Drugs that delay glucose absorption

α-Glucosidase inhibitors

Clinical effects

This class of drugs primarily targets postprandial hyperglycaemia. It reversibly inhibits the brush border glucosidases and results in a redistribution of carbohydrate absorption from the upper portion of the gut to the extended surface area covering the whole length of the small intestine (Balfour and Mcavish, 1993).

Clinical studies have shown a decrease of HbA_{1c} of 0.5 to 1.0%, a decrease of fasting blood glucose of 10 to 20% and a decrease in postprandial glucose of 30 to 50% (Holman et al., 1999).

Addition of acarbose to type 2 diabetic subjects pretreated with insulin, metformin, or sulphonylureas causes a reduction of HbA1c levels between 0.5 and 0.8%. This beneficial effect seems to last for at least 3 years as has been recently shown by UKPDS (UKPDS 33, 1998). During the last 3 years of this long-term trial, 379 patients were additionally treated with acarbose in a placebo-controlled design. This resulted in a mean reduction of the HbA_{1c} by 0.5% in the group of patients who still took acarbose after 3 years. This significant effect was sustained over the 3 year period.

Adverse effects

Acarbose is well tolerated at low doses (25mg), but at higher doses, symptoms of carbohydrate malabsorption and gastrointestinal discomfort are common (Chiasson et al., 1994).

Major adverse effects associated with acarbose therapy are mainly gastrointestinal including flatulence and abdominal discomfort resulting from malabsorption with consequent increased fermentation of carbohydrates. Depending on the acarbose dosage used (300–900 mg/day), the frequency of gastrointestinal effects was as high as 56–76% (placebo, 32–37%) in early studies. When the new recommendations for the use of of α-glucosidase inhibitors were introduced viz., low starting dose of 50 mg/day, slow increase of dosage over weeks to a maximum dose 100 mg three times daily, the incidence of gastrointestinal adverse effects were reported to be as low as 7.5%. Furthermore, it has been shown that the incidence of gastrointestinal side effects decreased with long-term treatment (Chiasson et al., 1994).

2.2.2 Insulin sensitizers

Biguanides

Clinical effects
Metformin alone

Metformin, the most commonly used biguanide, reduces hepatic gluconeogenesis, thereby reducing basal hepatic glucose output while enhancing glucose uptake by the peripheral tissue, mainly skeletal muscle. The precise cellular mechanism of action of metformin is still not entirely understood. While several cellular mechanisms have been described, no single unifying site of action has been identified. Nevertheless, it is generally undisputed that metformin has no effect on the pancreatic β-cell or on stimulating insulin secretion. Mild increases in glucose-stimulated insulin secretion after metformin treatment are thought to be the result of reduced glucose toxicity on the β-cell secondary to improved glycaemic control (Katzung and Karam, 1998).

Metformin has several potential advantages including a favourable effect on plasma lipid profile. There is a decrease in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol levels (Bailey, 1993). A slight increase in the high density lipoprotein (HDL) cholesterol level has been observed. Metformin is often associated with loss of body weight (UKPDS 34, 1998).

UKPDS 34 (1998), showed that metformin is particularly effective in overweight type 2 diabetic subjects. In essentially all clinical studies, the improvement of hyperglycemia with metformin occurred in the presence of unaltered or reduced blood insulin concentrations indicating the potential of metformin as an insulin-sensitizing or insulin-mimetic drug.

Studies have consistently shown that metformin lowered fasting blood glucose levels by about 3.3 to 3.9 mmol/L and HbA_{1c} by about 1.5 to 2 percent (DeFronzo and Goodman, 1995)

Metformin in combination

Metformin is also used in combination with other antihyperglycemic agents. Because of metformin's unique mechanisms of action, a synergistic effect on glycaemic control has been observed in combination with sulphonylureas (Marena et al., 1994), thioglitazones and insulin where a dose-sparing effect was consistently demonstrated (Schneider et al., 1999). Interestingly, in patients in whom sulphonylurea therapy has failed to achieve satisfactory glycaemic control, the combination of bedtime NPH insulin with metformin was found to be most advantageous when compared with other regimens viz., insulin alone, sulphonylurea alone or a combination of insulin and sulphonylurea. A decrease in HbA_{1c} was achieved without significant weight gain in patients on a combination of metformin and insulin (Yki-Jarvinen et al., 1999).

Adverse effects

Mild gastrointestinal disturbances are the most common side effects. Lactic acidosis, although rare, is the most serious side effect of metformin treatment (Katzung., 1998). Selby et al. (1999) showed that in 9,875 patients, only one case of probable lactic acidosis was observed in 20 treatment months. The incidence of lactic acidosis with metformin is 10 to 20 times lower than with phenformin. This is explained by the necessity to hydroxylate phenformin before renal excretion, a step that is genetically defective in 10% of Caucasians (Oates et al., 1983). In contrast, metformin is excreted unmetabolized. In addition, metformin neither increases peripheral lactate production nor decreases lactate oxidation, making lactate accumulation unlikely (Selby et al., 1999).

Contraindications

The contraindications of biguanides, as reviewed by Matthaei et al. (2000) are:

renal disease, hepatic disease, cardiac or respiratory insufficiency, severe infection, alcohol abuse, history of lactic acidosis, pregnancy and the use of intravenous radiographic contrast.

Thiazolidinediones (TZD's)

Examples of the above class of agents include the following: troglitazone (withdrawn), pioglitazone and rosiglitazone.

Clinical effects

These agents increase insulin sensitivity at skeletal muscles and adipose tissue. Their effects are believed to be mediated through selective activation of peroxisome proliferator activated receptor γ (PPAR-γ) [Saltiel and Olefsky, 1996].

TZD's alone

The use of TZDs as monotherapy has been associated with a decrease of HbA_{1c} by 0.5 to 0.8% and when combined with insulin therapy, the drop in HbA_{1c} ranges from 0.7 to 1.4%. Between 20 to 50% of patients may not respond to this class of drugs especially patients with low insulin levels. However, type 2 diabetic patients who are obese and hyperinsulinaemic respond well to TZD therapy (Saltiel and Olefsky, 1996).

TZD in combination

While thiazolidinedione monotherapy is less effective compared with sulphonylureas or metformin, combinations with other forms of pharmacological treatment appear to be more promising.

The addition of various doses of troglitazone (200 – 600 mg) to the sulphonylurea compound Glynase® (glibenclamide) in patients with secondary sulphonylurea failure has been shown to reduce fasting blood glucose by 4.38 mmol/L and HbA_{1c} by 2.65% (absolute numbers). This additive effect with sulphonylureas was also reported for pioglitazone (Schneider et al., 1999).

Reports showing a marked reduction in exogenous insulin requirements in insulin-treated obese patients are in keeping with the concept that thiazolidinediones enhance insulin action. Troglitazone reduced HbA1c by 1.3% below placebo, while insulin dosage was reduced by 30% (Schwartz et al., 1998). Similarly, addition of pioglitazone in insulin-pretreated patients with type 2 diabetes resulted in an improvement of glycaemic control compared to patients treated with insulin only (Rubin et al., 1999).

The combination of thiazolidinediones with metformin also showed significant additive effects (Fonseca et al., 1999). One study suggested that troglitazone improved peripheral insulin sensitivity while metformin preferentially acted on the liver in an insulin-mimetic or insulin-sensitizing way (Inzucchi et al., 1998). However, this study unexpectedly demonstrated that metformin had no effect on glucose production when added to troglitazone therapy.

Adverse effects

Adverse effects of both rosiglitazone and pioglitazone are oedema and fluid retention (Beebe and Patel, 1999)

The most commonly reported side effect with rosiglitazone is upper respiratory tract infection. Pioglitazone has been associated with significant, as yet unexplained, elevations of creatine kinase (Matthaei et al., 2000).

Although early studies showed that troglitazone was generally well tolerated, reversible increases in liver enzymes more than 3 times the upper limit of normal occurred in 1.9% of troglitazone-treated patients compared to 0.6% of placebo-treated patients. At this point the treatment of 20 patients were discontinued because of liver function abnormalities (Watkins and Whitcomb, 1998). However, prescription on a larger scale has led to 43 known cases of severe liver damage associated with troglitazone resulting in 28 deaths (FDA, 1997). It is unclear the extent to which the liver damage in those patients resulted from the drug per se i.e., whether the hepatotoxicity was substance specific (PPAR-γ-mediated) or idiosyncratic. Recently, cases of hepatocellular injuries were reported in patients taking rosiglitazone (Freid et al., 2000). However, the causal relationship is open to question. In large cohorts, transaminases were not found to be significantly higher with rosiglitazone compared with placebo (Salzman and Patel, 1999). On the basis of the available data, there is currently no evidence of hepatotoxicity with pioglitazone.

The use of a combination of thiazolidinedione and sulphonylurea is associated with an increase in body weight to as much as 5.9 kg after 12 months use (Inzucchi et al., 1998).

The potential disadvantage of TZDs is the increased incidence of liver injury (Imura, 1998). Clinical trials suggest that rosiglitazone and pioglitazone may produce less hepatotoxicity than troglitazone, but liver function testing is still recommended. Rosiglitazone, in contrast to troglitazone, does not induce cytochrome P450 3A4 metabolism and is thus likely to have fewer drug-drug interactions (Balfour and Plosker, 1999). It is to be noted that troglitazone has been withdrawn from the market due to hepatotoxicity.

Contraindications

Contraindications to the use of thiazolidinediones include hepatic disease and cardiac failure (Imura, 1998).

2.2.3 Insulin secretagogues

Meglitinide analogues

This is a new class of insulin secretagogue similar to SU's. Their actions are mediated through ATP-regulated potassium channels but via different binding sites on the β-cells. Unlike SUs, they have a "quick-on, quick-off action" that theoretically offers improved postprandial control and reduces the occurrence of postprandial hyperglycaemia.

The meglitinides decrease FBG by 3.3 mmol/L and HbA_{1c} by 1.7-1.9 % compared with placebo. Body weight gain occurs with repaglinide treatment. The risk of inducing hypoglycaemia low, but further therapeutic trials are needed to evaluate their safety and efficacy and to determine their place in therapy (Mayerson and Inzucchi, 2002).

D-phenylalanine derivatives

Nateglinide (Starlix®) which is not a meglitinide, appears to have a faster onset and termination of action than repaglinide but with a reduced efficacy. It reduces postprandial hyperglycemia and it complements the action of metformin and thiazolidinediones in type 2 DM. It is safe and well-tolerated. Hypoglycaemia and related symptoms occur less frequently with nateglinide (0.3%) than with repaglinide (approximately 13%). Its pharmacokinetic profile, β-cell specificity, targeting of postprandial glucose and efficacy, alone and in combination with other agents makes it an ideal agent for mealtime insulin replacement therapy (Kalbag et al., 2001).

Sulphonylureas

A comprehensive discussion on this group of oral hypoglycaemic agents follows later in this Chapter.

Potential agents

Potential drugs under investigation for the treatment of type 2 diabetes include the following :

Glucagon-like peptide-1 (GLP-1)

GLP-1 increases meal-stimulated insulin secretion by binding to GLP-1 receptors on the β-cell membrane. It has insulinotropic effects, glucagon inhibitory effects and it delays gastric emptying. It primarily targets postprandial hyperglycaemia but fasting hyperglycaemia can also be reduced. These peptides should be given parentally or bucally due to their short plasma half-life of less than 5 minutes. More stable agonists are being developed for oral administration. Dipeptidyl peptidase IV (DDIV) inhibitors are also being investigated as potential antidiabetic agents. These agents inhibit the degradation of GLP-1 by reducing its catalysis by DPPIV) (Wiedeman and Trevillyan, 2003).

Vanadium

Vanadium has been shown to have antihyperglycaemic activity in type 2 diabetic patients. Vanadium compounds decrease endogenous glucose production, increase peripheral glucose disposal and reduce lipolysis, but the effect was found to be relatively mild in type 2 diabetic patients (Matthaei et al., 2000).

Etomoxir

Etomoxir, an inhibitor of carnitine palmitoyl transferase 1, has been shown to have antihyperglycaemic activity in type 2 diabetic patients by inhibiting hepatic gluconeogenesis and decreasing plasma triglyceride concentrations. The antigluconeogenic effect is slow to reverse, resulting in difficulties in reversing hypoglycaemia (Matthaei et al., 2000).

Amylin

Amylin is a hormone secreted by the pancreatic β-cells in response to hyperglycaemia. Serum amylin levels are very high in hyperinsulinaemic individuals and very low in individuals with low insulin secretory capacity, for example type 1 diabetic patients. Amylin inhibits gastric emptying and, to a lesser extent, suppresses glucagon secretion. Its short half life and its tendency to aggregate precludes it from being pharmacologically useful. Pramlintide, an amylin agonist, circumvents some of these difficulties and ongoing clinical studies, both in patients with type 1 and type 2 diabetes, indicates that it significantly reduces postprandial hyperglycaemia (Matthaei et al., 2000).

2.3 Treatment of Type 2 Diabetes Mellitus

Four major studies (DCCT, VASCM, UKPDS, Kumamoto) have demonstrated that intensive therapy decreases both macro and microvascular complications. The goals of current management should therefore not only address glycaemic endpoints but also control risk factors i.e., hyperglycaemia and hyperinsulinaemia, for the prevention of complications. The challenge is to optimize glycaemic control with the minimum number of available agents at the least possible cost.

2.3.1 Therapeutic Goals

Various therapeutic goals have been developed for the management of type 2 DM. These are represented in the following tables. In South Africa, the therapeutic goals are similar to that of the American and European guidelines as listed in table 11, 12 and 13 respectively. It is noted that the acceptable limits are set at a higher level for South Africa (SEMDSA guidelines, 2002). This will have consequences on the development of complications based on the major trials mentioned above and the study by Ousman and Sharma (2001). While aggressive and stringent glycaemic control is the ideal, economic and financial constraints are a barrier to this goal in developing countries like South Africa (SEMDSA guidelines, 2002).

Table 11: Therapeutic goals for the management of diabetes in South Africa (SEMDSA guidelines, 2002)

Parameter	*SEMDSA/ SAMJ*	
	Optimal	*Acceptable*
FBG (mmol/L)	4-6	6-8
PPG (mmol/L)	5-8	8-10
BMI (kg/m²) male	20-25	25-27
BMI (kg/m²) female	19-24	24-26
Waist (cm) male	---	<94
Waist (cm) female	---	<82
HbA_{1c} (%)	Normal	<2% above
BP (mm Hg)	<140/90	140/90-160/95
Cholesterol (mmol/L)	<5.2	5.2-6.5
HDL (mmol/L)	>1.1	0.9-1.1
Triglycerides (mmol/L)	<1.7	1.7-2.2

Table 12: Therapeutic goals for the management of diabetes in the USA

American Diabetic Association (ADA)	
HbA_{1c}	7%
LDL-cholesterol	<2.6 mmol/L
BP	<130/80
BMI	<27 and ideally <25
FBG	<4.4-6.7 mmol/L
Bedtime glucose 5-7.78 mmol/L	

Table 13: Therapeutic goals for the management of diabetes as proposed by the European Diabetes Policy Group (1999)

Microvacsular risk	Low risk	Arterial risk
HbA_{1c} (%)	6.5	>6.5
>7.5		
Venous blood glucose		
Fasting/preprandial	6.0	>6.0
7.0		
Self monitored blood glucose		
Fasting/ preprandial	5.5	>5.5
>6.0		
Postprandial (peak)	<7.5	7.5
>9.0		
Lipids	Low risk	at risk
high risk		
Total serum cholesterol	<4.8	4.8-6.0
>6.0		
LDL cholesterol	<3.0	3.0-4.0
>4.0		
HDL cholesterol	>1.2	1.0-1.2
<1.0		
Triglycirides	<1.7	1.7-2.2
>2.2		
*All values are in mmol/L		
Blood pressure (mm Hg)	Low risk <140/85	

Measures of Glycaemic Control

- **Blood glucose monitoring**

The development of test-strips suitable for self-monitoring of blood glucose levels has revolutionised diabetes management. Most test-strips contain glucose-oxidase and a dye which reacts with hydrogen peroxide generated by the oxidation of glucose in the blood sample. The colour generated can be read visually against a colour chart or electronically by a reflectance meter. Generally, blood glucose readings can be made at different time-points on different days so that a pattern is established. This is particularly helpful in optimizing insulin doses for type 1 DM. In type 2 DM, fasting values are useful but self- monitoring should also include postprandial and random blood glucose levels. Patients should always test whenever they feel unwell, and during the night if nocturnal hypoglycaemia is suspected. The accuracy of self blood glucose monitoring depends on accurate technique as well as the type of reflectance meter used.

- **Glycosylated (Glycated) haemoglobin and fructosamine**

Glucose reacts with the valine residue on the β-chain of the adult haemoglobin (HbA) and the resulting glycosylated haemoglobin (HbA_{1c}) can be separated from native haemoglobin and measured by electrophoresis. HbA_{1c} is a stable, clinically useful subcomponent of HbA and is a measure of the average glycaemia during the life-span of the red blood cell viz., glycaemic control over the preceding 120 days.

Non-diabetic HbA_{1c} values are about 5-6% of total HbA (SEMDSA 2002). HbA_{1c} measurements are a useful index of medium-term glycaemic control but are invalidated if red-cell turnover is disturbed or if abnormally migrating haemoglobin variants are present.

Serum albumin is also glycosylated and can be measured by the 'fructosamine' reaction. Albumin turnover is faster than haemoglobin and therefore fructosamine indicates mean glycaemia during the preceeding 1-2 weeks. Current assays, while cheaper than those for glycosylated haemoglobin, are unreliable and less reproducible (Fluckiger et al., 1987).

The Impact of Glycaemic Control on Complications

Ousman and Sharma (2001) demonstrated that the incidence of clinical complications of type 2 DM was significantly associated with glycaemia viz. that there is a direct relationship between the risk of complications of DM and the degree of hyperglycaemia over time. These investigators also showed that there was no threshold of glycaemia above which the risk of complications no longer increased, nor was there a threshold below which the risk no longer decreased. The risk of each of the complications evaluated rose with increasing mean HbA_{1c}. In particular, at near-normal HbA_{1c}, the risk of myocardial infarction (MI) was 2-3 fold that of microvascular complications.

Ousman and Sharma (2001) also showed the dramatic decrease in the risk of complications for every 1% reduction in mean HbA_{1c} as follows:

- 21% reduction in death related to DM
- 21% reduction in all-cause mortality
- 37% reduction in microvascular complications
- 43% reduction in amputations
- 43% reduction in death from peripheral vascular disease

2.3.2 Management of diabetes mellitus

Non-pharmacological Management

Theoretically, diet, exercise and lifestyle modification can prevent the progression to, and of, type 2 DM if the decline in insulin secretion can be prevented. If these measures are insufficient to prevent the early emergence of postprandial hyperglycaemia then therapy should be initiated as described in the text below.

Pharmacological Management

Type 1 diabetes is characterized by an absolute deficiency of insulin. Pharmacotherapy is thus aimed at replacing this deficiency with exogenous insulin. This thesis will concentrate on the management of type 2 diabetes mellitus.

Type 2 diabetes is characterized by a relative deficiency of insulin and insulin resistance. Pharmacotherapy is aimed at increasing secretion of insulin from β-cells, improving insulin sensitivity and delaying the absorption of carbohydrates. In the evolution of type 2 diabetes there is a progressive rise and a subsequent decline in insulin secretion. With these changes in insulin secretion, there is an initial increase in postprandial hyperglycaemia followed by fasting hyperlycaemia.

There are many pharmacological approaches to the management of DM, each based on mechanism of drug action, patient characteristics and glycaemic targets.

Monotherapy

The relative efficacy of the oral hypoglycaemic agents used as monotherpy are presented in table 14. The sulphonylureas and metformin appear to produce the greatest benefit in terms of HbA_{1c} reduction and decrease in fasting blood glucose. Other oral agents tend not to differ markedly in their individual efficacies. Since there is little to choose between them, the selection of any individual OHA would depend on cost, patient profile and side effects.

Table 14: Relative efficacies of oral Monotherapy Agents (Medical Association Communications, 2001)

Monotherapy	Reduction of HbA_{1c} (%)	Reduction of Fasting Blood Glucose (FBG) (mmol/L)
Sulphonylurea	1.5-2	3.3-3.85
Metformin	1-2	3.3-4.29
Pioglitazone	0.6-1.9	3.02-3.3
Rosiglitazone	0.7-1.8	3.02-3.3
Repaglinide	0.8-1.7	1.65-2.2
Acarbose	0.5-1	1.1-1.65

The UKPDS 34 (1998) described a 7% secondary failure rate with monotherapy. Primary and secondary failure with sulphonyureas have been discussed. Patients who do not reach the goal of normoglycaemia on monotherapy should be introduced to combination therapy to achieve target control.

From a practical point of view, fasting and postprandial blood glucose values can be used as a guide to management. Table 15 lists factors predisposing patients to secondary failure to monotherapy.

Table 15: A summary, based on the literature, of predisposing factors to secondary failure to Monotherapy

- Decreasing β-cell function
- Obesity
- Non-adherence to treatment
- Lack of exercise
- Intercurrent illness

Combination Therapy

- **Combination of Oral Hypoglycaemic Agents**

One of the main conclusions from UKPDS 49 (1999) is that combinations of treatments will routinely be needed for type 2 diabetes (Holman et al., 1999). The advantages of combination therapy is that better glycaemic control can be achieved with a combination of two drugs that work at different sites. In addition, there are fewer side effects with lower doses of two different drugs than with a large dose of one drug. Furthermore, if these two drugs are combined in one form, e.g., Glucovance®, a combination of glibenclamide and metformin, then compliance may be improved. Glycaemic outcomes need to be considered when embarking on combination therapy. The potential benefits of combination therapy is presented in table 16 below.

Table 16: Glycaemic outcomes of combination therapy (De Fronzo et al., 1999)

Regimen	Reduction in HbA_{1c} (%)	Reduction in Fasting Blood Glucose (mmol/L)
Sulphonylurea and metformin	1.7	3.60
Sulphonyurea and troglitazone	0.9-1.8	2.78-3.33
Sulphonyurea and pioglitazone	1.2	2.78
Sulphonylurea and acarbose	1.3	2.22
Repaglinide and metformin	1.4	2.22
Pioglitazone and metformin	0.7	2.22
Rosiglitazone and metformin	0.8	2.78
Insulin and oral agents	Open to target	Open to target

Bell and Ovalle (2000) demonstrated that patients who did not achieve glycaemic control on SU monotherapy and had been initiated on insulin therapy, could be transferred back to oral combination therapy with metformin and a SU. The successfully converted patients were better controlled on a combination regimen than on insulin alone. On the basis of this study, it was concluded that adequate glycaemic control can be maintained for an average of 7.8 years on combination therapy following failure using SU monotherapy.

The success of glibenclamide-metformin combinations has been confirmed by other clinical studies. Erle et al. (1999) studied 40 type 2 diabetics who received combined glibenclamide (5mg, 7.5mg or 10 mg/day) plus metformin (800mg, 1200mg or 1600 mg/day) as preconstituted, fixed combinations, or glibenclamide alone (5mg, 10mg or 15 mg/day). Metabolic control was achieved with fixed combinations of low-dose glibenclamide plus metformin, compared to higher doses of glibenclamide alone. The FDA, in the USA, and the Medicines Control Council (MCC) in South Africa approved the use of Glucovance®, a combination of glibenclamide and metformin (1.25 mg/250 mg; 2.5 mg/500 mg; 5mg/500 mg) for use in type 2 DM. Xixing et al. (2001) who added rosiglitazone to SU in Chinese type 2 diabetics previously not controlled by SU alone, demonstrated a decrease in HbA_{1c} levels (-1.4% to -1.9%).

Combination of rosiglitazone and metformin (Avandamet®) has also received approval by the FDA for the treatment of type 2 diabetes. However, long term, extensive clinical usage will determine its efficacy.

It has been shown that the addition of a TZD after failure of a combination of metformin and a SU reduced HbA_{1c} levels to within therapeutic range (Ovalle and Bell, 1998). This effect is thought to be due to the rejuvenating effect of the thiazolidinediones on the pancreatic β-cells rather than the insulin-sensitizing effect of the thiazolidinediones. The decision to implement a triple regimen must be based on sound pharmacological principles and must be economically viable as an alternative to introducing insulin therapy.

- **Combination Insulin and Oral Hypoglycaemic Agents**

Patients who persistently present with a HbA_{1c} greater than 8% and a fasting blood glucose greater than 7.77 mmol/L should be considered to be in secondary failure. Table 17 tabulates the causes and signs of secondary failure from the literature.

Table 17: Causes and signs of Secondary Failure

Signs	FBG > 7.77 mmol/L (>6.66 mmol/L? HbA_{1c} > 8% (>7%?)
Causes	Decreasing β-cell function Nonadherence to treatment Obesity Insufficient exercise Intercurrent illness

In a multicentre study (eight centres) 826 patients with type 2 diabetes were randomized shortly after diagnosis to initiate treatment with diet alone, insulin alone, or a sulphonylurea alone. Insulin was added when fasting blood glucose was persistently >108 mg/dL/5.99 mmol/L (Riddle et al., 1992). The sulphonylureas used were glipizide or chlorpropamide, possibly because earlier in the trial, glibenclamide was associated with more hypoglycaemic episodes. Insulin was given first as ultralente alone, with mealtime regular insulin added as needed. Both pharmacotherapeutic regimens out performed diet alone. However, the regimen mandating the timely addition of insulin to a sulphonylurea proved superior to insulin alone. The median HbA_{1c} over 6 years was 0.5% lower with the progressive regimen and was associated with similar weight gain but less hypoglycaemia compared with insulin alone. The advantage in glycaemic control was quantitatively very similar to that seen in smaller and shorter studies which also compared sulphonylurea/insulin therapy with aggressively titrated insulin monotherapy (Riddle et al., 1992).

Oral hypoglycaemic agent-insulin combinations are still not very widely used because such combinations have been hindered by lack of a clear physiologic rationale. It has been proposed that injected long-acting insulin can improve overnight glucose control sufficiently to reduce glucose toxicity and lipotoxicity, thereby allowing a sulphonylurea to maximize its effect of potentiating mealtime insulin secretion. A second proposal is that chronic use of sulphonylureas increases the contribution of endogenous insulin secretion to regulation of basal glucose production, leading to greater glycaemic stability. This effect can reduce the exogenous insulin requirements by 20-50% but cannot be adapted to varying needs especially during exercise, when mobilization of insulin from subcutaneous depots can increase inappropriately (Riddle et al., 1992).

These mechanisms might apply not only to sulphonylureas, but also to metformin and thiazolidinediones, when they are combined with insulin therapy. A recent physiologic study by Yu et al. (1999) of concurrent use of metformin or troglitazone with very intensive, continuous subcutaneous insulin infusion, showed that the insulin dosage declined by 30% with metformin and 50% with the thiazolidinedione, whereas C-peptide levels were unchanged. In these conditions of increased tissue responsiveness to insulin, appropriate modulations of endogenous insulin secretion is more effective in attenuating both increases and decreases of blood glucose. With metformin, there is less risk of hypoglycaemia, while with thiazolidinediones, which act mainly at muscle and fat, a reduction of postprandial hyperglycemia is as important. The ability of metformin to improve the effectiveness of multiple injections of insulin for type 2 diabetes was shown in a small 6-month clinical study by Ales-Santa et al. (1999). In this study, optimized treatment achieved HbA_{1c} levels of 6.5% with metformin plus insulin but only 7.5% with placebo plus insulin. Another advantage of using metformin with insulin is the limitation of the weight gain usually seen with intensive insulin therapy.

One study compared the ability of bedtime NPH insulin versus bedtime glargine to re-establish optimal glycaemic control while one or two oral agents were continued. A preliminary report by Rosenstock et al. (2001) included data from more than 300 patients and pooled data from the two randomized treatment groups. This report concluded that remarkably good control is possible using an oral agent plus basal insulin strategy. Mean HbA_{1c} was reduced from 8.6% at baseline to 6.9% after 18 weeks of titration of insulin dosage.

Therefore in summary, the management of type 2 DM should start with dietary change, exercise and lifestyle modification. Initial and subsequent therapy must consider patient weight, blood glucose levels, symptoms, level of activity, co-morbid illnesses and concommitant medication. Initial monotherapy for the overweight patient may be metformin or a thiazolidinedione. In the case of the lean patient, SUs would be the first choice. Failure of monotherapy would necessitate combination therapies as discussed above. The addition of a third drug should be based not only on benefits in glycaemic control but also on patient acceptance and costs.

3 A review of the pharmacology of the sulphonylureas with particular emphasis on glibenclamide

The potential of the sulphonylamides, the forerunners to the sulphonylureas as hypoglycaemic agents, began with a serendipitous observation by Marcel Janbon. He noticed that patients treated with a sulphonamide for typhoid fever developed symptomatic hypoglycaemia (Feldman, 1985). Further investigations on dogs confirmed that the compound produced hypogylcaemia in fasted normal dogs but not in pancreatectomised dogs. It was therefore concluded that the agent probably stimulated insulin release from islets of the normal fasted dogs and that this insulin was responsible for the development of hypoglycaemia.

The first effective hypoglycaemic sulphonylurea, carbutamide, was introduced into clinical study in Germany in 1955 and later in the United States of America. However, because of the high frequency of toxic effects, particularly on the bone marrow, it was withdrawn in the United States. Thereafter, the first safe and effective sulphonylurea, tolbutamide, was introduced into clinical practice in 1956. Three other sulphonylureas viz., chlorpropamide, acetohexamide and tolazamide were introduced in the next decade as treatment of DM. These four agents are known as the first-generation sulphonylureas [Marble et al., (1897); White and Cambell., (1980); Lebowitz and Melander., (1997)].

Glibenclamide (glyburide) and glipizide, classed as the second-generation sulphonylureas, were introduced into clinical practice in Europe in 1969 and 1973, respectively. Others that make up this group include gliclazide, glimepiride, and gliquidone (see Table 18).

The second-generation sulphonylureas have much greater potency per milligram than their first-generation counterparts. Glibenclamide, which has the generic name glyburide in the United States, became the most widely prescribed sulphonylurea in Europe and worldwide. Extensive studies both in Europe and the United States demonstrated that glibenclamide is an effective and safe medication for the treatment of certain patients with DM (Feldman, 1985). The name glibornuride has frequently, but erroneously, been applied to glibenclamide. In this dissertation the name glibenclamide will be used.

3.1 Chemistry

The sulphonylureas are chemically related more closely to the sulphonamide antibiotics and to a lesser extent to the thiazide diuretics as all three groups are para-substituted arylsulphonamides (Feldman, 1985).

Structural modification of the second-generation sulphonylureas has resulted in a marked increase in hypoglycaemic potency as compared to the first-generation agents. Thus, 5 mg of glibenclamide is equivalent to 1000 mg of tolbutamide, 500 mg of acetohexamide, 250 mg of chlorpropamide and 250 mg of tolazamide (Feldman, 1985). This increased potency is not due to a pharmacokinetic effect of slow metabolic inactivation or renal excretion, but is an intrinsic property of the molecule. Whilst SU's appear to have similar principle pharmacodynamic properties, their clinical effects may differ due to variations in chemical structure causing dissimilarities in selective β-cell binding and thus potency. The 1000:1 potency ratio between the least and the most potent SU parallels the difference in selective binding to β-cells. However, this diversity in potency does not signify a corresponding difference in clinical efficacy (Feldman, 1985). Figure 4 illustrates the chemical structure of glibenclamide.

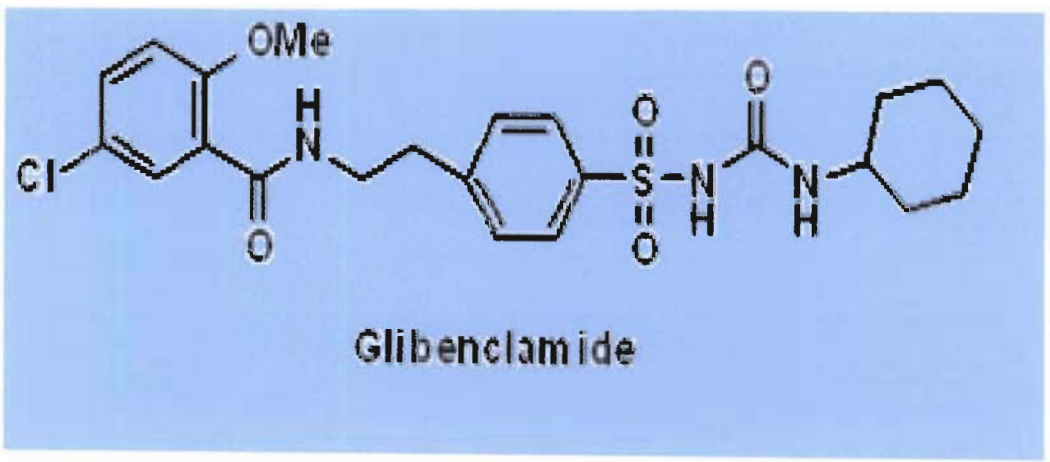

Figure 4: **The chemical structure of glibenclamide (Feldman, 1985)**

3.2 Pharmacodynamics

Mechanism of hypoglycaemic action

The mechanisms of SU-induced hypoglycaemia remain to be fully elucidated. Probable changes in pancreatic insulin and glucagon secretion and peripheral insulin receptor populations are involved. Post-receptor aspects of tissue response to insulin remain to be elucidated and may contribute considerably to the debate. In addition, the regulators of insulin receptor populations and the mediators of insulin responsiveness at the cellular level remain to be clarified.

At present there are currently 3 postulated mechanisms of action for sulphonylureas:

- release of insulin from pancreatic β-cells
- reduction of serum glucagon concentration
- an extrapancreatic effect to potentiate the action of insulin on its target tissues

Insulin release from the β-cells:

Sulphonylureas bind to a specific receptor that is associated with a potassium channel in the β-cell membrane. This binding inhibits the efflux of potassium ion through the channel and results in depolarization. This depolarization opens a voltage-gated calcium channel and results in calcium influx and the release of pre-formed insulin. It must be noted that insulin synthesis is not stimulated and may even be reduced by sulphonylureas. Insulin secretion is only stimulated by sulphonylureas if there is a sufficient mass of β-cells. Thus, a reduction of β-cells may result in inadequate insulin release on stimulation (Katzung et al., 1998).

Reduction of serum glucagon concentration

Chronic administration of sulphonylureas to type 2 diabetic patients reduces serum glucagon concentrations and this may contribute to the hypoglycaemic effect of these drugs. The mechanism of this inhibitory effect on glucagon has not been elucidated (Katzung et al., 1998).

Potentiation of insulin action on target tissues

Evidence suggests that increased binding of insulin to tissue receptors occurs during sulphonylurea administration, but this is a secondary beneficial metabolic effect resulting from reduced glycaemia or low fatty acid levels (Katzung et al., 1998).

Additional effects of Sulphonylureas

- Increased Insulin Action

SU therapy is associated with increased tissue sensitivity to insulin action. This may be a primary effect but also a consequence of the increased access to insulin and the ensuing reduction of hyperglycaemia following increased exposure to insulin (Sartor et al., 1987).

- Reduced Hepatic Extraction of Insulin

Early studies suggest that reduced hepatic extraction of insulin secreted from the pancreas may promote an increased systemic availability of insulin. This has been demonstrated for glipizide and glibenclamide (Groop et al., 1987). This could be a primary or secondary effect as effective diet regulation seems to reduce hepatic insulin extraction. Thus, the effect may be a consequence of glucose reduction and improved insulin action in the liver.

- Effects on Platelets and Fibrinolysis

While several reports suggest that SUs may reduce platelet adhesion and platelet aggregation, it is likely that the anti-platelet effects are secondary to blood glucose reduction produced by such drugs. Glipizide and gliclazide may be able to increase fibrinolytic activity, while chlorpropamide is associated with low fibrinolytic activity (Sartor et al., 1987).

- Effect on Basement Membranes

In subjects with impaired glucose tolerance, glipizide reportedly reversed the increase in membrane thickness that may be a sign of diabetic microangiopathy (Sartor et al., 1987).

- Effect on Insulin Receptor Population

A primary defect in type 2 DM is peripheral insulin resistance (Flier et al., 1979) which is associated with a decreased number of high-affinity insulin binding sites or insulin receptors in peripheral tissues viz., monocytes, adipocytes and erythrocytes.

Beck-Nielsen. (1979) studied 9 patients over a one year period of continuous treatment, 5 with diet alone and 4 with diet plus glibenclamide. The drug treated group showed a significantly greater increase in the number of insulin receptors but this was not dose-related when the dosage was changed at 3-month intervals.

Beck-Nielsen et al. (1979) showed that the short term change in receptor number was similar in both diet- and drug-treated groups, while the responsiveness to insulin increased much more in the drug-treated group. Similarly, blood glucose control appeared to be equally as good following one year of treatment with either drug plus diet or with diet alone, despite significantly higher numbers of monocyte insulin receptors in the drug-treated group.

Summary of mode of action of SUs

SU's bind to receptors in the plasma membrane of insulin-secreting β-cells of the pancreas causing closure of K^+-ATP channels. This gives rise to voltage changes, calcium influx and subsequently, the release of insulin. SU receptors are also found in blood vessels, the heart and brain. While the clinical relevance of these receptors is unclear, there is evidence to suggest that their activation may increase peripheral vascular resistance and exacerbate myocardial ischaemia. Glimepiride seems to be more β-cell selective than glibenclamide and the first generation SUs. In humans, glibenclamide, not glimepiride, attenuates diazoxide-induced vasodilatation (Melander et al., 1998).

The precise mechanism of the long-term pancreatic and extra-pancreatic effect of SUs is uncertain.

Effect of sulphonylureas on glycaemia and microvascular end points

This class of drugs is effective in reducing blood glucose levels in patients with type 2 diabetes and are generally used when exercise and dietary therapy fail to control hyperglycaemia. Research conducted by the UKPDS group revealed that the sulphonylureas, chlorpropamide and glibenclamide, were both more effective than dietary treatment alone, in reducing HbA_{1c} levels in patients with type 2 diabetes mellitus. The median HbA_{1c} levels over 10 years were 6.7%, 6.7%, 7.1% and 7.9% for chlorpropamide, glibenclamide, insulin and dietary therapy, respectively. In addition, the UKPDS group found that intensive treatment with chlorpropamide, glibenclamide or insulin produced a 25% reduction in the risk of microvascular end-points as compared to dietary therapy. There was no observed difference in the effect produced by these 3 agents (UKPDS 33, 1998).

Adverse effects of sulphonyureas

Hypoglycaemia

Sulphonylureas carry the risk of causing severe hypoglycaemia which in turn is associated with the risk of death or serious neurological defect. In the UKPDS (1998), the percentage of patients experiencing major hypoglycaemic episodes per year were 0.7%, 1.0%, 1.4% and 1.8% with dietary treatment, chlorpropamide, glibenclamide and insulin, respectively. The corresponding percentage of patients experiencing any hypoglycaemic episode in these treatment groups were 10%, 16%, 21% and 28%, respectively (UKPDS 33, 1998). Chlorpropamide and glibenclamide carry the greatest risk of severe prolonged hypoglycaemia due to their relatively long duration of action. A decrease in drug elimination that may be due to renal impairment further increases the risk of severe hypoglycaemia (Krentz, 1994).

Hypoglycaemia is an exaggeration of the SUs desired pharmacologic effect. Seltzer, (1972) in a review of drug-induced hypoglycaemia, found that the agents responsible for hypoglycaemia were sulphonylureas (47%), alcohol (37%) and salicylates (3%). Of the 212 cases involving SU therapy, there were 120 for chlorpropamide, 49 for tolbutamide, 21 for carbutamide, 9 for acetohexamide, 7 for glibenclamide and 6 for tolazamide. Hypoglycaemia induced by chlorpropamide required the longest period of glucose infusion before it resolved because of its long half life. The small number of cases of glibenclamide-induced hypoglycaemia probably reflects the caution following the experience with chlorpropamide

Two other studies by Lewis et al. (1975) showed that 1.6% of 5033 patients and 3.1% of 3209 patients receiving glibenclamide experienced hypoglycaemia. In a multicentre study in the United Kingdom, the percentage of patients who developed mild hypoglycaemia were 62% on insulin, 31% on glibenclamide and 7% on chlorpropamide (Multi-center study, 1983). Seltzer (1972) reported hypoglycaemia with glibenclamide use in 6% of 285 patients. In this study, the initial daily doses were 5-15 mg of glibenclamide which were increased to a maximum of 30 mg daily.

A study done in Sweden from 1972 to 1981 showed that of 57 cases of glibenclamide-induced hypoglycaemia that were reported, there were 10 deaths and 22 protracted hypoglycaemic episodes (12-72 hours). Factors identified as contributing to the hypoglycaemia were age greater than 75 years (21% were over 80 years old), and hepatic and renal impairment. The median dose of glibenclamide in the hypoglycaemic patients and a random sample of non_hypoglycaemic patients was comparable viz.,10 mg (Seltzer, 1972).

Cardiac effects

There are concerns regarding the cardiovascular safety of sulphonylureas. Animal experiments have highlighted these concerns. SUs block the ATP-sensitive K^+ channels in the β-cells of the pancreas, but also block the opening of these channels in other tissues, including the myocardium. Glibenclamide is taken up into cells readily and this may give rise to an increased risk of cardiac complications. In addition, glibenclamide has a longer lasting and irreversible effect on membrane potential. Glimepiride is a more selective K^+ channel blocker but its effect on the cardiovascular system remains to be elucidated (O'Keefe et al., 1999)

While the University Group Diabetes Programme (UGDP, 1970) found tolbutamide to produce a cardiovascular mortality rate 2.5 times that of patients treated with diet alone, the UKPDS 33 (1998) group did not show any increase in cardiovascular events with intensive glycaemic control. Berger et al. (1999) concluded that sulphonylureas may exert adverse cardiovascular effects in patients with ischaemic heart disease and hence, did not advocate their general use in this group of patients.

Other adverse effects (O'Keefe et al., 1999)

The first generation sulphonylureas have a low frequency of side effects. These side effects include gastrointestinal discomfort (nausea, anorexia), psoriasis and skin eruptions. Chlorpromide has the highest reported frequency of side-effects (6.2 and 8.5%), followed by glibenclamide (1.5 and 3.6%) and glipizide (1.6%)

The more serious side effects that have been reported with first-generation sulphonylureas include:

- cholestatic jaundice and hepatocellular disease. Liver disease decreases the metabolism of glibenclamide and the recommendation is that the dose of glibenclamide should be reduced (not > 20mg/day).
- red cell aplasia and haemolytic anaemia. Chlorpropamide is implicated more than other first generation sulphonylureas. Serious toxicity associated with glibenclamide has been extremely uncommon.
- Since glibenclamide is chemically related to sulphonamides, cross allergenicity is a possibility.
- Occasionally patients receiving sulphonylureas develop symptomatic dilutional hyponatraemia. This is a drug-induced syndrome of inappropriate antidiuretic hormone seen in 4 -6.3% of patients receiving chlorpropamide, 0.9% receiving tolbutamide and 1.9% receiving glibenclamide. Hyponatraemia is more likely to develop in elderly patients taking chlorpropamide together with a thiazide diuretic.

Hepatic and Renal Disease

Liver disease not only inhibits the metabolism of glibenclamide but also impairs hepatic gluconeogenesis which makes diabetics with cirrhosis more susceptible to glibenclamide-induced hypoglycaemia. Since the literature is sparse on the evaluation of the pharmacokinetics of glibenclamide in patients with liver disease, it is therefore prudent to avoid treating diabetic patients who have significant liver disease with glibenclamide.

The kidneys play an important role in the elimination of several first-generation sulphonylureas, 30% in the case of chlorpropamide (Pettplerre et al., 1972). In the case of glibenclamide which undergoes both hepatic and renal excretion, it is less likely to accumulate in patients with moderate renal impairment. In studies using ^{14}C-labelled glibenclamide or radioimmunoassay (Kuhnie et al., 1982), glibenclamide elimination was found to be unaffected in patients with moderately impaired and normal renal function. This finding was confirmed by Jonsson et al. (1998) who demonstrated that neither glibenclamide nor its two major metabolites, 4-trans-hydroxyglibenclamide and 3-*cis*-hydroxyglibenclamide accumulated in diabetic patients with impaired renal function. The metabolites of glibenclamide, 4-trans-hydroxyglibenclamide and 3-*cis*-hydroxyglibenclamide (Feldman, 1985) have minimal hypoglycaemic activity (0.25% and 2.5%), respectively, after oral administration in the rabbit. The hypoglycaemic activity of these metabolites has not been established in humans.

Neither glibenclamide nor its two metabolites accumulated in DM subjects with impaired renal function (Jonsson et al., 1998). Animal studies of the effect of renal impairment on the pharmacokinetics and pharmacodynamics of glibenclamide do not clarify the picture.

In view of the above evidence, glibenclamide should be used with caution in patients with moderate renal compromise and not at all in patients with severe renal impairment.

Drug Interactions (Marble et al., 1987)
Alcohol

Flushing reactions (disulfiram reaction) with the ingestion of alcohol occur almost exclusively in patients taking chlorpropamide (34%). This phenomenon has not been described in patients on glibenclamide. Possible explanations for this is that glibenclamide does not inhibit aldehyde dehydrogenase or that its plasma concentration is not high enough to inhibit this enzyme.

Alcohol and tolbutamide are both metabolised by hepatic microsomal enzyme systems. Since there is a decrease in plasma half-life of tolbutamide after the ingestion of alcohol, it is more difficult to maintain therapeutic plasma concentrations of tolbutamide in this state. Interactions between alcohol and tolbutamide are not well documented (Feldman, 1984).

Agents that antagonize the hypoglycaemic action

Drugs that may antagonise the action of first generation sulphonylureas, such as thiazide diuretics, glucocorticoids, oestrogens and phenytoin, will probably also antagonise the actions of second-generation sulphonylureas.

Agents that potentiate the hypoglycaemic action (Marble et al., 1987)

Some mechanisms by which drugs potentiate the action of sulphonylureas include:

- displacement of the sulphonylurea from albumin binding sites
- alterations of renal excretion
- hepatic metabolism of sulphonylureas
- insulin-like action on glucose transport into tissues
- stimulation of pancreatic insulin secretion

Drugs that are highly protein bound such as salicylates, warfarin and phenybutazone, will displace sulphonylureas from albumin binding sites. This will result in a higher plasma concentration of the free drug thereby exerting a greater hypoglycaemic effect. The large, non-polar chemical group of glibenclamide prevents it from being displaced from its albumin binding site by ionic agents such as aspirin, dicumarol and phenylbutazone. Caution however needs to be exercised when using these drugs in clinical situations to establish if in fact this interaction does not occur.

Phenylbutazone may potentiate the action of glibenclamide by decreasing the renal excretion of its metabolites without altering its metabolism.

Primary and Secondary Failure

The following patient characteristics are sometimes predictive of a positive clinical response to sulphonylureas:

- The patient is not diagnosed with diabetes until after the age of 40
- Duration of diabetes is less than 5 years
- Patient is close to, or above, his or her ideal body weight
- There has been no prior insulin treatment or insulin requirement for glycaemic control is less than 40 units per day
- The fasting blood glucose is less than 10 mmol/L

Failure of SU therapy can be either primary or secondary.

Primary failure of therapy is defined as treatment failure that occurs in patients who did not ever achieve good control of diabetes with a sulphonylurea. The causes of primary failure include inappropriate patient selection, poor dietary compliance, poor compliance in taking medication and unresponsiveness to the drug (Davidson et al., 1970; Camerini-Davalos et al., 1962).

Secondary failure occurs in those patients who achieve good control of diabetes initially and later lapse into poor control. The causes of secondary failure include those indicated for primary failure as well as temporary metabolic stress. It has been shown that between 25-50% of patients with primary or secondary failure to a first-generation sulphonylurea achieved satisfactory glycaemic control with glibenclamide therapy (Davidson et al., 1970). Some patients on glibenclamide also experience primary or secondary failure.

In a study by Camerini-Davalos et al. (1962) an 18% primary failure rate and a 22% secondary failure rate was noted in patients treated with tolbutamide. It was found that only 3.7% of the patients had true secondary failure after causes such as disregard for diet, poor drug compliance and temporary metabolic stress were eliminated. In another study by Gunderson (1975), of 3500 patients, 8.4% and 4.8% were found to have primary and secondary failure, respectively. Of these, 42% were treated with first-generation sulphonylureas, 9% received phenformin and 49% required insulin therapy. It was therefore concluded that primary and secondary failure rates with glibenclamide are comparable with other sulphonylureas. Overall, between 20-30% of patients treated with SUs may present with primary or secondary failure.

Indications for Sulphonylureas (Marble et al., 1987; Katzung et al., 1998)

SU's, including glibenclamide, are only effective in those diabetic patients who still possess some capacity for endogenous insulin production. SU's should be used in type 2 DM patients when non-pharmacological treatment modalities and the use of non-insulinotropic anti-diabetic agents (acarbose, metformin and thiazolidinediones) are insufficient to achieve therapeutic goals. Insulinotropic agents are not first line drugs in overweight or obese type 2 diabetic patients because they cause further weight gain. Sulphonylureas represent first line drugs in non-obese type 2 diabetic patients whose main pathophysiological problem is impaired insulin secretion. Success is more likely in those type 2 diabetics with little or no tendency to ketoacidosis and who, if they are receiving insulin, would have a requirement of no more than 20, or at most, 30 units of insulin daily (UKPDS 34, 1998).

Contraindications (Marble et al., 1987; Katzung et al., 1998)

SU's are contraindicated in patients with:

- type 1 diabetes
- during acute infections, particularly with fever
- those undergoing major surgery

Clinical comparisons between SUs

The second generation SUs such as glibenclamide, have an increased hypoglycaemic activity, 50 to 100 times more potent on a weight to weight basis than the 1st generation agents. They also offer the advantage of fewer drug interactions.

In a placebo controlled crossover study by Groop et al. (1987) the clinical efficacy of glibenclamide and glipizide were compared over two 6-month periods. While there were great inter individual differences in the final daily dose, the mean final doses were similar (about 15mg for both drugs). Most patients took the medication 3 times daily. This is contrary to the once a day dosing recommended for glibenclamide.

Draeger et al. (1996) compared the efficacy of glibenclamide and glimepiride in the treatment of type 2 diabetic patients. They concluded that both drugs were well tolerated and that glimepiride once daily provided equivalent metabolic control at a lower dosage (1-8mg) as compared to glibenclamide (2.5-20.0mg).

When dosage is individualised as governed by the effect on fasting blood glucose, there is little or no difference in clinical efficacy between sulphonylureas i.e., the SU's are qualitatively similar but quantitatively different.

3.3 Pharmacokinetics

Sulphonylureas differ in intrinsic activity, potency and pharmacokinetics (Lebovitz and Melander, 1997) and are primarily metabolized by the liver and excreted in the urine. Table 18 below outlines the comparative pharmacological profile of the sulphonylurea agents.

Table 18: Pharmacokinetic properties of oral hypoglycaemic drugs (Marble et al., 1987; White and Cambell, 1986)

Drug	Duration (hr)	Dose range (mg)	Protein binding[a] (%)	Plasma half-life (h)	Volume of distribution (L/kg)	Elimination[a]	Contribution of metabolites to activity
Sulphonylureas Acetohexamide	2-24	250-1500	≈75	3.5-11	0.2	Hepatic metabolism to hydroxyhexamide (active; then to dihydroxyhexamide (inactive). Renal excretion of active metabolite.	Hydroxyhexamide more potent than parent drug but only very small amounts remain as this metabolite
Chlorpropamide	24-72	100-500	88-96	24-42	0.15	Variable hepatic metabolism (active metabolites). Renal excretion of unchanged drug (6 to 60%) and less active metabolites	Probably minimal (metabolites eliminated very rapidly)
Glibenclamide[a] GlibenlamideM*	12-24 12-24	1.25-20 1.25-10	99	6-10	0.2	Hepatic metabolism and biliary excretion. Renal excretion of less active metabolites.	trans-hydroxyglibenclamide and 3-cis-hydroxyglibenclamide have low potency (Feldman, 1985).
Glibornuride[a]			95	5-12	0.25	Hepatic metabolism and biliary excretion. Renal excretion of less active metabolites.	Probably nil
Gliquidone[a]	8-10	15-60				Hepatic, renal excretion of inactive metabolites	
Gliclazide[a] Gliclazide MR*	10-15	40-320 30-120	85-97	6-14 12-20	0.25-0.3	Hepatic metabolism. Renal excretion of unchanged drug (< 20%) and metabolites	?

Glipzide [a] Glipizide ER*	16-24 24	40-320	97-99	3-7	0.2	Hepatic metabolism. Renal excretion of inactive metabolites.	Probably nil
Glisoxepide[a]	5-10	2-16	93	1.4-5.3	0.07	Hepatic metabolism and renal excretion of unchanged drug (50% and metabolites.	?
Tolazamide	12-24	100-1000	94	≈7		Hepatic metabolism. Renal excretion of less active metabolites.	Small (6 major metabolites are formed , but only 3 are hypoglycaemic, and mildly so)
Tolbutamide	6-12	500-3000	95-97	4-10	0.15	Hepatic metabolism. Renal excretion of less active metabolites.	Nil (hydroxytolbutamide active but formed in small amounts)
Sulphonamidopyrimidines Glymidine (glycodiazine)			89	2.6-5.6		Hepatic metabolism (active metabolite). Renal excretion of active metabolite	About 15 to 30% excreted as demethyolated metabolite (as active as parent drug)

[a]'Second generations' drug.
ER*- Extended release
MR*- Modified release
M*-Micronised

Absorption

While early formulations of glibenclamide showed bioavailabilities of 45%, current formulations are virtually 100% bioavailable. Peak plasma concentrations are reached within 2 to 6 hours after ingestion in the fasting state. Dietary fibre was shown to decrease the plasma concentration of glibenclamide by 50%. The rate of, or completeness of absorption is not affected by food (Jackson and Bressler, 1981). Kaiser and Forist (1975) suggested that absorption from one formulation follows zero-order kinetics at least in normal subjects. After a single dose, peak serum concentrations in volunteers occurs at about 1.5 hours after oral administration of a tablet (Adams et al., 1982; Groop et al., 1985) and 0.5 to 2 hours after a solution (Pearson et al., 1986). Table 19 summarises the pharmacokinetics of glibenclamide following oral dosage.

Chlorpropamide is the slowest and the longest acting SU, while glipizide is one of the fastest- and shortest acting. Glibenclamide is slower in onset than glipizide because it is more slowly and incompletely absorbed in the non-micronised form. This slower onset is demonstrated when glibenclamide and glipizide are infused at equal rates and at similar plasma concentrations (Groop et al., 1987).

Optimal reduction of postprandial hyperglycaemia occurs if glibenclamide is ingested 30 minutes prior to a meal because of the time required for gastrointestinal absorption. However, the potential danger of hypoglycaemia necessitates that the drug be given immediately before or with meals so as not to compromise compliance. Sartor et al. (1982) reported that the addition of glibenclamide to a standardized breakfast, lunch and dinner would enhance plasma immunoreactive insulin (IRI) concentrations and reduce blood glucose concentrations as compared to meals taken without the drug.

Distribution

The SU's are highly protein bound, mainly to albumin, varying from about 75% for acetohexamide to 99.5% for glimepiride. There are also differences in the volume of distribution (Vd) ranging from 0.07 L/kg for glisoxepide to 0.25-0.3 L/kg for gliclazide 0.13 L/kg for glimepiride and 0.2 L/kg for glibenclamide. Glibenclamide produces fewer protein-binding related drug interactions than the first generation SUs. This is due to lower plasma concentrations and non-ionic binding capacity [Marble et al., (1987); White and Cambell., (1986)].

Metabolism and elimination

All SU's undergo varying degrees of hepatic metabolism and biliary and renal excretion. Tolbutamide, glibenclamide, glipizide and gliclazide are eliminated by hepatic metabolism. Although they can be used with caution in patients with impaired renal function, insulin therapy is preferred to prevent severe hypoglycaemic episodes that have been reported in such patients treated with SU's (Katzung, 1998).

Characteristics of elimination of SUs influence their use in patients with impaired renal function or liver disease. Compounds that are eliminated mainly by hepatic metabolism (tolazamide, tolbutamide, glibenclamide), carry the increased risk of symptomatic hypoglycaemia if significant liver disease is present, particularly if other drugs which inhibit their metabolism are given at the same time. In the case of highly albumin-bound drugs such as tolbutamide, clearance of unbound drug may not change in liver disease and alteration of dosage may not be necessary. Enzyme-inducers on the other hand, can accelerate the metabolism of SUs such as tolbutamide and thus interfere with diabetes control.

Glibenclamide is metabolised almost completely in the liver to two hydroxy derivatives and one unidentified metabolite. The cyclohexyl ring is hydroxylated by the liver to form 4-trans-hydroxyglibenclamide and 3-cis-hydroxyglibenclamide. These compounds possesed only 0.25% and 2.5% the hypoglycaemic potency of glibenclamide, respectively, when evaluated after oral administration in rabbits (Feldman, 1985).

The excretion of these hydroxy metabolites appear to be the same in humans as in rabbits with equal amounts being excreted in the urine and bile. This pattern of excretion (dual route) is unusual as almost all the metabolites of the first-generation SU's are excreted in the urine only.

The elimination half-life varies from 3-5 hrs for glibenclamide to 35 hours for chlorpropamide. The time to steady state varies from 5 to 9 days and therefore dosage adjustment should not be made before this time. Approximately 50% of a dose of glibenclamide is excreted in the urine as metabolites and the remainder in the faeces (Feldman, 1985). The elimination half-life varies from 1.4 to 2.3 hours in healthy subjects (Ings et al., 1981; Matsuda et al., 1983) while the half-life increased to 2.7 hours at a dose of 2.5 mg daily and 2.9 hours at 5 mg daily during long term treatment (Matsuda et al., 1983).

A 'third phase' of elimination of glibenclamide with a half-life of 10 hours in 6 healthy subjects has been reported (Ferner and Chaplin, 1987). They suggested that this may reflect either the elimination of metabolites measured by a nonspecific assay, or the elimination of glibenclamide from the 'deep' compartment. Severe renal impairment (creatinine clearance = 5 ml/min/1.7m^2) in one subject delayed the elimination of glibenclamide, increasing its half-life to 11 hours (Pearson et al., 1986). However, in other subjects with creatinine clearances ranging from 29 to 131 ml/min/1.7m^2, the half-life varied from 2.0 to 5.0 hours.

3.3.1 Summary of Glibenclamide pharmacokinetics

The pharmacokinetic parameters of glibenclamide following oral administration are summarised in table 19 below.

Table 19: Glibenclamide pharmacokinetics following oral administration

Drug	Reference	Subjects	Condition	Dose (mg)	C_{max} ng/mL ± SD	T_{max} h ± SD	$T_{1/2}$ h ± SD
micronised	(Neuvonen and Kistot, 1991) (Kivisto et al., 1993)	healthy	fasting	1.75	117 ± 32	1.8 ± 1.2	0.78 ± 0.45
micronised	(Sartor et al., 1982), (Ayanoglu et al., 1983)	healthy	fasting	2	138 ± 44.3	1.84 ± 0.44	1.35 ± 0.54
non-micronised	(Sartor et al., 1982), (Ayanoglu et al., 1983)	healthy	fasting	2.5	93 ± 60	2.77 ± 0.51	1.97 ± 0.98
Gilemid®		healthy	fasting	2.5	27.9 ± 3.2	3.8 ± 1.7	-
Daonil®	(Ayanoglu et al., 1983), (Yamato et al., 1969)	healthy	food	2.5	66.2 ± 32.4	3.8 ± 1.1	2.4 ± 0.8
non-micronised	[Neuvonen and Kistot, 1991],(Fleishaker and Phillips, 1991)	healthy	fasting	2.5	34 ± 11	3.8 ± 2.1	2.4 ± 5.1
micronised	(Fleishaker and Phillips, 1991), (Sartor et al., 1982)	Type 2	fasting	5	179 ± 82.8	4.9 ± 2.5	8.3 ± 3.9
suspension	(Coppack., 1990), (Fleishaker and Phillips, 1991)	healthy	fasting	5	398 ± 40.9	0.75 ± 0.42	3.3 ± 2.7
Daonil®	(Ings et al., 1981), (Kivisto, 1993)	healthy	fasting	5	292 ±168.5	3.4 ± 1.3	1.8 ± 0.8
Daonil®	(Ings et al., 1981), (Kivisto et al., 1993)	healthy	food	5	292 ± 64	2.6 ± 0.8	1.6 ± 0.3
Daonil®	(Sartor et al., 1982)	healthy	fasting	5	143 ± 33	2.35 ± 1.22	1.59 ± 0.41
solution		healthy	food	5	308 ± 120	2.2 ± 1.1	1.5 ± 1.1
Daonil®	(Compendium, 1994), ((Sartor et al., 1982)	healthy	food	5	189.6 ± 42.9	3.0 ± 0.9	1.8 ± 0.7
Gn®	(Compendium, 1994), (Sartor et al., 1982)	healthy	food	5	85.5 ± 27.9	3.6 ± 0.7	1.8 ± 0.9
Gt®	(Compendium, 1994), (Sartor et al., 1982)	healthy	food	5	50.3 ± 29.7	4.0 ± 1.1	1.9 ± 0.8
Euglucon®	(Yamato, 1969)	healthy	food	5	176 ± 34.8	3.94 ± 1.18	-
Glibenclamide BP	Coppack, 1990	Type 2	fasting	10	241 ± 154	2.1 ± 0.7	-
Glibenclamide BP	Coppack, 1990	Type 2	Food	10	262 ± 152	2.7 ± 0.9	-
Glibenclamide BP	Coppack, 1990	Type 2	Fasting	20	354 ± 93	3.2 ± 2.4	-
Glibenclamide BP	Coppack, 1990	Type 2	Food	20	360 ± 139	3.5 ± 1.3	

Key:

C_{max}- Maximum concentration
T_{max}- time to reach maximum concentration
$T_{1/2}$- half-life

Synopsis of selected pharmacokinetic studies of glibenclamide

Studies on the pharmacokinetics and pharmacodynamics of glibenclamide have increased our understanding of its clinical use in diabetes mellitus. These studies have focused on the use of the drug in different age groups, different ethnic groups and different disease states. A synopsis of some of these studies is presented here.

Jaber et al. (1994) compared the effect of acute dosing with chronic dosing of glibenclamide on pharmacokinetic parameters. They administered 2.5mg glibenclamide as an oral solution to 20 type 2 DM patients aged between 40-70 years. The results are presented in table 20 below.

Table 20: Glibenclamide pharmacokinetic parameters (mean ±SD) at 0, 6 and 12 weeks (Jaber et al., 1994)

Week	Tmax (h)	Cmax (ng/ml)	ke (h^{-1})	$t_{1/2}$ (h)	Cl (L/hr)	Vd (L)
0	1.9 ±1.5	177±75	0.209±0.095	4.0±1.9	3.8±1.3	20 ±9
6	1.1 ±0.9	268 ±240	0.077±0.047	13.7±10.5	2.6±1.3	41±27
12	1.7±1.3	278±146	0.08±0.047	12.2±8.2	3.2±2.1	51±51

Key:
Tmax- time to maximum concentration
Cmax- maximum concentration
Ke- elimination rate constant
$t_{1/2}$- half life
Cl- clearance
Vd- volume of distribution

Significant prolongation in the elimination half-life (4 to 13 hours) and an increased V_d (from 20 to 51L), with a relatively constant CL was observed with chronic dosing. Thus the pharmacokinetics of glibenclamide differs with acute and chronic administration. These changes suggest the possibility of glibenclamide accumulation during chronic dosing with no apparent increase in the incidence of adverse effects. The insulinotropic effect of glibenclamide was maintained during long term therapy, which would exclude the possibility of exhaustion or glibenclamide-induced desensitization of pancreatic β-cells. The authors conclude that these findings support the initiation of glibenclamide at the lowest possible doses. Although this study, published in 1994, reported the merits of initiating glibenclamide at low dose, subsequent studies did not follow through the recommendation. However, a major limitation of this study and hence its conclusion was that it was a single dose study with no dose escalation. The dose escalation study (in this dissertation), attempts to correct this limitation and confirm this finding.

Niemi et al. (2002) studied the role of genetic variability on the pharmacokinetics of glibenclamide in healthy volunteers (Table 21). The pharmacokinetic parameters of glibenclamide were not significantly changed in healthy volunteers with the CYP2C9*1/2* genotype. But in those individuals with CYP2C9*3 allele, the total AUC was 280% that of subjects with the CYP2C9*1 genotype. It can be concluded from the above results that genetic polymorphisms of CYP2C9 markedly affect the pharmacokinetics (AUC and C_{max}) of glibenclamide. This could result in a prolonged and more intense hypoglycaemic activity or action which could result in hypoglycaemia in susceptable individuals.

This study was conducted in a very small number of healthy volunteers and examines the affect of genetic polymorphism/differences on the metabolism of glibenclamide. Extreme differences in pharmacokinetics in patient populations might translate into pharmacodynamic differences. Therefore the individual pharmacokinetic parameters of patients should be examined in relation to pharmacodynamic variability. Generalisation of the results of this study is restricted by the small sample size.

Table 21: Mean (range) pharmacokinetic variables for glibenclamide in healthy volunteers with CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*1/3 or CYP2C9*2/*3 (Niemi et al., 2002)**

	CYP2C9*1/*1 (n=2)	CYP2C9*1/*2 (n=3)	CYP2C9*1/*3 or CYP2C9*2/*3 (n=2)
Cmax (ng/mL)	80.4 (54.7-109.4)	64.0 (46.8-123.0)	136.0 (135.6-136.4)
Tmax (h)	1.0 (1.0-1.5)	1.5 (1.0-1.5)	2.5 (2.0-3.0)
$T_{1/2}$	1.7 (1.5-1.9)	2.0 (1.6-3.0)	2.6 (2.3-2.8)
AUC (0-12) (ng.h/mL)	221.6 (206.3-284.2)	221.1 (154.0-410.8)	594.4 (558.0-630.8)
AUC (0-8) (ng.h/mL)	223.9 (208.1-287.9)	225.3 (155.7-434.8)	626.9 (579.6-674.1)

Key:

Cmax- maximum concentration
Tmax- time to maximum concentration
$T_{1/2}$- half life
AUC- area under the curve

Jonsonn et al. (2000a; 2000b) studied the pharmacokinetics of glibenclamide in Caucasians and Chinese type 2 diabetic patients after oral (2000a) and intravenous administration (2000b) respectively. The aim was to evaluate the role of ethnicity on pharmacokinetics of glibenclamide. The results are presented in table 22 and 23 below.

Table 22: Pharmacokinetic variables of 2.5mg glibenclamide given orally during an oral glucose tolerance test (Jonsonn et al., 2000a)

	Caucasians (n=10)	*Chinese (n=10)*
	Mean (Range)	*Mean (Range)*
C_{max} (ng/mL)	69 (21-153)	82 (41-146)
Tmax (h)	2.0 (1.0-4.5)	4.5 (1.5-6.5)
$T_{1/2}$ (h)	7.09 (2.14-27.8)	4.63 (2.77-11.24)
AUC (ng.h/mL)	440 (200-684)	513 (392-661)

Key:

Cmax: maximum concentration
Tmax- time to maximum concentration
$T_{1/2}$- half life
AUC- Area under the curve

Table 23: Pharmacokinetics after 1.25 mg IV glibenclamide in Caucasians and Chinese DM patients (Jonsonn et al., 2000b)

	Caucasians (n=10) *Mean (Range)*	*Chinese (n=10)* *Mean (Range)*
C_{max} (ng/mL)	376 (309-420)	368 (220-443)
Tmax (h)	2.0 (1.0-4.5)	4.5 (1.5-6.5)
Cl (L/hr)	4.41(3.38-8.11)	4.10 (2.91-5.18)
AUC (ng.h/mL)	283 (154-370)	305 (241-430)
Vss (L)	6.31 (4.68-7.68)	5.49 (4.04-9.55)

Key:
Cmax: maximum concentration
Tmax- time to maximum concentration
Cl- clearance
AUC- area under the curve
Vss- volume of distribution at steady state

There were no significant inter-ethnic differences in the pharmacokinetics of glibenclamide whether given orally or intravenously and the authors concluded that it would be appropriate to employ the same dosage regimens in these two population groups.

In the two studies cited above (Jonsonn et al., 2000a and 2000b), the tmax of glibenclamide was identical in spite of the different routes of administration, namely, oral and intravenous. A comparison of the AUC's (283/440= 0.643; 305/513= 0.594) quoted in these studies provides an estimate of the absolute bioavailabilty of approximately 78%, which is consistant with the literature.

Courtois et al. (1999) investigated the effect of age on the pharmacokinetics of 5 mg single dose glibenclamide (table 24 below) (single 5mg dose) whilst insulin therapy was continued as normal. There was no significant difference in the pharmacokinetics of glibenclamide between the young and the aged group. The half-life of glibenclamide (2.63 ± 0.75 vs 2.78 ± 0.55 hr) between the groups was comparable. The authors concluded that age does not affect the pharmacokinetics of glibenclamide after a single dose.

Table 24: Pharmacokinetic variables for glibenclamide (Courtois et al., 1999)

	Control subjects (42-59 years)	Subjects (71-75 years)
	n=6 (Mean ± SD)	*n=5 (Mean ± SD)*
C_{max} (microg/mL)	0.14 ± 0.01	0.10 ± 0.03
T_{max} (h)	2.58 ± 0.37	2.20 ± 0.34
$T_{1/2}$ (h)	2.63 ± 0.75	2.78 ± 0.55

Key:
Cmax- maximum concentration
Tmax- time to maximum concentration
$T_{1/2}$- half life

The pharmacokinetic of glibenclamide in diabetic patients with impaired (IRF) and normal (NRF) renal function was compared by Jonsson et al. (1998) and is presented in table 25 below.

In this study, a single dose of 7 mg of glibenclamide was administered to 11 diabetic patients with impaired renal function and 11 diabetic patients with normal renal function. It was found that neither glibenclamide nor its two major metabolites, 4-trans-hydroxyglibenclamide and 3-*cis*-hydroxyglibenclamide accumulated in diabetic patients with impaired renal function. AUC and C_{max} of glibenclamide were lower in the IRF group but Cl/f and k_e and k_a were higher (table 25). The significant difference between AUC, Cmax and Cl/f for glibenclamide between the groups may be an indication of higher free fraction of glibenclamide in the IRF group. This would result in an increased metabolic clearance of glibenclamide, resulting in lower Cmax and AUC values in the IRF group. While serum albumin levels were significantly lower in the IRF group, this cannot fully explain the above findings since only one patient had a serum albumin level below 30g/L, a concentration above which minimal alterations in glibenclamide binding occurs. The most probable elimination route for glibenclamide and its metabolites is biliary secretion.

Table 25: Mean (SD) pharmacokinetic variables of glibenclamide in diabetic patients with impaired (IRF) and normal (NRF) renal function after 7 mg orally (Jonsson et al., 1998)

	IRF	NRF
t lag (h)	0.46 (0.16)	0.43 (0.05)
ka (h^{-1})	3.55 (3.03)	2.68 (1.50
Tmax (h)	1.5 (0.5)	1.4 (0.2)
C_{max} (ng/mL)	302 (88)	463 (226)
ke (h^{-1})	0.387 (0.09)	0.307 (0.095)
V/f	17.2 (5.6)	13.4 (5.9)
Cl/f	6.31 (1.30)	3.70 (1.15)
AUC (ng.h/mL)	1153 (241)	2086 (707)

Key:
t lag- lag time
ka- absorption rate constant
Tmax- time to maximum concentration
C_{max}- maximum concentration
Ke- elimination rate constant
V/f- apparent volume of distribution
Cl/f- apparent clearance
AUC- area under the curve

Two studies (Rydberg, et al., 1997; Tracewell et al., 1998) estimated the pharmacokinetic parameters of glibenclamide using non-linear mixed effects model (NONMEM). The study by Rydberg et al. (1997) comprised 8 healthy Caucasians. The aim of the study was to describe the relationship between the serum concentrations of glibenclamide (3.5 mg IVI and oral after 3 months) and its two major metabolites (3.5 mg IVI) and its effects on blood glucose levels. The pharmacokinetic parameters estimated by using NONMEM are presented in table 26.

Table 26: Population pharmacokinetics parameters of glibenclamide in 8 healthy volunteers estimated by NONMEM (Rydberg et al., 1997)

Parameter	*Mean (CV%)*
K_{10} (h^{-1})	1.30 (16%)
K_{12} (h^{-1})	0.447 (15%)
K_{21} (h^{-1})	0.916 (3%)
V_1 (L)	3.63 (17%)
F	0.82 (29%)
Ka (h^{-1})	0.756 (60%)
T lag (h)	0.40 (13%)
EC_{20} (ng/mL)	87 (Parent and metabolites)
$EC_{ss\ 50}$ (ng/mL)	233 (Parent and metabolites)

Key:
K_{10}- elimination rate constant from compartment 1 to 0
K_{12}- elimination rate constant from compartment 1 to 2
K_{21}- elimination rate constant from compartment 2 to 1
V_1- volume of distribution of first compartment
F- bioavailabilty
Ka- absorption rate constant
T lag- lag time
EC_{20}-concentration producing 20% of maximal effect

This study shows that there is no direct relationship between sulphonylurea concentrations and blood glucose lowering effect (Rydberg et al., 1997). NONMEM analysis demonstrated that the longer half life, the lag time effect and the bioactive metabolites of glibenclamide reinforced the once daily dosing with the drug.

Tracewell et al. (1998) investigated the pharmacokinetics of glyburide (glibenclamide) in 51 well controlled type 2 DM patients in the daily dose range of 1.25 to 20 mg (table 27). The objective of the study was to test the hypothesis that inter-subject variability in the dose of the drug is due to patient differences in pharmacokinetics. A one-compartment model with first-order absorption and first-order elimination was used. The data were analysed using NONMEM. The parameters of concern were oral clearance (Cl/f), apparent volume of distribution (Vd/f) and absorption rate constant (k_a). The Vd (43.7L) was larger than reported for most studies. The oral Cl for a 75 kg individual was 3.9 L and 2.9 L, for the older and younger patients, respectively. These values are comparable to those reported elsewhere in this discussion (see above). There were no significant differences in the pharmacokinetics of glibenclamide between obese and non-obese patients.

Table 27: Population Pharmacokinetics of Glyburide in Patients With Well-Controlled Diabetes –NONMEM (Tracewell et al., 1998)

Estimate		*Std Error*
0.244	ka (h^{-1})	0.957
4.58	V(L)	43.7
0.00642	Cl (L/hr/Kg) <60 yrs	0.0387
0.00349	Cl (L/hr/kg) >60yrs	0.0525

Key:

Ka- absorption rate constant

V- volume of distribution

Cl- clearance

The majority of pharmacokinetic analyses yielding PK parameters quoted above were obtained from non-compartmental analysis. The major difficulty with this type of analysis is noted when one attempts to compare results across studies and populations. In only a small number of studies was an integrated data analysis approach utilized [Rydberg et al., (1997); Tracewell et al., (1998)]. These latter types of analyses lend themselves to improved interpretation and the ability to interpolate and sometimes extrapolate to doses that have not been formally tested.

3.3.2 Dose-exposure response relationship investigations for sulphonylureas

There is inadequate information on sulphonylurea dosage and its stimulatory effect on insulin secretion in human studies. There is even less information on the relationship between dose and response i.e., blood glucose lowering effect of SU's.

In the USA, the dose of glibenclamide was increased to up to 20 mg/day if treatment goals were not met. In South Africa it is not uncommon to see doses of 20 mg/day and sometimes greater prescribed. While this practice has little scientific support, it is practised on the assumption of a linear dose-response relationship in the case of glibenclamide.

The usual daily doses range from 2.5 to 20 mg/day. Most patients require between 5 to 10 mg/day with few benefiting from doses greater than 15 mg/day. A dose-related increase in duration of action was seen over the range 1.25 to 5 .0 mg. Once daily dosing equally controls blood glucose as twice daily dosing (Jackson and Bressler, 1981). Since the drug accumulates, dose adjustments should be made not sooner than at two week intervals.

Sartor et al. (1982) noted that ambulatory diabetic patients receiving glibenclamide therapy had a wide variation in serum drug concentrations that was not related to their daily dose or to the degree of control of the patients' hyperglycaemia. Similar observations have been made in ambulatory patients receiving tolbutamide or chlorpropamide (Bergman et al., 1980; Melander et al., 1978). Hospitalised patients receiving glibenclamide and chlorpropamide also have a wide range of serum SU concentration suggesting that poor compliance alone cannot explain this occurrence. It is possible that individual differences in the rate of metabolism may explain this phenomenon. Early studies have found no direct relationship between serum concentration of glibenclamide and its ability to reduce hyperglycaemia (Matsuda et al., 1983).

The maximum effect of glibenclamide does not appear to be different from that achieved with other sulphonylureas (Feldman, 1985). With long term dosing, Sartor et al. (1980) found no correlation between serum glibenclamide levels and fasting blood glucose levels among 37 diabetics. However Balant et al., (1977) noted a statistically insignificant trend towards lower postprandial blood glucose levels after 15 days of glibenclamide treatment than after the first dose of the drug in 6 diabetics.

A long-term study comparing glibenclamide and glipizide showed little or no improvement in blood glucose control at doses greater than 10 mg/day (Groop et al., 1987). In the case of glipizide, dose increases from 15 to 25 mg/day resulted in increased, rather than decreased, blood glucose levels (Wahlin-Boll et al., 1982). The authors concluded that there may be a narrow range of plasma concentrations below which SUs are ineffective and above which there is little additional beneficial effect.

It has been reported that the insulin-releasing effect of glipizide is more rapid than that of glibenclamide when the drugs are infused at the same rate. This suggests that there may be differences in the rates of tissue distribution and/or differences in the rates of binding of these two drugs to SU receptors on the β-cells. This may be clinically relevant because delayed release of insulin following a glucose load is an important factor in the pathogenesis of type 2 DM (Melander et al, 1998).

Groop et al. (1991) examined the relationship between plasma glibenclamide concentrations (after primed continuous intravenous infusion of glibenclamide) and insulin response and glucose metabolism in 9 healthy subjects. They found that glibenclamide increased insulin secretion and glucose disposal during both euglycaemia and hyperglycaemia. This effect was achieved at drug levels of 100-200 nM (0.0494-0.0998 mg/L) corresponding to an oral dose of less than 10 mg of glibenclamide.

Under hyperglycaemic conditions, plasma glibenclamide levels greater than 200 nM did not evoke any additional stimulation of insulin secretion or increase glucose disposal rate. In clinical practice, drug levels of 200 nM (0.0998 mg/L) is usually exceeded in many type 2 DM patients who do not become euglycaemic on low doses of glibenclamide.

Groop et al. (1991) concluded that this concentration of 200 nM corresponded to a maximum effective dose of less than or equal to 5mg glibenclamide. For oral administration, if the bioavailability is assumed to be 50%, then the maximum oral dose would be less than or equal to 10 mg. The bioavailability of the US formulation used in this study is incomplete, probably greater than 50%.

Under euglycaemic conditions, plasma glibenclamide had to be raised from 100 nM to 800 nM to achieve the same insulin response that was achieved by 100 nM glibenclamide under hyperglycaemic conditions. This was an experimental study in healthy subjects which may be difficult to extrapolate to chronic treatment in type 2 DM patients. However, this study demonstrates that the maximum acute effect of glibenclamide is achieved at lower doses than previously thought, due to its narrow range of operation.

Herman et al. (1994) assessed the antihyperglycaemic efficacy of glibenclamide (G) metformin (M) and their combination MG in 165 type 2 diabetic patients. Doses were titrated to a FBG of less than 6.7 mmol/L as the target. Success rates were higher on the MG low dose combination than on monotherapy. When M and G were combined FBG declined with increasing doses of M whereas G exerted most of its effect at low dose. This study lends weight to the effectiveness of low dose glibenclamide in the treatment of type 2 DM.

Jaber et al. (1994) in their investigations with 2.5 mg of glibenclamide, found that, since the insulinotropic effect of glibenclamide was maintained during long-term therapy, exhaustion of the β-cells or glibenclamide-induced desensitization does not occur. The authors conclude that these findings support the initiation of glibenclamide at the lowest possible dose.

In contrast, in a study by Peters et al. (1996) markedly symptomatic patients under 65 years of age were given 10 mg glibenclamide twice daily with a good clinical response. Their mean blood glucose levels fell from 25.4 to 11.2 mmol/l after one week of treatment while HbA_{1c} dropped from 18.1 to 8.1% after 4 months of therapy. It is suggested that the reason for this high dose in these severely hyperglycaemic patients might be due to the blunted absorption caused by pronounced hyperglycaemia.

Rydberg et al. (1997) in their study of the kinetic-dynamic relations after a single dose of glibenclamide in healthy volunteers, derived a parameter that represented the steady-state drug concentration associated with 50% maximal hypoglycaemic effect (CE_{ss50}). The derived CE_{ss50} for glibenclamide was 108 µg (0.108 mg). Rydberg et al. (1997) defined a minimum effective glibenclamide metabolite level at 30-50 ng/ml. They also found a significant decrease in glucose levels down to the 6 ng/ml level for patients given 1.25 mg of glibenclamide. These data suggest that glibenclamide and its metabolites are active at low concentrations.

Jonsson et al. (2001) in their study of glibenclamide in type 2 DM patients, found that during chronic therapy, the glibenclamide-stimulated release of insulin and proinsulin was more pronounced in patients on a low dose (less than 7.0 mg) than those on high dose (10.5 mg or more). They suggested that this was due to less impaired β-cells in those receiving low doses, or a reduced SU sensitivity (down-regulation) in those on high dosage.

To date, the package insert for DiaBeta® (glyburide USP, February 2003) states that the usual maintenance dose is in the range 1.25 to 20 mg daily. The maximum dose, it cautions, should not exceed 20 mg. The package insert for Daonil® (glibenclamide) limits the maximum daily dose to 15 mg.

The preceeding review of the literature illustrates the contrasting views of researchers and the clinical application of glibenclamide dosage. It is this discrepancy which forms the basis of this study.

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Patients and Methods

1 Clinical study methods

1.1 Patients

This study was conducted at the Diabetes Unit at Addington Hospital, a tertiary referral diabetes clinic situated in eThekwini/Durban, KwaZulu-Natal, South Africa. With the advent of democracy in 1994 and the abolition of segregated health care facilities, this predominantly white clinic began to mimick the demographics of the region in racial composition. Hence, it was chosen as a study site. In addition, the clinic has a captive diabetic population. For this dissertation the terminology to describe ethnicity is both colloquail and based on the censors document (2002) where individuals catergorised themselves as black referring to South Africans of African origin, coloured referring to those of mixed ethnicity, Indians referring to those of Indian origin and whites referring to Caucasians (www.info.gov.za/yearbook/2004).

1.2 Study design and study procedure

In this prospective, within subject, dose-escalation, pharmacokinetic-pharmacodynamic study, a minimum of twenty patients was needed to ensure a sample size that would result in an accurate representation of the pharmacokinetic-pharmacodynamic parameters of the study population. From the diabetic population attending the outpatients clinic, 33 after being briefed on the study, voluntarily consented to participate. All participants signed a consent (Appendix 2) and indemnity form (Appendix 3). Subjects were selected and withdrawn on the basis of the criteria listed below. A zero-dose study was performed for each patient.

Inclusion criteria

- Entry level: >20 years of age
- Fasting blood glucose level: > 9 mmol/L
- Signed informed consent

Exclusion Criteria

- Patients on insulin therapy.
- Allergy to sulpha drugs.
- Any contra-indications to multiple blood sampling e.g., poor venous access

Withdrawal Criteria

- Withdrawal of consent.
- Intolerance to glibenclamide e.g., allergy to sulphonamides during study
- Blood glucose level less than 3.5 mmol/L or signs and symptoms of hypoglycaemia during dosage escalation

Study Procedure

This study comprised 6 components:

- V0 baseline determinations
- V1 zero-dose
- V2 2500*µg glibenclamide
- V3 5000µg glibenclamide
- V4 10000µg glibenclamide
- V5 20000µg glibenclamide

*Glibenclamide doses are expressed in mg or µg

Visit 0/Day 0. Enrolment and washout period (V_0)

Baseline biochemistry including lipid profile, FBG, fructosamine, insulin levels, HbA_{1c}, full blood count (FBC), urea and electrolytes (UE) and liver function tests (LFTs) were performed. All oral hypoglycaemic drug therapy was stopped for 14 days viz. the two-week washout period between V_0 and V_1.

Visit 1/Day 14 (V_1)

This zero dose study was necessary to profile insulin and glucose in the absence of glibenclamide. A standardised breakfast to be consumed over a period of 10 minutes was administered. Blood samples (5 mls) were taken to measure the blood glucose and insulin response to this meal at 30 minute intervals for up to 120 minutes post-breakfast. A standardised lunch was administered four hours post-breakfast and blood samples were taken to measure the glucose and insulin response at 30 minute intervals for up to 120 minutes. No drug was administered during this part of the study.

After this zero-dose phase, the subject began the dose escalation component of the study. The starting dose of glibenclamide was 2.5 mg daily for 14 days (commencing on day 15) with escalating doses of 5 mg; 10 mg and 20mg per day at 14 day intervals thereafter.

Visit 2/Day 28 (V2)

On day 28 the subject presented to the Clinic at 7 am after an overnight fast and without having taken glibenclamide. Blood (5 ml) was drawn for FBG, insulin and fructosamine determinations. Glibenclamide 2.5mg was administered with 240 mL of water. This was followed 10 minutes later with a standardised breakfast, to be consumed over 10 minutes. Blood samples (5mls) were taken at the same time intervals as for the zero-dose phase, for insulin, glucose and glibenclamide measurements.

Four hours post-dose, a standardised lunch was administered and consumed over 10 minutes and blood sampling was repeated as for the post-breakfast phase. Thereafter the dose of glibenclamide was increased to 5 mg before breakfast to be commenced the following day (Day 29) for the next 14 days.

Visits 3, 4 and 5 (V_3, V_4, V_5 respectively)

The procedure for visit 2 was repeated with subsequent escalation of glibenclamide doses as indicted below:

Days 29-42: 5 mg/day (V_3) with repeat of PK/PD procedures as performed on day 28.

Days 43-56:10 mg/day (V_4) with repeat of PK/PD procedures as performed on day 42.

Days 57-70 dose 20 mg/day (V_5) with repeat of PK/PD procedures as performed day on 56.

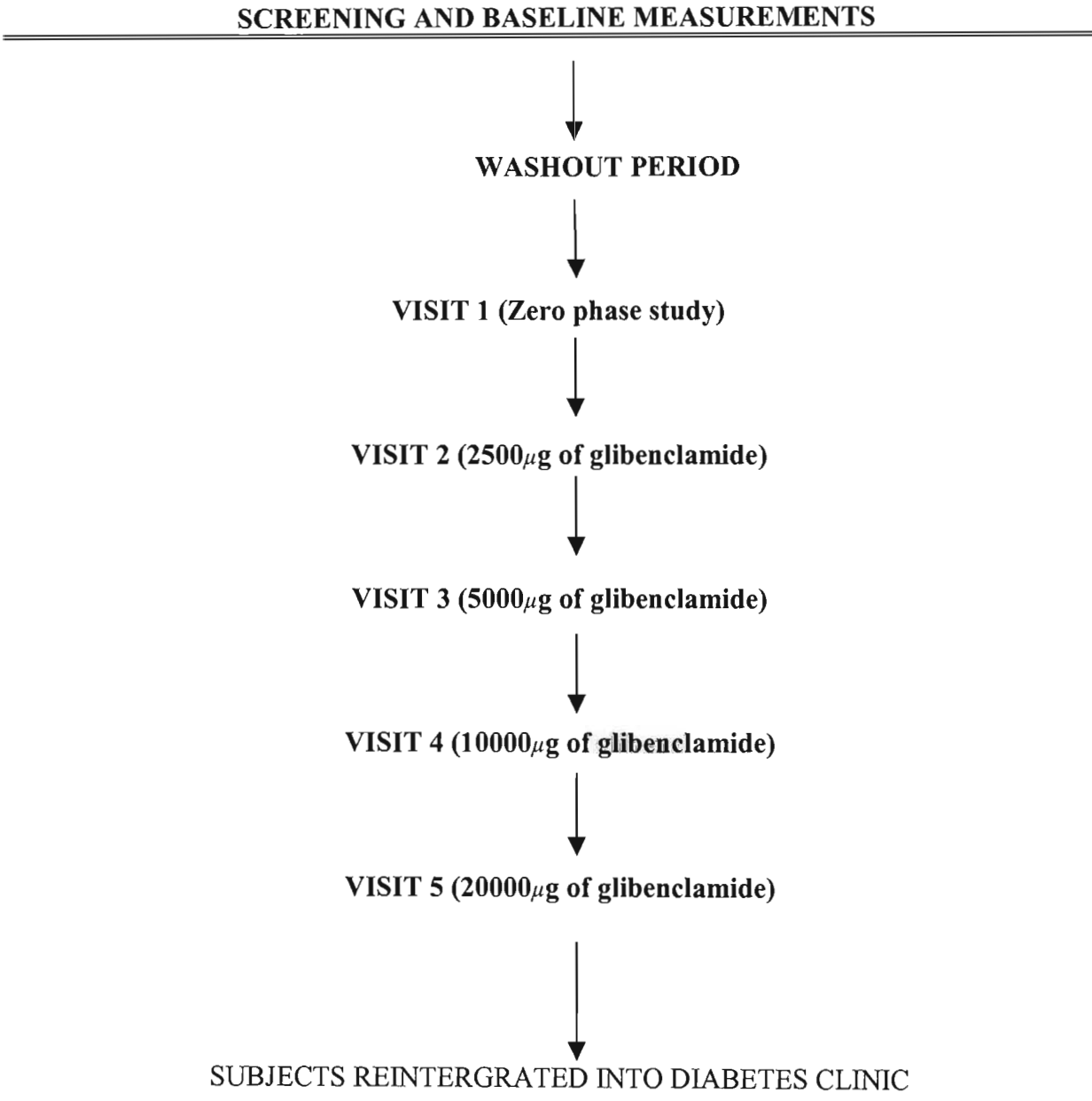
In summary, blood sampling times were: 0, 30, 60, 90, 120 minutes (post-breakfast sampling) and 240, 270, 300, 330, 360 and 420 minutes (post-lunch sampling) on days 14, 28, 42, 56 and 70 for doses 0, 2.5, 5.0,10 and 20 mg, respectively. A synopsis of the dose-dependent study is presented in table 28 and figure 7.

On completion of study all subjects were re-introduced into the pool of patients attending Addington Diabetes clinic for further follow up and continued treatment.

NB: Glibenclamide doses are represented in µg units for purposes of analysis and calculations.

Table 28: Synopsis of study procedures

Visit one (V_1)	Visit two (V_2)	Visit three (V_3)	Visit four (V_4)	Visit five (V_5)
Zero (0)-phase *Dose of Glibenclamide= 0*	*Dose of Glibenclamide= 2500μg*	*Dose of Glibenclamide= 5000μg*	*Dose of Glibenclamide= 10000μg*	*Dose of Glibenclamide= 20000μg*
Drug therapy stopped for previous 2 weeks (washout period)	Overnight fast	Repeat method as for visit two but with 5000μg of glibenclamide	Repeat method as for visit three but with 10000μg of glibenclamide	Repeat method as for visit three but with 20000μg of glibenclamide
Baseline measurements for lipids, FBG[1], fructosamine, insulin, LFT[2], Haematology, HbA_{1c}[3]	Bloods for FBG, insulin and fructosamine	Bloods for FBG, insulin and fructosamine	Bloods for FBG, insulin and fructosamine	Bloods for FBG, insulin and fructosamine
Administration of standardised breakfast	Administration of drug Administration of standardised breakfast	Administration of drug Administration of standardised breakfast	Administration of drug Administration of standardised breakfast	Administration of drug Administartion of standardised breakfast
Blood sampling for insulin and glucose at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours
Administration of standardized lunch	Administration of standardized lunch	Admin of standardized lunch	Administartion of standardized lunch	Administration of standardized lunch
Blood sampling for insulin and glucose at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours	Blood sampling for insulin, glucose and glibenclamide at 30 minute intervals for 2 hours
Subjects leave clinic with 2 week supply of 2500μg of glibenclamide	Subjects leave clinic with 2 week supply of 5000μg of glibenclamide	Subjects leave clinic with 2 week supply of 10000μg of glibenclamide	Subject leave clinic with 2 week supply of 20000μg of glibenclamide	Subjects reincorporated into Diabetes Unit and treated accordingly



*NB: 2 week interval between visits

Figure 5: Flow diagram of study*

Procedural and subject safety considerations

- Subjects were trained to perform daily pre-dose home glucose monitoring using the Haemoglucotest® strips by Roche® diagnostics, and record this in a diary.
- The 20 mg dose was administered as 10 mg in the morning and 10mg in the evening, 10 minutes before meals during the 14 day period between visits.
- Concomitant medication was continued throughout the study period and were itemized for each subject
- Post-meal blood sampling occurred at consistent times for all study subjects.
- All subjects were provided with 24 hour contact telephone numbers of the researcher as well as that of the clinician in charge of the Diabetes Unit.

Compliance

Compliance was assessed by pill counts, examination of diary entries and subject interview. Subjects were contacted telephonically to reinforce compliance and check on progress.

Diet

All subjects were counselled on appropriate diet by the dietician assigned to the Addington Diabetes Unit. Subjects were requested to record and report any major changes in dietary or exercise patterns during the study period.

During the study a standardised breakfast and standardised lunch was provided. The composition of each is presented below:

Standardised breakfast

2 Wheat biscuits (37.5 g Bokomo Weetbix®)

200 mls of 2% low fat milk

250 mls tea /coffee with/without 50 mls of 2% low fat milk

Standardised Lunch

2 slices brown bread with 18 g sweetmilk cheese (Melrose®) per slice with fresh lettuce

250 mls tea /coffee with/without 50 mls of 2% low fat milk

Drugs

Glibenclamide 5 mg tablets - Glycomin ® (Lennon Meds)

1.3 Ethics and confidentiality

Approval to conduct this study was obtained from the Ethics committee of the University of Durban-Westville. Permission to conduct this study was obtained from the Secretary for Health, KwaZulu-Natal Provincial Administration (Appendix 4).

The rights of subjects were honoured and all subject information was treated with the strictest degree of confidentiality. Subjects were identified by their initials only for record purposes. Refusal or withdrawal from the study did not jeopardize the management of the patient. At the end of the study all subjects were incorporated into the patient population of the Diabetic Unit for continued management.

All participants were briefed about the study in English or Zulu. They were given the opportunity to ask questions prior to signing consent forms

2 Bioanalytics

2.1 Glycated Haemoglobin

In vitro tests for the evaluation of glycaemic control over a 90-120 day period is made possible by measuring glycated haemoglobin, a stable minor haemoglobin (HbA_{1c}). It correlates well with blood glucose concentration over this period and hence is used as an objective mesasure of glycaemic control. All HbA_{1c} measurements were performed at the Chemical Pathology Laboratory at Addington Hospital using the ***Cobas Integra*** method (Roche Diagnostics). Glycated haemoglobin was performed only on entry to determine patient suitability for entry into the study. It was not used as a measure of glycaemic control and therefore not performed at the end of the 70 day study period.

Normal range: HbA_{1c} forms approximately 4-6% of total haemoglobin with a lifespan mirroring that of red blood cells (90-120 days).

2.2 Fructosamine

Glycated serum proteins have a shorter half life (19 days) than glycated haemoglobin and therefore provide a measure of short term glycaemic control. Fructosamine was measured in this study because dose adjustments were made every 14 days. Fructosamine is 1-amino-deoxyfructose. It was assayed at the Chemical Pathology Laboratory at Addington Hospital.

Normal range: 5-285 μmol/L. A change in fructosamine level can be used as a short term measure of glycaemic control.

2.3 Glibenclamide assay

After blood samples were collected, they were left to stand for 1 hour prior to centrifugation for 10 minutes at 5000 rpm. They were stored temporarily in an ice-box in a fridge for the duration of the specific study (approximately 10 hours) and then stored at -80°C until transportation by airfreight for assay at the Department of Pharmacology, University of Cape Town.

Glibenclamide was determined using a rapid high-performance liquid chromatography (HPLC). The assay is based on the procedure of Hamid et al. (1989). The process is described briefly. The serum samples were treated with acetonitrile to precipitate proteins. Flufenamic acid was added as an internal standard. After centrifugation, separation and reconstitution, the residue was dissolved and eluted from 5µm Spherisorb C-8 reverse phase column at ambient temperature. The mobile phase consisted of acetonitrile:water (45:55 v/v) at pH 3.7-3.8 and pumped at a flow rate of 2 ml/min. The effluent was monitored at 230 nm. The analysis time did not exceed 12 min. A peak height ratio (glibenclamide/flufenamic acid) and concentration displayed a linear relationship in the range 20-400 ng/ml. A regression equation of $y=0.0035x + 0.015$ ($r^2=0.9999$) was obtained for a typical calibration curve. The detection limit of glibenclamide in serum was 20 ng/ml and the mean recovery of drug from serum samples spiked with known amounts of glibenclamide was 96.77%. Coefficients of variation ranged from 1.6-4.0% (within-day) and 1.4-3.5% (between-day).

2.4 Glucose determination

The finger prick sampling for blood glucose evaluation was used. Blood glucose was measured with an Accutrend Alpha® glucometer. The glucometer was calibrated according to manufacturer's guidelines and the accuracy of the measure of blood glucose was evaluated by the Pathology Laboratory at Addington Hospital. The finger prick method was used because any deleterious decrease in blood glucose levels would be detected early and remedial measures instituted. With laboratory blood glucose assays there was a delay of approximately 2 hours. The accuracy of the Accutrend Alpha® glucose test was confirmed daily against the standard procedure used by the hospital laboratory.

2.5 Insulin assay

Insulin was quantitatively determined in serum at the Chemical Pathology Laboratory of the King Edward VIII Hospital, eThekwini. Serum insulin concentrations were measured with radio-immuno-assay (Pharmacia & Upjohn, Uppsala, Sweden).

The radioimmunoassay was based on a double antibody solid phase technique. The insulin sample was allowed to compete with a fixed amount of an ^{125}I labelled insulin for the binding sites on highly specific antibodies. The concentration of insulin was then determined by comparing its competitive capacity to that of insulin standards of known concentration.

A summary of the procedure as per the manual is described. Serum samples, Insulin ^{125}I solution and anti-insulin solution were incubated at room temperature. A double antibody suspension was added to separate bound and free insulin, followed by incubation at room temperature, centrifugation and decanting. The resulting solid phase pellet was then measured for radioactivity in a gamma counter. The amount of bound radioactivity is inversely proportional to the amount of insulin in the sample.

2.6 Biochemical and other tests

Liver function tests (LFT), urea and elecytrolytes and lipograms were performed at the Chemical Pathology Laboratory at Addington Hospital.

Liver function tests and urea and electrolytes assays were performed on a Synchron CX3 instrument using the Beckman kits and the lipograms were performed on the Synchron CX4/5/7/9 using Beckman kits.

3 Statistical methodologies

3.1 Data Collection and management

All data were recorded on a data capture form and then subsequently captured onto a spreadsheet. All data collected and computed during the course of this study was captured on Microsoft Excel® 2000 for Windows® 98. Entries on the spread sheet were checked for accuracy of data capture.

3.2 Descriptive statistics and statistical comparisons

SPSS® for Windows® was used for computation of descriptive statistics and statistical analyses.

Area under the curve (AUC) and Cmax data were log transformed to obtain normally distributed data. The transformed end points were then compared across groups, using ANOVA for a cross over design accounting for sequence of treatment subject (within a treatment sequence visit period and treatment).

Statistical tests used in this analysis included analysis of variance (ANOVA), independent samples t-test and Pearson correlation. Statistical significance was assumed at the $p<0.05$ level.

3.2.1 Evaluation of clinical benefit due to glibenclamide

Both FBG and PPG determinations were used to evaluate glycaemic control. FBG at time zero for all dose levels was recorded, PPG was determined as the maximum glucose concentration between the time windows 1.8 to 2.5 h (for the post breakfast PPG) and 5.5 to 6.5 h (for the post lunch PPG). These values were compared against the SEMDSA criteria for optimal and acceptable blood glucose concentrations.

3.2.2 Measures of insulin resistance

Indices of insulin sensitivity/resistance derived from fasting glucose and insulin concentrations reflect hepatic insulin sensitivity and basal hepatic glucose production.

The formula for HOMA and QUICKI are:

$$R_{HOMA} = \textbf{Fasting Glucose*Fasting Insulin /22.5}$$

and, QUICKI is: 1/[log fasting insulin + log fasting glucose]

When glucose is measured in mmol/L, the factor 22.5 is used and 405 when glucose is measured in mg/dl. HOMA is an index of insulin resistance (R_{HOMA}) which is the inverse of the corresponding index of sensitivity (S_{HOMA}) i.e., ($R_{HOMA}=1/ S_{HOMA}$). HOMA and QUICKI are related by the following equation:

$$\textbf{QUICKI} = 1/[\log(\textbf{HOMA}) + \log(22.5)]$$

The insulinogenic index is calculated as the ratio of the increment of insulin to that of blood glucose 30 min after glucose load and it provides a parameter of insulin response (Del Prato et al., 2002).

$$\textbf{II} = \frac{\Delta_{\text{Insulin (30-0 min)}}}{\Delta_{\text{Glucose (30-0 min)}}}$$

$$= \frac{\textbf{[30 min insulin level – fasting insulin level (µU/ml)]}}{\textbf{[30 min blood glucose – fasting blood glucose (mg/dL)]}}$$

Del Prato et al. (2002)

A modified II was used in this study, where the $AUC_{0\text{-}30min}$ insulin and $AUC_{0\text{-}30min}$ glucose (after breakfast) were used in place of the change in blood insulin and blood glucose between 0 and 30 minutes. This modified method (which requires confirmation and validation) was used because a precise 30 minute insulin glucose measurement was not always possible. In addition the standardised breakfast served in place of the glucose load. Therefore the modified II formula is:

$$\textbf{II'} = \frac{\textbf{AUC}_{\textbf{0-30min}}\ \textbf{insulin [µU/mL]}}{\textbf{AUC}_{\textbf{0-30min}}\ \textbf{glucose [mmol/L]}}$$

In this dissertation the term II' will be used to represent the modified insulinogenic index as calculated above, unless otherwise indicated.

This is a modified II, because it is a measure of the change in insulin from 0 to 30 min divided by the change in glucose from 0-30 min after a standardised meal. All subjects received the same meals and all subjects are there own controls and therefore this modified II can be used as a measure of insulin response.

3.3 Pharmacokinetic and pharmacodynamic methods

Glibenclamide concentration versus time data was analysed using non-compartmental methods as well as compartmental methods.

The non-compartmental analysis (NCA) was performed using WinNonlin® Professional Version 4.01, 2002 (Pharsight Corporation, California, USA). The computed PK measures and their definitions are listed in Table 29.

Table 29 Non-compartmental pharmacokinetic measures and their definitions

Abbreviation	*Definition*
AUCINF	AUC from the time of dosing extrapolated to infinity
AUClast	Area under the curve from the time of dosing to the last measurable concentration
Cl/f	Total body clearance for extravascular administration =Dose/AUCINF
Clast	Concentration corresponding to Tlast.
Cmax	Maximum observed concentration, occurring at Tmax.
t-half	Terminal half-life
Tlast	Time of last measurable (non-zero) concentration.
Tmax	Time of maximum observed concentration
Vz/f	Volume of distribution based on the terminal phase

Area under the curve (AUC) was calculated using the linear trapezoidal rule. In view of the short sampling period (approximately 8 hours), any non-zero pre-dose sample was substituted as the final 24-hour sample. During graphical exploration of the data, multiple peaks were noted. In view of this, data points for calculation of the terminal slope were manually selected rather than using the default WinNonlin algorithm.

Pharmacokinetic and pharmacodynamic (PKPD) analyses and modeling were performed using nonlinear mixed effects modelling as implemented in the software NONMEM (Globomax LLC, USA and NONMEM Project Group, University of California, San Francisco). A more complete description of the modeling analyses is presented in the Results section.

4 References

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www.info.gov.za/yearbook/2004.

Results and Discussion

1 Demographics

1.1 Introduction

Patients attending the Diabetes Unit at Addington Hospital are mainly African, Coloured and Indian in the age group characteristic of type 2 dabetics.

1.2 Results

Thirty three patients between 39 and 73 years of age, who met the inclusion criteria were screened prior to participation in this study. Of these, 24 consented to participate in this study. The remaining 9 comprised of 2 who declined entry into the study, 5 who did not return for baseline measurements after being screened and 2 who presented histories indicative of poor compliance.

Two subjects did not complete all the dose escalation steps. Subject 16 could not proceed to a dose escalation beyond 5 mg/day due to the presence of symptoms of hypoglycaemia and subject 24 was lost to follow up after the 2.5 mg dose of glibenclamide. Data from these 2 subjects was excluded from the non-compartmental analyses. There were no further withdrawals and no adverse effects were reported.

All 22 subjects who completed the full dose escalation steps were thereafter incorporated into the regular pool of diabetic patients attending the Diabetes Unit. The study population described above is represented in Table 30, Table 31 and in Figure 6 below.

Table 30 Patients screened and enrolled into the study

	Male	*Female*	*Total*
Screened	4	29	33
Enrolled	2	22	24
Lost to follow up	1	0	1
Withdrawal	0	1	1
Completed all dose escalation steps	2 (9%)	20 (91%)	22

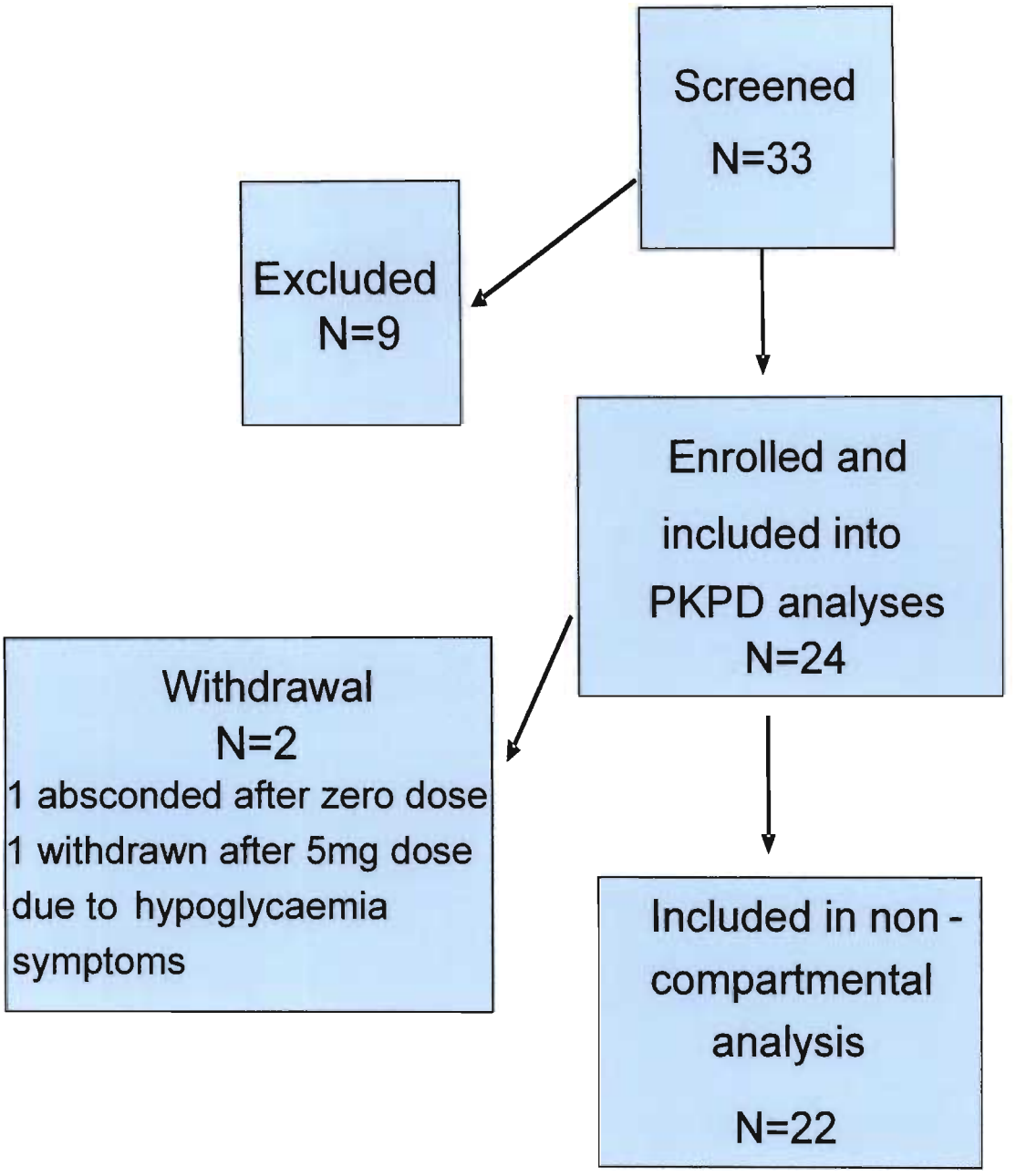


Figure 6: **Flow diagram showing study and analysis population**

Table 31 **Demographic profile of study population (n22) and cohort characteristics at entry into the study**

Variable	*Mean*	*±SD*	*Minimum*	*Maximum*
Age (yrs)	54.1	9.2	39	73
Weight (kg)	71.1	14.1	42.0	107.8
Height (cm)	156.4	8.6	145.0	173
BMI (kg/m^2) Males (n 2) Females (n 20)	 26.48 29.93	 5.64 6.71	 22.49 19.05	 30.46 46.88
FBG (mmol/L)	15.39	3.83	9.90	21.8
FBI (µU/mL)	13.92	6.87	3.0	24.7
HbA_{1c} (%)	12.18	3.83	8.1	18.5
Fructosamine (µmol/L)	466.86	92.49	310.0	680.0
Cholesterol (mmol/L)	5.8	1.2	4.0	8.2
LDL cholesterol (mmol/L)	3.9	0.95	2.2	5.9
HDL cholesterol (mmol/L)	1.1	0.26	0.8	1.8
Triglycerides (mmol/L)	1.6	0.89	0.5	4.0
ALT (U/L)	25	12.6	10.0	59.0
Creatinine (µmol/L)	64.1	10.4	48.0	91.0
Ethnicity	African 6 (27.3%)	Coloured 2 (9.1%)	Indian 14 (63.6%)	
Gender	Female 20 (91%)	Male 2 (9%)		
Duration of DM (years) **Mean Duration of DM (±SD) years**	0-5 6-10 >10 8.95 (4.5)	n 8 n 6 n 8		

The demographics of the study population is shown in Table 31. The age range of the final study cohort was 39-73 years with a mean (± SD) of 54.1 (± 9.2) years, with the majority (20) being female. The race distribution was African 27.3 % (n = 6), Coloured 9.1% (n = 2) and Indian 63.6% (n = 14). The average duration of diabetes in this population was 8.95 (±1.45) years.

The mean (±SD) mass and HbA_{1c} of the study population were 71 (±14.1) kg and 15.39 (±3.83) %, respectively. Based on body mass index, 30 % of subjects were of normal weight, 21 % were overweight and 49% were obese. The mean baseline (±SD) serum creatinine was 64.1 (±10) µmol/L. The total cholesterol, triglycerides, low density lipoproteins, high density lipoproteins was 5.8 (±1.2), 1.6 (±0.89), 3.9 (±.95) and 1.1 (±0.26) mmol/L, respectively. The mean baseline (±SD) ALT was 25 (±12.6) U/L and the mean baseline glucose (±SD) on entry was 15.39 (±3.8) mmol/L. The mean baseline insulin (±SD) was 13.92 (±6.87) µU/mL.

Concomitant disease distribution

Hypertension was present in 50% of the subjects in the study. Of these, 3 subjects had concomitant angina. All other concomitant diseases are outlined in Table 32 below.

Table 32 Concomitant diseases in subjects included in the study

Disease	Number
Hypertension	8
Hypertension with angina	3
Postmenopausal syndrome (PMS)	2
Rhinitis	1
Hyperlipidaemia	1
Systemic Lupus Erythematosis (SLE)	1
Musculo-skeletal	4
Gout	1

Concomitant Medication

The following medications as outlined in Table 33 were used concurrently by the subjects during the study period.

Table 33 Concomitant medications used by subjects in this study

Medication	*Number*
Aspirin	3
Isosorbide dinitrate	3
Indapamide	4
Diltiazem	1
Hydrochlorthiazide	4
Ibuprofen	2
Isradipine	2
Atenolol	2
Calcium gluconate	2
Conjugated oestrogen	2
Beclomethasone aqueous nasal spray	1
Chlorpheniramine	1
Hydrochlorthiazide/triamterine	1
Indomethacin	2
Amlodipine	1
Perindopril	1
Fluvastatin	1
Benzbromarone	1
Imipramine	1
Carbimazole	1
Diclofenac injection	1
Thyroxine	1
Captopril	1

Hydrochlorthiazide was used by 5 subjects (alone or in combination with other therapy) in doses ranging between 12.5 and 25 mg per day. Non-steroidal anti-inflammatory drugs (NSAID's) were used in 7 subjects and one subject was on a lipid lowering drug, fluvastatin.

1.3 Discussion

This **prospective dose escalation study** was conducted in type 2 diabetic subjects. Each subject in the study received the whole spectrum of doses. This within subject dose escalation design has the advantage over **a parallel study** design in that each subject is characterized at each dose level. In parallel design studies average dose responses are used to characterize individual subjects. Therefore parallel study designs do not account for intraindividual differences in dose responses leading to extreme variation in doses.

The **endpoints** in this study were **FBG, FBI, PPG, PPI**, serial glucose and insulin evaluations. Serial insulin and glucose evaluations were performed to obtain a fuller picture of variations and exposure throughout the study period i.e., 8 hours. Ideally, characterization of these variations should be evaluated over a 24 hour period. Additionally, earlier and more frequent sampling for insulin and glucose would have allowed measurements of acute insulin response (AIR). It is recommended that future studies should include this in the study design.

Fructosamine is a measure of glycaemic control in the preceeding 2 weeks and thus served as a measure of glycaemic control with each dose during the two week dosing interval. Therefore, fructosamine serves as a useful barometer when changes need to be considered over a short term i.e., 2-3 weeks. In the case of **HbA_{1c}**, which is the gold standard of glycaemic control, a duration of 2-3 months is needed.

FBG and PPG are relevant measures not only for the study but because they are both appropriate and clinically relevant measures in the management of diabetic patients.

Blood **insulin measurements**, although expensive, are useful in determining the insulin resistance status of the study population. Insulin resistance dictates and guides the rational pharmacological treatment of diabetes mellitus. It may thus be prudent to include insulin measurements in selected patients to guide pharmacotherapy.

Blood sampling was adequate to profile changes in blood glucose, blood insulin and blood glibenclamide concentrations, simultaneously. The sampling process was adequate to describe both fasting and postprandial glucose and insulin profiles. However, the terminal phase of glibenclamide elimination was largely estimated because an inadequate number of samples were taken in the terminal phase. A 24 hour sample may have overcome this limitation. This was not possible as subjects saw this as an inconvenience.

Type 2 diabetes mellitus is one of the most common chronic diseases in the world, affecting 12% of the adult population aged 40-74 years in the United States of America (ADA, 2003). The prevalence of type 2 DM in South Africans rises sharply from **age** 35-40 years and increases with advancing age, with a 3-fold prevalence by age 60 years (Motala et al., 2001). In this study the ages ranged from 39-73 years, which correlates with both national and international studies and shows that type 2 diabetes is mainly a disease of the older patient.

Courtois et al. (1999) investigated the effect of age on the pharmacokinetics of glibenclamide and concluded that age does not affect the pharmacokinetics of glibenclamide after a single 5 mg dose. Therefore it may be concluded that population age in this study is not a contributory factor for pharmacokinetic variation.

The sample population (who completed the dose escalation study) comprised of 91 % (n20) **females** and 9 % (n = 2) **males**. Mayet (2003), Robertson (1998) and Omar et al. (1988) in their studies of diabetes in South African type 2 diabetics, reported that females comprised approximately 76 % of their study populations. As with both local and international studies, females formed the majority (91%) of the final study cohort in this trial.

This study cohort comprised 27% **African** (n6), 9% **Coloured** (n2) and 64% **South Africans of Indian origin** (n14) patients. In South Africa, it is estimated that 5-8% of Africans, 8-10 % of Coloureds, 13-18% of South Africans of Indian origin and 3.5-4% of whites are diabetic (Naicker, 2002; Trutter 1998). The high incidence of diabetes in South Africans of Indian descent has been reported by Motala et al. (2003). In the past few years there has been an increase in the reporting of type 2 DM in Africans due mainly to urbanisation, increasing obesity, and the removal of restrictive apartheid legislation thus allowing African patients the freedom to be treated at previously whites-only state hospitals. The high incidence of type 2 DM in Asians has been well documented by Omar et al. (1988). However, this study population is not truly representative of South African demographics, but closely mirrors the incidence of type 2 DM in the greater eThekwini/Durban area which has the largest population of Indians in South Africa.

The **mean duration of diabetes** (±SD) in this population was 8.95 (± 4.5) years. This is a referral diabetes clinic and therefore *de novo* diabetics are not likely to present. Most patients attending this clinic are established diabetics who require specialized care in the management of their diabetes.

According to studies of **BMI** in South African type 2 diabetics, Kalk (2001) found that 45 % of females and 15 % of males had BMIs greater than 30 kg/m^2, and 44 % of males and 70 % of females had BMIs between 25 and 29 kg/m^2 respectively. Based on these findings, approximately 70% of this study population were overweight and 50% obese.

Reaven (1998) estimated that approximately 80% of type 2 diabetic patients are obese. In the study by Jaber et al. (1994) comparing the pharmacokinetics and pharmacodynamics of short and long term glibenclamide therapy in type 2 diabetes (NIDDM), the mean BMI of the sample population was 31.5 ± 9.2 kg/m^2, similar to this study population (29.1 ± 6.0 kg/m^2). These findings compare favourably with studies by Motala et al. (2001) and are thus a reasonable reflection of the diabetic population in terms of body mass. With increasing BMI, adiposity and age, insulin resistance-related metabolic syndrome increases (Ford et al., 2002). The mean BMI of this study cohort strongly suggests a tendency for patients to present with insulin resistance and has been confirmed by measuring this insulin resistance using the HOMA and QUICKI techniques.

Concomitant disease: hypertension is approximately twice as common in diabetic patients as in the non-diabetic population. Hypertension was present in 50% (n = 11) of the sample population while angina was present in 3 of these hypertensive patients. The high incidence of hypertension as reported by the ADA (1999b) [between 30 and 60 %], is reflected by this study sample. Mayet (2003) in her study of type 2 diabetic patients found that 42 % of type 2 diabetic patients were hypertensive. Hypertension in combination with dyslipidaemia, and obesity has been labelled the 'Metabolic syndrome' or 'Syndrome X', a cluster of clinical conditions due to insulin resistance. Each of these conditions conveys significant cardiovascular risk which becomes substantial when present as such a cluster. This study population is characterised by the whole spectrum of insulin resistance viz., hypertension, dyslipidaemia and obesity, all of which are the metabolic abnormalities associated with type 2 diabetes.

Concomitant medications were used concurrently by the patients throughout the study period. Of these, 8 have been documented to affect blood glucose levels.

Non-steroidal anti inflammatory drugs (NSAID's) were used in 7 subjects. Only one subject was on a lipid lowering drug, fluvastatin, probably due to the fact that the prescription of statins is restricted to patients attending the Ischaemic Heart Disease (IHD) clinic at this study site (Addington Hospital).

The following diuretics were used by the study population: triamterene 50 mg in combination with hydrochlorthiazide 25 mg (Dyazide®), hydrochlorthiazide 25 mg and indapamide 2.5 mg. Hydrochlorthiazide was used alone, or in combination, in 5 subjects in doses ranging between 12.5 and 25 mg per day. Thiazide diuretics have been implicated in inducing glucose intolerance in diabetic patients with an incidence of up to 30 %. High doses (greater than 12.5 mg) are more likely to induce hypokalaemia which has been implicated in decreasing insulin secretion and thereby aggravating the glucose intolerance (Pandit et al., 1993; Luna and Feinglos, 2001). These diuretics are one of the cheapest group of drugs used in the management of hypertension and it is recommended as first line antihypertensive therapy in the African patient (JNC 6, 1997). However, half or quarter of the dose would be more beneficial because, while the antihypertensive effect can be maintained, there would be a decrease in metabolic side-effects (glucose intolerance and hyperlipidaemia) [McNeil and Sloman, 1987]

Although indapamide has been reported to be less likely to induce glucose intolerance, recent reports have suggested that this may not be the case. The substitution of indapamide for thiazides (for the reasons stated above), may not be totally safe as there are reports of metabolic side-effects due to indapamide (Osei et al., 1986).
Atenolol, a beta blocker, may inhibit insulin release leading to glucose intolerance. However, marked hyperglycaemia is not common with beta blockers, although they may inhibit glycogenolysis and cause hypoglycaemia. Added to these conflicting effects, β-blockers mask the symptoms of hypoglycaemia, in particular, tachycardia (Pandit et al., 1993).

The calcium channel blockers (isradipine, amlodipine, diltiazem) generally do not produce severe hyperglycaemia, although the potential does exist (Pandit et al., 1993). These drugs were used for the treatment of concomitant diseases such as hypertension and ischaemic heart disease (IHD) in this study cohort.

Angiotensin converting enzyme (ACE) inhibitors (captopril, perindopril), individually and as a group, have neutral effect on blood glucose in diabetic patients (Pandit et al., 1993). ACE inhibitors are preferred in diabetic patients because of their beneficial effect on renal function and the cardiovascular system (O'Brien and Bulpitt, 1997).

Low dose aspirin, as used by subjects in this study, rarely produces hypoglycaemia. Salicylates in daily doses of 4-6 grams are potent inducers of hypoglycaemia (Pandit et al., 1993).

In animal studies, indomethacin and ibuprofen did not significantly affect glibenclamide induced hypoglycaemia (Sharma et al., 1981). However, in some patients, these NSAIDs were co-administered with thiazide diuretics. This is a pharmacodynamic interaction, where the antihypertensive effect of thiazides can be blocked by NSAIDs due to the inhibition of vasodilatory prostaglandins (Brater, 1985).

The effects of glucocorticoids on carbohydrate metabolism in susceptable patients are dose related and are most often seen with the systemic use of these drugs (Pandit et al., 1993). The use of low dose nasal beclomethasone is less likely to produce this effect because of its low systemic bioavailabilty.

Thyroxine has been reported to increase blood glucose. It may be the result of depletion of insulin reserves and increase in hepatic glucose production (Pandit et al., 1993).

Calcium gluconate, carbimazole, benzbromarone, chlorpheniramine, nitrates, and lofepramine and imipramine, have neutral or no clinical effects on glucose profiles. There are no reports of fluvastatin affecting blood glucose. The role of lipid lowering drugs in a diabetic population has been discussed in the section on lipids above.

The effect of sex hormones on carbohydrate metabolism remain complex and controversial. Natural oestrogens, like conjugated oestrogen (Premarin®), can improve glucose tolerance and insulin action. Oestrogen increases insulin binding in adipocytes, hepatocytes and other plasma membranes (Pandit et al., 1993).

Type 2 diabetes is associated with a cluster of conditions viz., hypertension, insulin resistance, obesity and dyslipidaemia. The pharmacotherapy of these co-morbid diseases in this study shows that each disease was treated independently and to the exclusion of diabetes. A number of the antihypertensive drugs use in this study population are known to affect blood glucose levels, however, the pharmacodynamic interactions of these drugs are not an absolute contraindication for use in DM patients. Fluctuations in blood glucose, although not ascribed to these drugs, are usually treated by adjustment of the dose of the OHAs. This practice may have contributed to the use of high doses of glibenclamide in type 2 diabetic patients in the KZN area.

HbA_{1c} was measured at entry as an inclusion criterion and was not used as a measure of glycaemic control in this pharmacokinetic/pharmacodynamic study of glibenclamide. The mean HbA_{1c} (±SD) in this study population was 12.18 % (±3.83). This is appreciably higher than the recommended acceptable value of 7 % (ADA, 1999). High HbA_{1c} levels are a reflection of poor glycaemic control and has been shown to be a marker for microvascular complications and macrovascular complications which begin at HbA_{1c} of 5% (UKPDS 35, 2000). The risk of complications increases for each percentage increase of HbA_{1c} (UKPDS 35, 2000). A 0.9% reduction in HbA_{1c} significantly reduces long term complications (UKPDS 33, 1998). The major studies (DCCT, UKPDS) target HbA_{1c} to less than 7%. However, this study population was poorly controlled as reflected by the high mean HbA_{1c} levels.

Fructosamine levels are a measure of glucose control over a 2-3 week period, as opposed to HbA_{1c}, which is a measure of glycaemic control over a 2-3 month period. Fructosamine levels are potentially useful when changes in treatment plan need to be evaluated over a short term (2-3 week). Fructosamine levels indicate, in a more timely fashion than a HbA_{1c}, how well changes to a treatment plan are working and whether other changes should be considered. Fructosamine levels do not detect wide swings in blood glucose levels or reflect postprandial glucose excursions (Kennedy and Merimee, 1981).

There was a statistically significant decrease ($p = 0.01$) in fructosamine levels as the dose of glibenclamide increased from 0 to 20000 µg i.e., all doses reduced fructosamine (2.72% and 9.36%) but only the 20000 µg dose produced a significant reduction ($p=0.01$; 9.36%) when compared to 0 dose.

In a comparative study of HbA_{1c} and fructosamine in the diagnosis of glucose tolerance abnormalities, Guillausseau et al. (1990) found that neither were suitable for diagnosis of mild abnormalities of glucose tolerance. Results of this study indicate a statistically significant decrease in fructosamine levels with increasing doses of glibenclamide. However, the decrease of fructosamine from zero dose ranged from 12.72 (2.72 %) to 43.67 (9.36%) µmol/L, which translates to a difference from 0.56 to 1.94 mmol/L of glucose (Bartol, 2000). As the mean fasting blood glucose at entry was 15.39mmol/L in this study cohort, the clinical impact of this change would be negligible.

This study indicates that changes in fructosamine are not robust enough to replace daily monitoring of fasting and postprandial blood glucose for changes in dose adjustment and/or treatment plan.

Type 2 DM is associated with micro- and macrovascular diseases, hypertension, insulin resistance and dyslipidaemias, which are a part of the cluster of conditions of the metabolic syndrome. This study population presented with **hypercholesterolaemia,** hypertriglyceridaemia, high LDL and low HDL, all of which are risk factors for cardiovascular diseases. Macrovascular complications due to atherosclerosis are the major cause of mortality in diabetic patients (Taskinen, 1995). Despite the fairly high prevalence of hyperlipidaemia in this study cohort, only one subject was on lipid lowering therapy. This is due to the very high cost of lipid lowering drugs (statins in particular). In addition, prescription of these drugs is limited to patients attending the IHD clinic at this study site. While this practice may not be uniform in all state institutions, it does indicate a conservative approach to the management of a major co-morbid condition in type 2 diabetics at this hospital.

In a recent study comparing the effectiveness of Humulin L® and Monotard HM® on glycaemic control, the mean (± SD) cholesterol was 5.98 (±0.48) and 5.72 (±0.18), respectively (Mayet, 2003). The present study population, with a mean cholesterol of 5.8mmol/l, was representative of the diabetic population as reported by Mayet. However, this value is higher than the recommended 5.2 mmol/L optimum by SEMDSA (2002) (<5.2), and within the 'at risk' range (4.8-6.0 mmol/L) as reported by the European Diabetes Policy Group (1999).

The mean HDL of 1.1 mmol/L for the study population was less than the acceptable range of >1.7 mmol/L (SEMDSA, 2002) but within the 'at risk' group of 1.0-1.2 mmol/L. (European Diabetes Policy Group, 1999). South African norms are less stringent than their European counterparts. In this study sample, only one subject was on lipid lowering therapy (fluvastatin).

The mean fasting triglycerides (1.6 mmol/L) for the study population was above the optimal (<1 mmol/L) but below the below the acceptable range of 1.7-2.2 mmol/L recommended by SEMDSA (2002) and the European Diabetes Policy Group (1999), respectively. Mayet (2003) in her study of type 2 diabetes, the mean (± SEM) triglycerides was 4.57 (±1.30) and 2.12 (±0.18) respectively. Mayet's study population was obese (BMI 31.3) as compared to this study population, BMI of 26.48 (males) and 29.93 (females).

The mean LDL of 3.9 mmol/L was greater than the acceptable value of < 2.6 mmol/L (ADA and Canadian) but within the 'at risk' level of 3.0-4.0mmol/L (European Diabetes Policy Group, 1999).

At entry all subjects fulfilled the inclusion criteria for normal **hepatic and renal function.** The mean ALT (± SD) was 25 (±12.6) U/L and was within the normal range of 10-60 U/L. The mean (± SD) creatinine clearance of the study population was 64.1 (± 10.4) μmol/L and was within the normal range of 46-98 μmol/L.

Glibenclamide undergoes hepatic metabolism and renal and biliary excretion [Feldman, (1985); Ings et al., (1981); Matsuda et al., (1983)]. Since all subjects in this study presented with normal hepatic and renal function at entry, it can be concluded that changes in pharmacokinetics and pharmacodynamics will not be due to dysfunction of these aforementioned organs.

2 Insulin Resistance and Acute Insulin Release

2.1 Introduction

Insulin resistance is a core metabolic defect in type 2 diabetics and its determination may help in identifying individuals at high risk of both diabetes and cardiovascular diseases. Insulin resistance is determined by HOMA-IR and QUICKI.

The insulinogenic index (II) is a mesure of acute insulin response which is a primary defect in type 2 diabetics.

2.2 Results

The modified II (II') is calculated using change in mean AUC $_{(insulin\ 0\text{-}30min)}$ divided by change in mean AUC $_{glucose\ 0\text{-}30min.}$ These values are presented in Table 34 and 35 below.

Table 34: Change in mean AUC $_{(insulin\ 0\text{-}30min)}$ (Acute Insulin Response-AIR)

Glibenclamide (µg)	*Mean AUC $\Delta_{0\text{-}30\ insulin} \pm SD$ (µU/mL.h)*		*p-value*
0	9.1579	4.21533	
2500	12.5641	6.38248	0.043
5000	14.9279	9.76992	0.348
10000	12.0409	7.49357	0.278
20000	13.2852	7.24603	0.579

The change in mean AUC $_{insulin\ 0\text{-}30min}$ between dose 0 and 2500µg was statistically significant (p=0.043). The change between all other doses was not significant (p= 0.348, 0.278 and 0.579 between doses 2500-5000µg, 5000-10000µg and 10000-20000µg respectively).

Table 35: Change in mean AUC $_{glucose\ 0\text{-}30min}$

Glibenclamide (µg)	Mean $\Delta_{0\text{-}30\ glucose}$ (mmol/L. h)	±SD	*p*-value
0	8.1327	1.97371	
2500	6.9152	1.62823	0.031
5000	6.9154	1.82522	1.00
10000	5.8799	2.08042	0.087
20000	6.0773	2.08932	0.755

The change in mean AUC $_{glucose\ 0\text{-}30min}$ between dose 0 and 2500 was statistically significant (p=0.031). The change between all other doses was not significant (p = 1.00, 0.087 and 0.755 between doses 2500-5000, 5000-10000 and 10000-20000 respectively). These AUC's were used to calculate the modified insulinogenic index (II').

The response of measures of insulin resistance (Modified Insulinogenic Index, HOMA-IR and QUICKI) to increasing doses of glibenclamide are presented in table 36 and graphical representations in figures 7, 8, 9 and 10 below.

Table 36: Indices of insulin sensitivity and insulin resistance

Dose	II'		HOMA-IR		QUICKI	
		SD		SD		SD
0	1.24	0.73	3.17	1.44	0.24	0.03
2500	1.98	1.17	3.76	2.1	0.24	0.03
5000	2.33	1.57	4.5	3.2	0.23	0.03
10000	2.46	1.9	3.9	4.27	0.25	0.03
20000	2.49	1.53	3.46	2.12	0.25	0.04

Key II': Modified Insulinogenic index

HOMA-IR: Homeostatic Model Assessment of insulin resistance

QUICKI: Quantitative Insulin-sensitivity Check Index

QUICKI is calculated using *pmol*/mL for insulin and mmol/L for glucose.

The Insulinogenic Index (II') is significantly different between doses if dose 0 is included. However, there is no statistically significant difference between doses if zero is excluded from the analysis.

In this study the II' is lower than the 9.22 reported by Suzuki et al. (2003) for subjects with normal glucose tolerance (NGT). All doses of glibenclamide improve the II' value ($p= 0.026$) when compared to baseline (0 µg of glibenclamide) but there is no significant difference in effect between the various doses i.e., 2500-5000, 5000-10000, 10000-20000 µg (see results).

In this study population, the mean HOMA (refer to table) ranged from 3.17-4.5 which is higher than the normal value of 1.57 ± 0.87 reported by Lichnovska et al. (2002).

At dose 0 µg, the mean QUICKI (± SD) is 0.24 ± 0.03 (refer to table 36) and at dose 20000 µg it is 0.25 ± 0.04. Lichnovska et al. (2002) reported a mean value of QUICKI of 0.366 ± 0.029 in healthy subjects of both genders. Using this as a normal value, the QUICKI, in this study is approximately 70% below normal. The very low mean QUICKI (0.24 ± 0.03) on entry in this study population indicates the high level of insulin resistance present. This reduced QUICKI value was seen across the dose range as there was no statistically significant difference in QUICKI as the dose of glibenclamide was increased from 0 to 20000 µg.

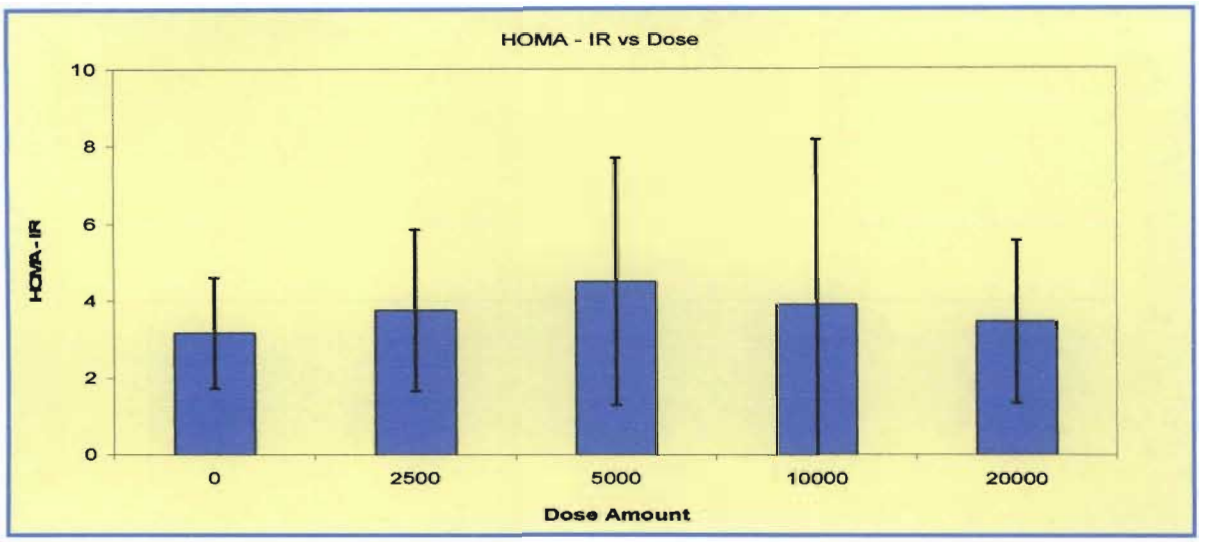


Figure 7: Dose vs HOMA-IR

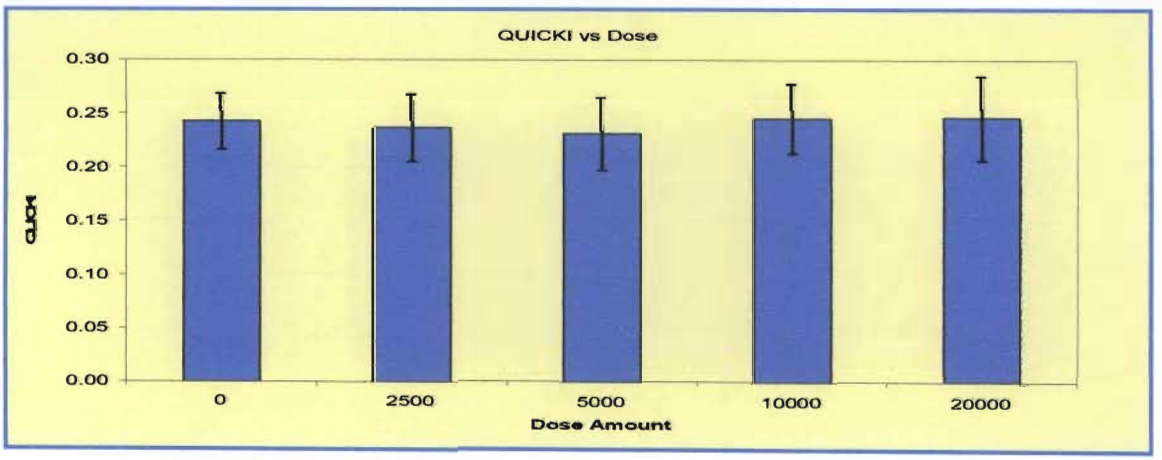


Figure 8: Dose vs QUICKI

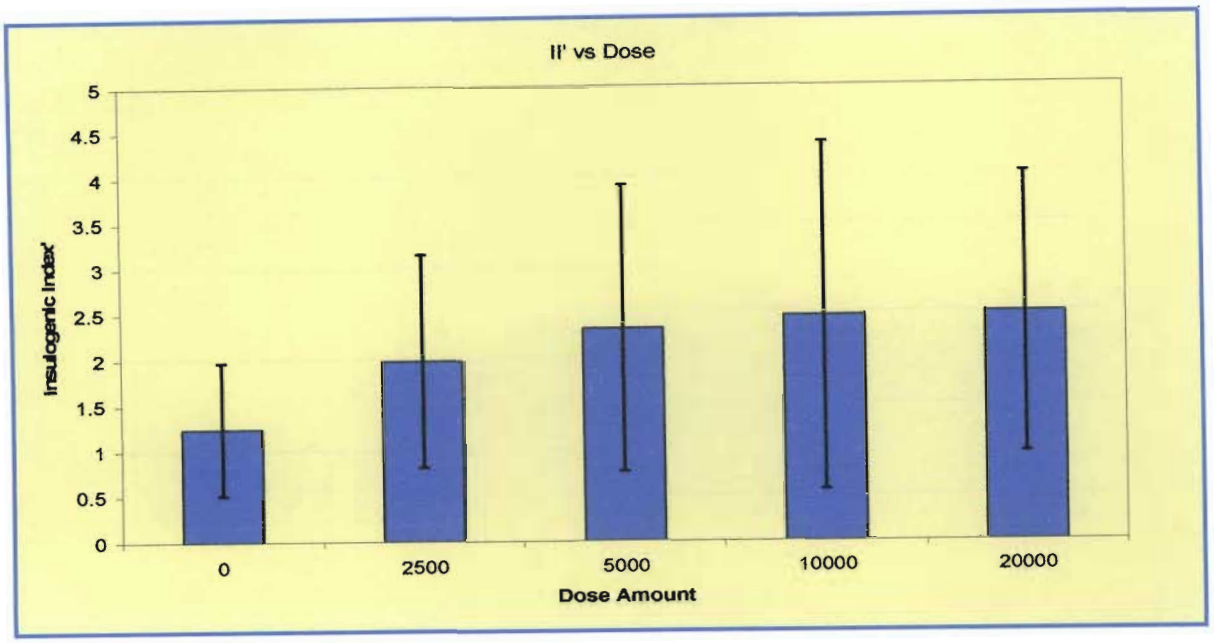


Figure 9: **Dose vs Modified Insulinogenic Index (II')**

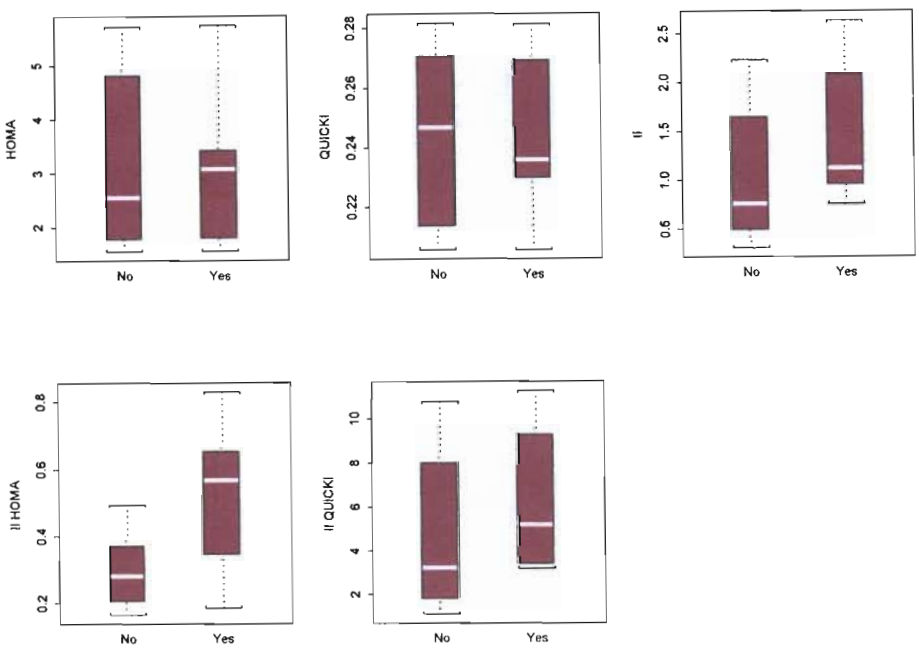


Figure 10: **Boxplots of Insulin sensitivity indices and responder status. The clear horizontal bar in the centre of the box shows the median; the box encloses the inter-quartile range i.e., 50% of the data. The whiskers show the interval of values outside the box and values far outside are represented by horizontal dashes**

2.3 Discussion

Assessment of insulin sensitivity is of paramount importance because it is widely accepted to be a core metabolic defect in subjects with type 2 diabetes mellitus. Glucose tolerance is an expression of the efficiency with which homeostatic mechanisms restore glycaemia to basal levels. The homeostatic response includes an increase in the insulin levels and also insulin dependent processes. Therefore an estimate of insulin sensitivity is possible if glucose and insulin concentrations are measured.

The hyperinsulinaemic euglycaemic glucose clamp is the gold standard in the measurement of insulin sensitivity, but it is invasive and technically complex. QUICKI and HOMA are much simpler and require less technical skills i.e., they require only fasting blood insulin and fasting blood glucose levels. Insulin sensitivity is less accurate in cases of greatly deteriorated β-cell function and/or marked hyperglycaemia (Anderson et al., 1995; Radziuk, 2000). QUICKI is said to provide a reproducible and robust estimate of insulin sensitivity that shows excellent linear correlation with the gold standard clamp measurement and has similar variability and discriminant power (Katz et al., 2000).

Type 2 DM represents an extreme of insulin resistance. Insulin resistance is a condition in which the response to the hormone is muted and the body must then produce excess insulin to maintain healthy blood glucose concentrations. This condition is also called low insulin sensitivity.

The study evaluates the effect of increasing doses of glibenclamide on insulin sensitivity by using ***two*** different measures namely, HOMA and QUICKI.

In this study, the mean **HOMA** ranged from 3.17 to 4.5 which is higher than the normal value of 1.57 ± 0.87 reported by Lichnovska et al. (2002). This indicates that the insulin resistance of the study population is pronounced. The effect of increasing doses of glibenclamide on HOMA, shows no statistically significant change ($p=0.785$). The implications of this finding is that glibenclamide in increasing doses (from 0 to 20000 µg) in this short term study, does not decrease insulin resistance. If this finding is confirmed in long term studies, then glibenclamide and possibly all other SUs would not protect patients against the co-morbid diseases (the triad) associated with insulin resistance and hence DM.

The HOMA-IR model performs well in comparison with the hyperglycaemic clamp and the frequently sampled IVGTT (intravenous glucose tolerance test) or the oral glucose tolerance test (OGTT). However, HOMA-IR scores obtained in different studies cannot be compared unless the insulin assay is standardised. Furthermore, insulin sensitivity evaluated by HOMA-IR is less accurate in cases of greatly deteriorated beta cell function and/or hyperglycaemia (Radziuk et al., 2000).

In this study population at dose 0 μg the mean (± SD) **QUICKI** was calculated to be 0.24 ± 0.03 and at dose 20000 μg it was 0.25 ± 0.04. Lichnovska et al. (2002) reported a mean QUICKI of 0.366 ± 0.029 in healthy subjects of both genders. Using this as a normal value, the QUICKI, in this study was approximately 70% that of the normal value. The very low mean QUICKI (0.24 ± 0.03) on entry in this study population reflects the high level of insulin resistance. This reduced QUICKI value was seen across the dose range as there was no statistically significant difference in QUICKI as the dose of glibenclamide was increased from 0 to 20000 μg. This lack of change in QUICKI indicates that increasing doses of glibenclamide do not favourably influence insulin resistance as measured in this study population.

QUICKI provides a reproducible and robust estimate of insulin sensitivity which shows excellent correlation with the gold standard clamp measurement. This method works best in persons without diabetes, and caution must be used when interpreting results in type 2 diabetes (Katz et al., 2000).

Although the derivation of QUICKI is more empirical, similar considerations apply as for HOMA-IR: it is more accurate when glycaemia is near normal and β cell function has not deteriorated greatly (Radziuk, 2000).

Insulin resistance determination by the HOMA and QUICKI methods work well in non-diabetic patients but caveats in diabetic patients exist. Katz et al. (2000) in introducing the QUICKI method, did not select a cut off point defining insulin resistance but rather presented a continuum of insulin sensitivity with their data suggesting an arbitrary cut-off of 0.3. Our data supports this cut-off of 0.3 for insulin resistance since all our diabetics presented with mean (± SD) QUICKI values of 0.24 ± 0.03 at dose 0.

HOMA and QUICKI are calculated on FBI and FBG. Hence, IR can be easily determined at diabetic outpatient clinics. However, the reliability and sensitivity of these indices are dependent on the duration of diabetes and the degree of hyperglycaemia. This study population had a mean FBG and FBI on entry of 15.39 mmol/L and 13.92μU/mL respectively with an average duration of diabetes of 8.9 years. Therefore, these measures of IR may have limited application in this study population.

Low **insulinogenic index** and low acute insulin release (AIR) are two defects that may play a pathogenic role in impaired glucose regulation and hence in the pathology of DM (Jensen et al., 2002). Insulinogenic index provides a parameter of insulin response and insulin release. Development of diabetes occurs more frequently in individuals with low values of insulinogenic index than in normal responders (Del Prato et al., 2002).

In this study the II' was low in all subjects. All doses of glibenclamide improve the II' value ($p= 0.026$) but there is no difference in effect with increasing doses. This implies that all doses of glibenclamide increase the early phase insulin secretion or AIR (0-30 mins), however increasing doses of glibenclamide, when compared to each other, do not significantly increase the II. While the maximum effect on II occurs at a dose of 20000 μg (II'= 2.49), this increase is not statistically significant from 2500 μg (II'=1.98) of gibenclamide.

The change in mean AUC $_{\text{insulin 0-30min}}$ between dose 0 and 2500 μg was statistically significant (p=0.043). The change between all other doses was not significant (p= 0.348, 0.278 and 0.579 between doses 2500-5000, 5000-10000 and 10000-20000 μg respectively). Mean $AUC_{\text{insulin 0-30min}}$ is an indirect measure of acute insulin release (AIR) and beta cell function. Therefore doses of glibenclamide greater than 2500μg provide little further benefit on acute insulin release (AIR) and beta-cell function. It can be conclude that doses greater than 2500μg are not likely to improve AIR.

In the study by Jensen et al. (2002) diabetic subjects with a reduced insulinogenic index (36%) were compared to patients with normal glucose tolerance (NGT) (100%) and impaired glucose tolerance (IGT) (64%). The decreased II' in the present study is indicative of decreasing glucose tolerance and hence decreasing beta cell function as shown by Jensen et al. (2002). The modified II adopted in this study is in agreement with the methodology adopted by Del Prato et al. (2002) and may be used when precise 30 minute insulin plasma levels are not available and a standardised meal is used instead of a oral glucose tolerance test (OGTT). Although the euglycaemic hyperinsulinaemic clamp is the gold standard in measuring II, the indices used in this study underline a correlation which may be used as a guide to quantifying insulin secretion. However, reproducibility of this II' (modified methodology) needs further investigation for confirmation.

The significance of the above findings are that the secretagogue glibenclamide, does not change insulin resistance, and that other agents alone, or in combination, may need to be used. Drug therapy targeting insulin resistance is discussed below.

Type 2 diabetes is a heterogenous disorder due to prevalent insulin resistance associated with deficient insulin secretion, or to a prevalent defect of insulin secretion associated with impaired insulin action (Del Prato et al., 2002). Insulin resistance is accepted to be a major risk factor in the aetiology of type 2 diabetes mellitus, hypertension, dyslipidaemias, atherosclerotic valvular disease and maybe a risk factor for coronary heart disease (Radikova, 2003). In this study population of type 2 diabetic patients, all subjects were insulin resistant based on the HOMA and QUICKI evaluations (albeit with their limitations).

In the **treatment of diabetes**, drug therapy must target the pathophysiological hallmarks of type 2 diabetes mellitus viz., insulin resistance, pancreatic β-cell dysfunction and endogenous glucose production.

The findings of this study show that increasing doses of glibenclamide do not significantly decrease or alter insulin resistance. This was determined by the HOMA and QUICKI methods. SUs, when used as monotherapy in the hyperinsulinaemic phase of type 2 diabetes promote further weight gain and results in the vicious cycle of insulin resistance, hyperinsulinaemia, hyperphagia, further weight gain and worsening of the already present insulin resistance. Thus, insulinotropic agents are not recommended as first line drugs in overweight or obese type 2 DM patients, as has been shown by UKPDS 34 (1998). Such patients may benefit by the use of α-glucosidase inhibitors which decrease postprandial hyperglycaemia when used as monotherapy. In type 2 DM patients with fasting hyperglycaemia, α-glucosidase inhibitors are less effective but may be used in combination with other agents such as SUs or metformin or insulin (Holman et al., 1999).

Metformin is the drug of choice as monotherapy in insulin resistant, overweight type 2 diabetic patients (Matthaei et al., 2000). The addition of meformin to SUs has a synergistic effect on glycaemic control and may be an ideal combination with glibenclamide in this study population because it reduces insulin resistance. Metformin is indicated in insulin resistant states in type 2 DM. Thiazolidinediones (TZDs) are also indicated in patients where insulin resistance, rather than insulin deficiency, is the leading pathogenic mechanism (Matthaei et al., 2000).
These agents i.e., α-glucosidase inhibitors, metformin or thiazolidinediones, utilise endogenous hyperinsulinaemia to improve insulin action and do not impede the goal of weight reduction by further increasing hyperinsulinaemia and insulin resistance which is seen with the insulinotropic agents such as SUs e.g., glibenclamide.

Insulin resistance as shown in this study is a common finding in type 2 diabetes mellitus. Glibenclamide is ineffective in decreasing insulin resistance in this diabetic population. This population is more likely to benefit from the addition of insulin sensitizers e.g., thiazolidinediones and metformin which target insulin resistance.

3 Dose exposure response relationships evaluated using conventional methodologies

3.1 Data used for dose-exposure-response relationship

There were twenty two subjects in the data set who contributed a total of 3005 observations (1100 glucose, 1088 insulin and 817 glibenclamide)

Blood samples were taken at 10 time points for each subject at each dose. Sample times were categorized into 10 groups as follows in table 37 below:

Table 37: Time points for blood sampling

Sample	*Time range (hours)*
1	0-0.45
2	0.46-1
3	1.1-1.5
4	1.6-2
5	2.1-4
6	4.1-4.5
7	4.6-5
8	5.1-5.5
9	5.6-6
10	>6

3.1.1 Graphical exploration of glibenclamide concentration versus time data

The spaghetti plots presented in figure 11 below represent the individual profiles of the twenty two subjects for each dose. There is an increase in glibenclamide concentrations with dose. There is linearity between dose and plasma glibenclamide concentration.

Figure 11: Graph of glibenclamide concentration versus time profiles for all subjects by dose group. Each line within a panel represents one subject

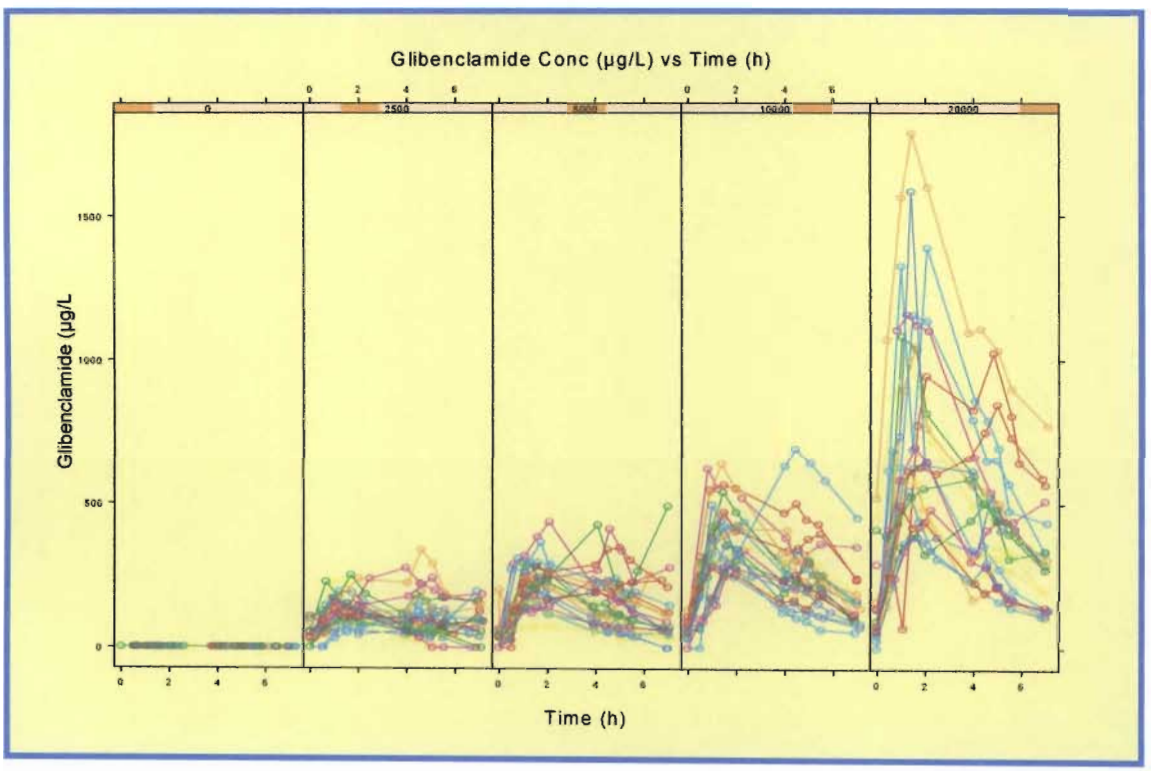


Table 38 demonstrates the effect of increasing glibenclimide doses on mean plasma glibenclamide concentration

Table 38: Glibenclamide levels

DAMT* (µg)	Mean plasma concentration of drug (ng/mL)	± SD
0	0.00	0.00
2500	95.76	4.28
5000	147.94	6.61
10000	258.36	9.98
20000	478.97	21.88

DAMT*= Dose amount

Inspection of figure 12 and table 38 shows the linear increase in mean glibenclamide concentration as drug dose increased.

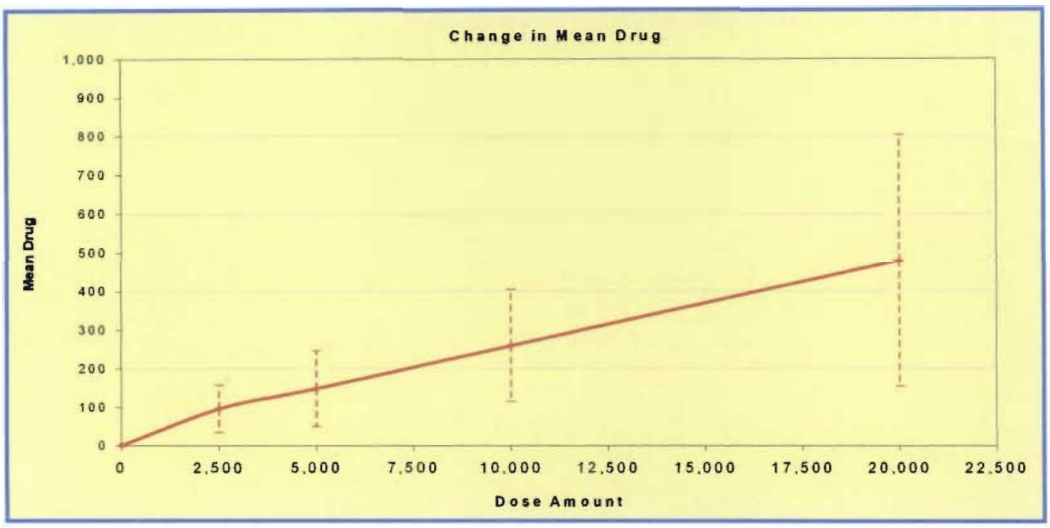


Figure 12: Change in mean plasma glibenclamide levels with dose escalation

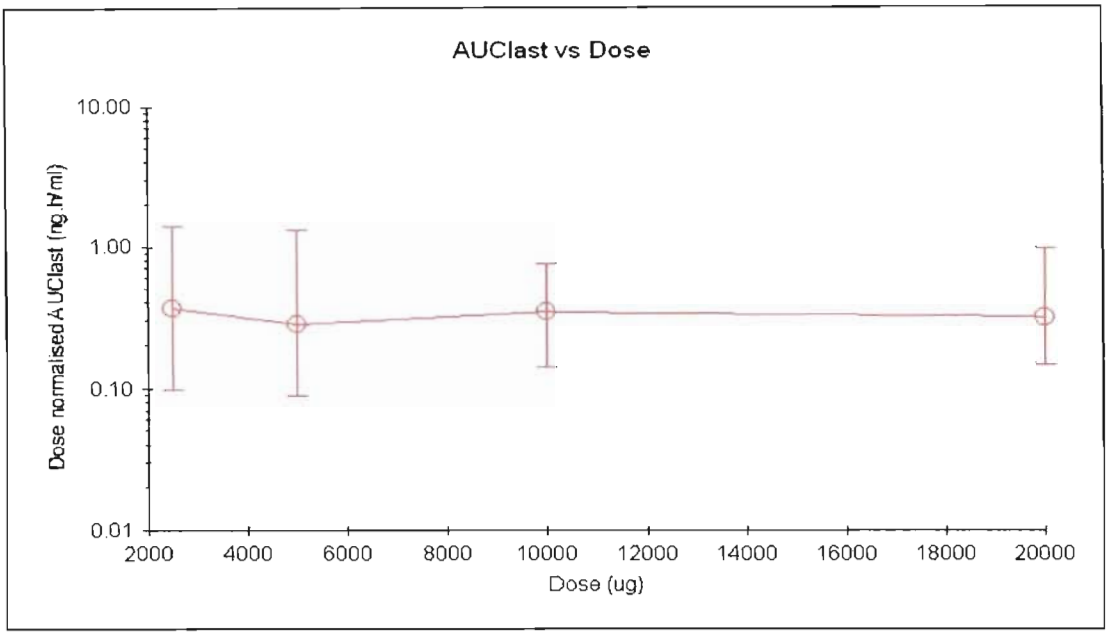


Figure 13: **Graph of dose normalized AUClast versus dose. Open circles show the geometric mean and the vertical lines span the minimum and maximum concentrations in 22 subjects**

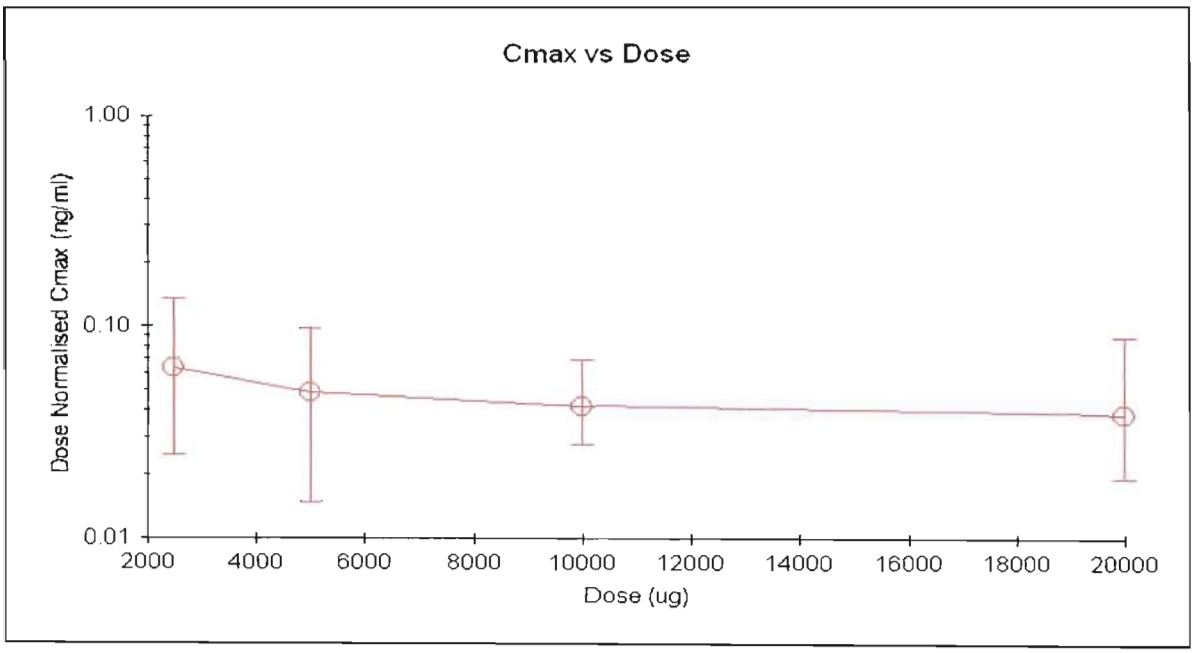


Figure 14: **Graph of dose normalized Cmax. Open circles show the geometric mean and the vertical lines span the minimum and maximum concentrations in 22 subjects.**

3.2 Dose-response and dose-exposure-response relationships

Figure 15 below provides a synopsis of the relationship between dose, glibenclamide concentration, glucose and insulin. The mean glibenclamide concentration increased in a linear fashion as dose increased. The relationship between increasing insulin and decreasing mean glucose is valid from 0-2500 µg. While the insulin concentration increases from doses 2500-5000 µg, there is little change in mean glucose. At dose 5000-10000 µg, while insulin decreases there is no appreciable change in blood glucose. At drug doses from 10000 to 20000 µg, insulin levels plateau and blood glucose levels exhibit little change.

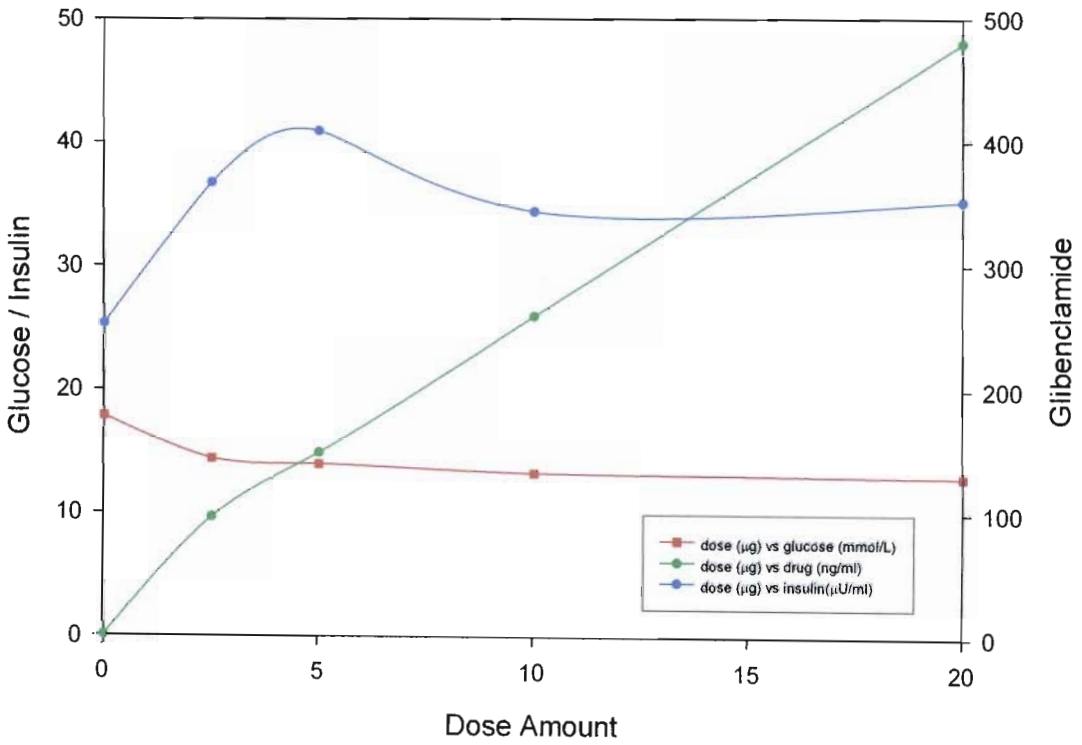


Figure 15: The relationship of dose, glucose, insulin and glibenclamide concentration

The Cmax of **glibenclamide** increases in a linear manner as the dose increases (from inspection of figure 16). This means that a doubling of dose produces a proportionate increase in Cmax of glibenclamide. In the case of **glucose**, there is an initial decrease in Cmax from dose 0 to 5000µg, followed by minimal decrease between 5000 and 10000 µg, and very little thereafter to 20000µg.

The Cmax of **insulin** increases from dose 0 to 5000µg, followed by a steady decrease to 10000µg, with minimal decrease thereafter to 20000µg.

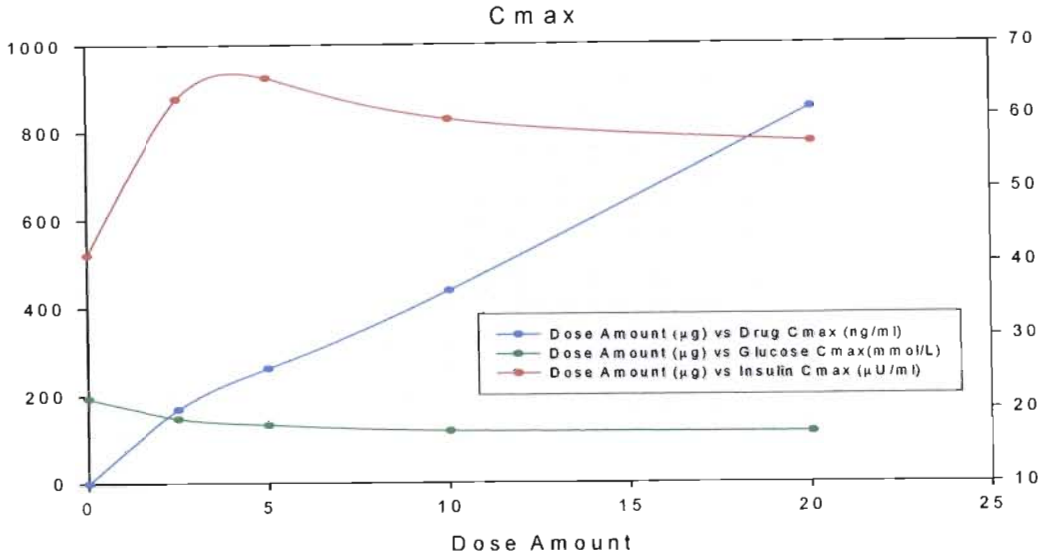


Figure 16: **The relationship between dose amount of glibenclamide and the Cmax of insulin, glucose and glibenclamide**

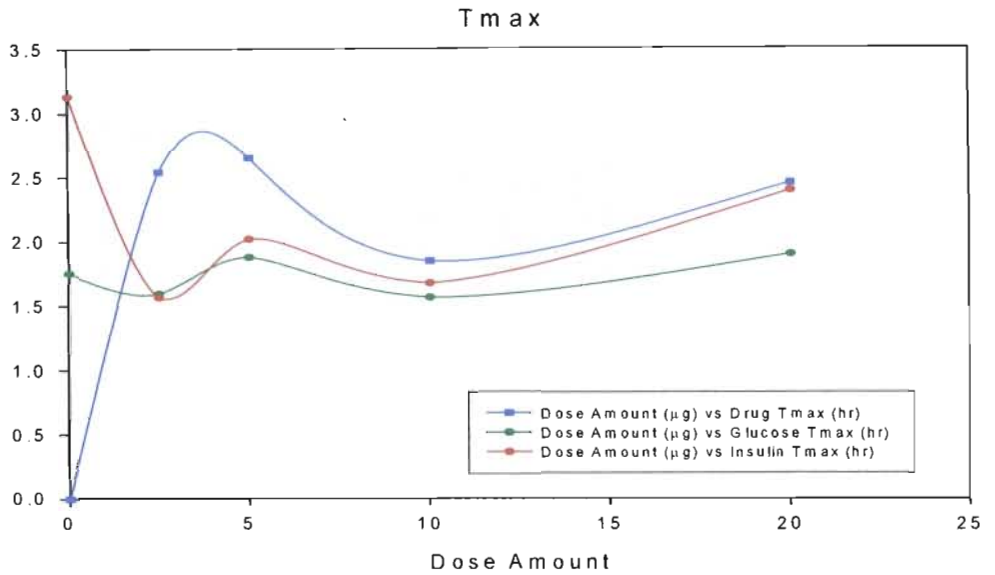


Figure 17: **The relationship between dose amount of glibenclamide and the Tmax of insulin, glucose and glibenclamide**

The Tmax of **glibenclamide** displays a parabolic relationship with dose amount as shown in the figure 17 above. The Tmax of **insulin** and **glucose** follow a similar relationship, with a parallel relationship from dose 2500 µg to 20000 µg. At zero dose insulin peaks at 3.13 hours compared to 1.57, 2.02, 1.68, and 2.39 hours after 2500µg, 5000µg, 10000µg and 20000µg. The times at which glibenclamide peaks, does not coincide with the peaking of glucose and insulin.

Table 39: AUC at various time intervals for glucose and insulin with increasing doses of glibenclamide

Dose µg		AUC0-2hrs				AUC4-6hrs				AUC0-8hrs			
		Partial AUC		*Plasma conc		Partial AUC		*Plasma conc		Partial AUC		*Plasma conc	
		Ins µmol/L.h	Gluc mmol/L.h	Ins µmol/L	Gluc mmol/L	Ins µmol/L.h	Gluc mmol/L.h	Ins µmol/L	Gluc mmol/L	Ins µmol/L.h	Gluc mmol/L.h	Ins µmol/L	Gluc mmol/L
0	Mean	54.01	38.28	27	19.14	50.8	34.04	25.4	17.02	201.37	138.65	25.17	17.33
	SD	27.27	8.18			24.54	8.23			83.03	32.32		
2500	Mean	86.89	32.4	43.44	16.2	69.52	26.39	34.76	13.2	281.55	110.71	35.19	13.83
	SD	48.53	7.23			35.23	10			145.48	35.16		
5000	Mean	94.25	31.34	47.12	15.67	82.29	25.64	41.15	12.82	318.5	103.07	39.81	12.88
	SD	60.41	7.52			43.58	8.15			173.46	30.4		
10000	Mean	77.82	29.77	38.91	14.88	66.76	24.37	33.38	12.18	252.78	97.29	31.6	12.16
	SD	48.25	7.84			29.19	8.9			119.81	31.49		
20000	Mean	79.83	29.12	39.92	14.56	71.15	24.12	35.57	12.06	270.92	91.41	33.87	11.43
	SD	47.03	8.48			37.61	9.57			143.03	26.16		

*Average concentration was calculated by dividing corresponding AUC by relevant time interval i.e., 2 4 and 8 hours hours.

Statistical analysis of the results in table 39 above, show that there is a significant difference in the $AUC_{0\text{-}2h}$, $AUC_{4\text{-}6h}$, $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}8h}$ for insulin between doses 0-2500 µg (p=0.007), 5000-10000 µg (p=0.033) and 0-2500 µg (p=0.056) and 5000-10000 µg (p=0.026) respectively.

3.2.1 Glucose as the pharmacodynamic response

The spaghetti plots in figure 18 and 19 below represent the individual profiles of the twenty two subjects for each dose. There is a decrease in glucose concentration from 0 to between 5000 and 10000 µg glibenclamide.

Figure 18: **Spaghetti plot of glucose (mmol/L) vs time (hour) by dose for all subjects. Each panel from left to right shows zero, 2.5mg, 5mg, 10mg and 20mg respectively. Each subject shown as a separate line.**

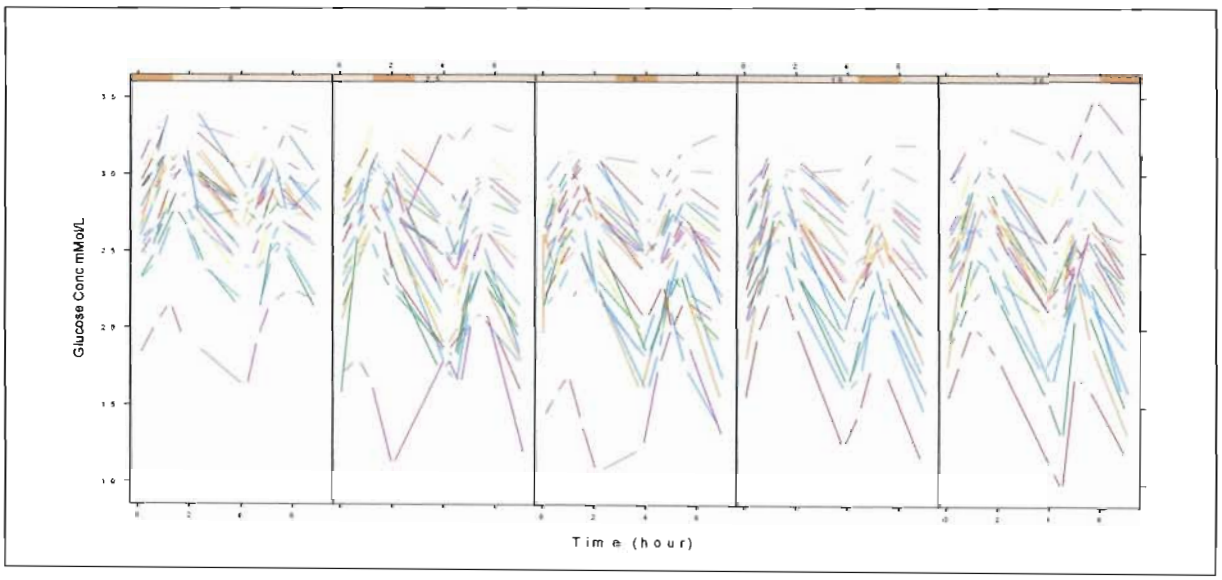
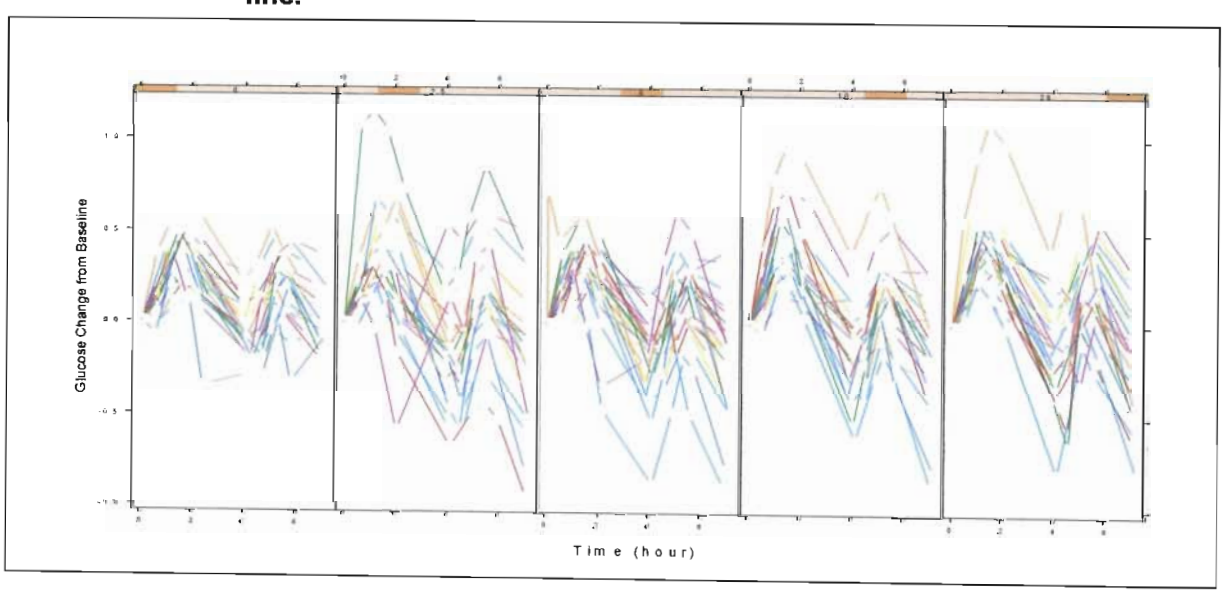


Figure 19: **Spaghetti plot of glucose (change from baseline) vs time (hour) by dose for all subjects. Each panel from left to right shows zero, 2.5mg, 5mg, 10mg and 20mg respectively. Each subject shown as a separate line.**



Mean Glucose levels

Table 40: Change in mean glucose levels between dose increments with escalating drug dose

Dose range (µg)	*Change in mean glucose (mmol/L)*	*p Value*
0-2500	-3.49	≤0.001*
2500-5000	-0.44	0.345
5000-10000	-0.72	0.123
10000-20000	-0.33	0.468

Key: * *p* Value < 0.05 statistically significant

All doses of glibenclamide from 2500µg to 20000µg decreased mean blood glucose when compared to zero dose. The percentage decrease of mean blood glucose from zero dose was 19.61%; 22.10%, 26.01% and 27.98% for 2500µg, 5000µg, 10000µg, and 20000µg, respectively. The decrease in glucose from dose 0-2500 µg is statistically significant ($p \leq 0.001$). The decrease in glucose is not statistically different for doses 2500-5000µg, 5000-10000µg and 10000-20 000µg. Figure 20 below is a graphic representation of the changes in mean glucose level with escalating drug dose.

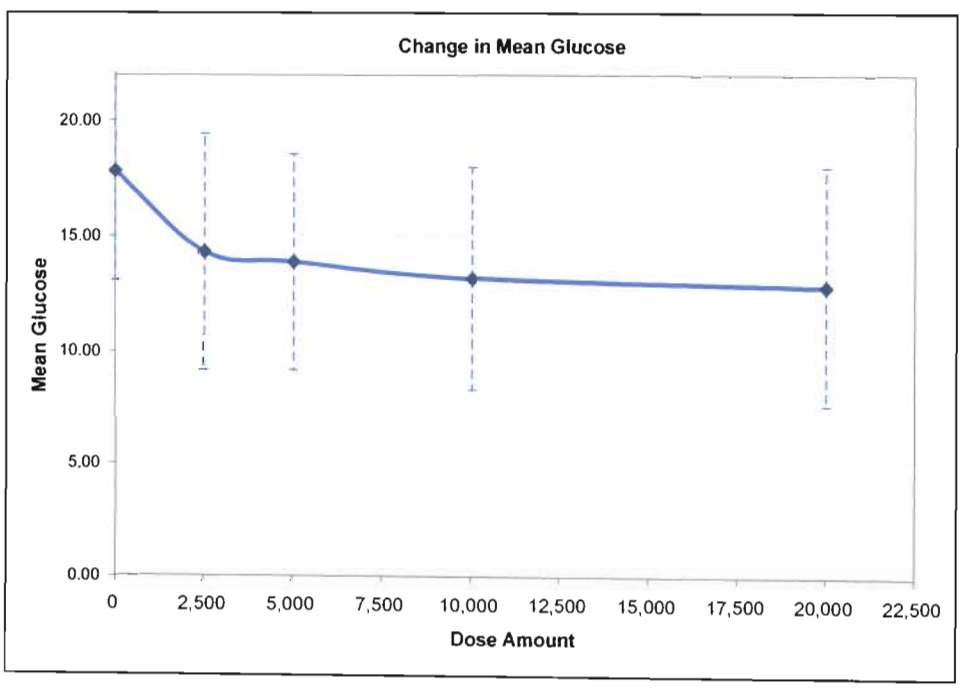


Figure 20: Change in mean glucose (mmol/L) with dose (µg) escalation

Fasting blood glucose

Table 41: Dose vs mean FBG

Dose of Drug (μg)	*Mean FBG (mmol/L)*	*(±SD)*	*p value*
0	15.4	3.84	
2500	12.8	3.51	0.024*
5000	12.7	3.88	0.919
10000	11.5	3.84	0.320
20000	11.2	4.15	0.782

* Statistically significant

Table 41 and figure 21 show the effect of glibenclamide dose escalation on mean FBG. The difference in mean FBG between dose 0 and 2500 μg is statistically significant (p=0.024). However, there is no statistical difference in mean FBG between doses 2500, 5000, 10000 and 20000 μg.

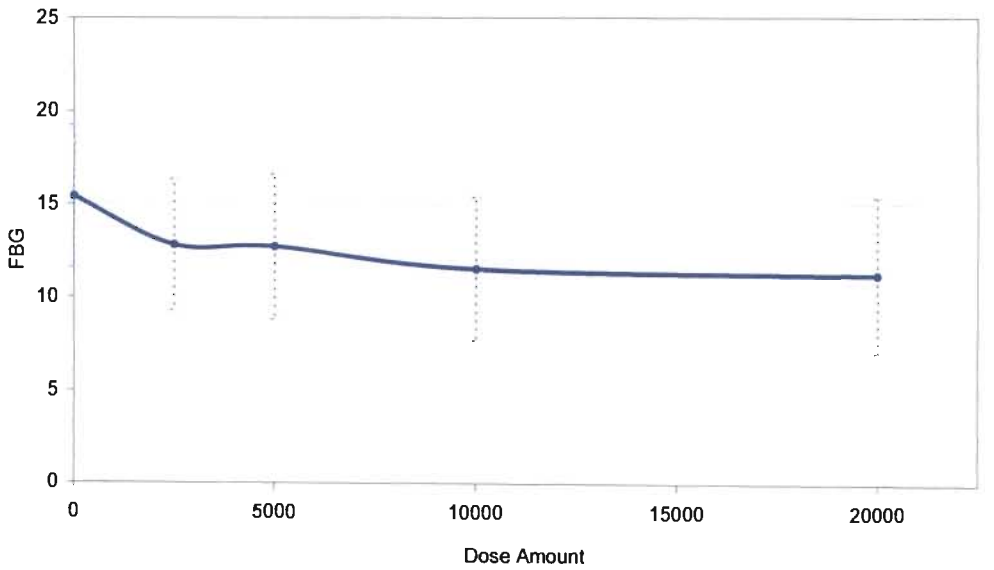


Figure 21: Fasting blood glucose vs Dose

Post-prandial glucose

Table 42: Dose vs mean single point glucose determination (mmol/L)

	Mean Postprandial BG (mmol/L)			
Dose of glibenclamide (µg)	*2hr*	*(±SD)*	*6 hr*	*(± SD)*
0	20.81	4.55	17.88	4.42
500	16.87	4.43	15.38	5.43
5000	16.55	4.38	13.38	4.79
10000	15.51	4.26	12.65	4.37
20000	15.36	4.69	13.22	6.47

The change in mean postprandial (2 and 6 hour) blood glucose is not statistically significant at all doses (p>0.05). Furthermore there is no statistically significant difference between doses.

When the 2 hour mean glucose level is compared to that obtained at 6 hours, the mean glucose level is statistically higher at time 2hr for doses 0 (p=0.016), 5000µg (p= 0.020) & 10000µg (p=0.027). However, there was no statistically significant differences at 2500µg (p= 0.270) and 20000 µg (p=0.162). However, 57% of the subjects (presented in table 47) show a decrease in PPG.

AUC glibenclamide vs FBG, PPG and glucose AUC

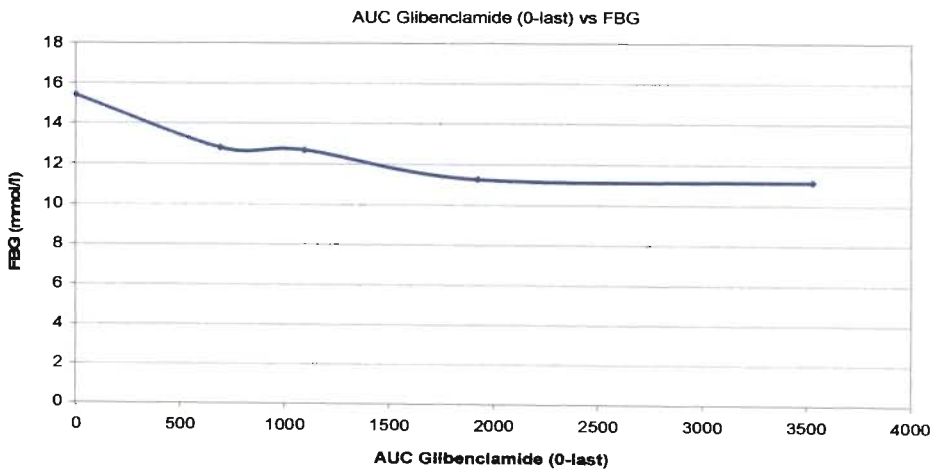


Figure 22: AUCglibenclamide vs FBG

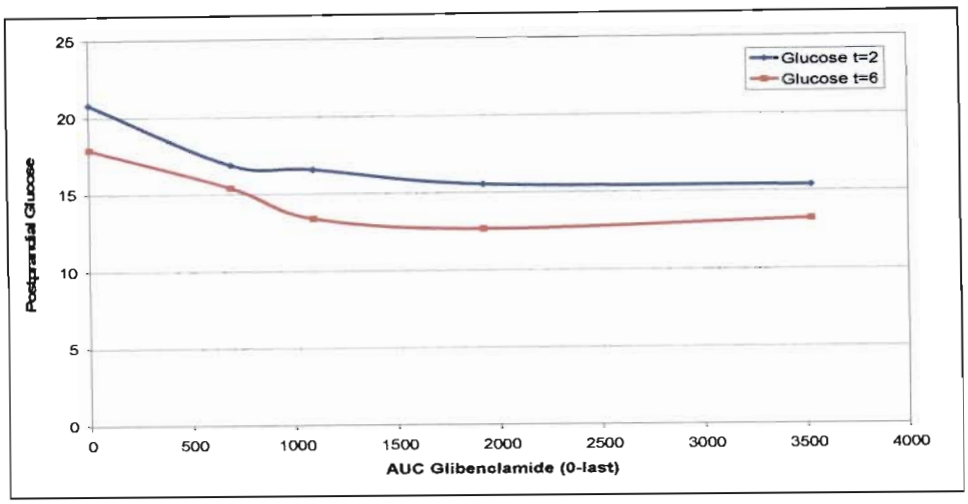


Figure 23: `Effect of glibenclamide AUC on post-prandial glucose

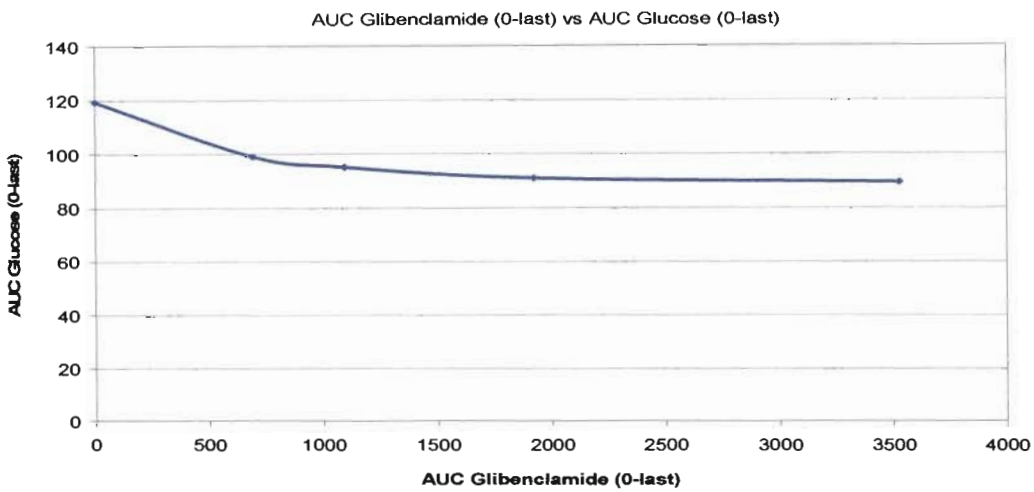


Figure 24: Effect of glibenclamide AUC on glucose AUC

The effect of AUC glibenclamide on FBG, PPG, and AUC glucose is presented above in figures 22, 23 and 24 respectively. There is a statistically significant difference between AUC glibenclamide corresponding to dose 0 and 2500µg and FBG (p=0.024) and a statistically significant difference on AUCglucose (p=0.028) corresponding to the same dose. There are no differences at all other AUC's of glibenclamide for FBG, AUCglucose and PPG (both 2 and 6 hours).

3.2.2 Fructosamine as the pharmacodynamic response

Table 43 and figure 25 below outline the changes in mean fructosamine levels with escalating doses of glibenclamide.

Table 43: Drug vs mean fructosamine concentration

Drug dose (µg)	Mean fructosamine (µmol/L)	Change from dose 0	
0	466.72		
2500	454	-12.72	(2.72%)
5000	437.28	-29.49	(6.32%)
10000	436.03	-30.69	(6.58%)
20000	423.05	-43.67	(9.36%)

Table 42 above shows the change in fructosamine from dose 0 to 20000 µg. shows the correlation between the change in fructosamine with increasing doses of glibenclamide. There was a statistically significant decrease in mean fructosamine ($p=0.001$) as the dose of glibenclamide increased from 0 to 20000 µg. The decrease in fructosamine between doses ranged from 2.72% to 9.36%. There was no statistically significant change in fructosamine between doses of glibenclamide.

Pearsons' correlation indicates that a significant ($p<0.05$) inverse relationship exists between fructosamine and DAMT, however the linear trend is weak.

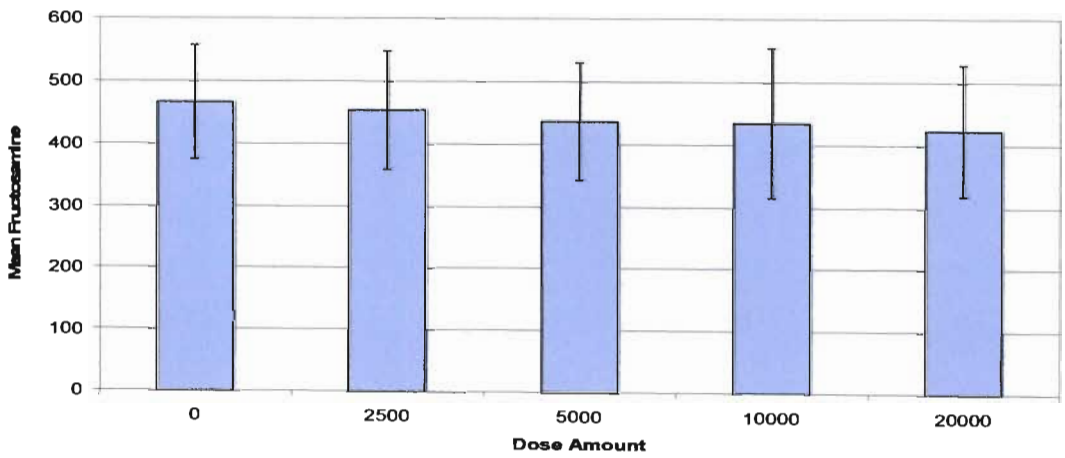


Figure 25: Changes in mean fructosamine with increasing doses of glibenclamide

3.2.3 Insulin as the pharmacodynamic response

The spaghetti plots in figure 26 below represent the individual profiles of the twenty two subjects for each dose. There is an increase in insulin concentrations with dose from 0 to 5000µg glibenclamide. Thereafter there is no apparent increase in insulin secretion with dose.

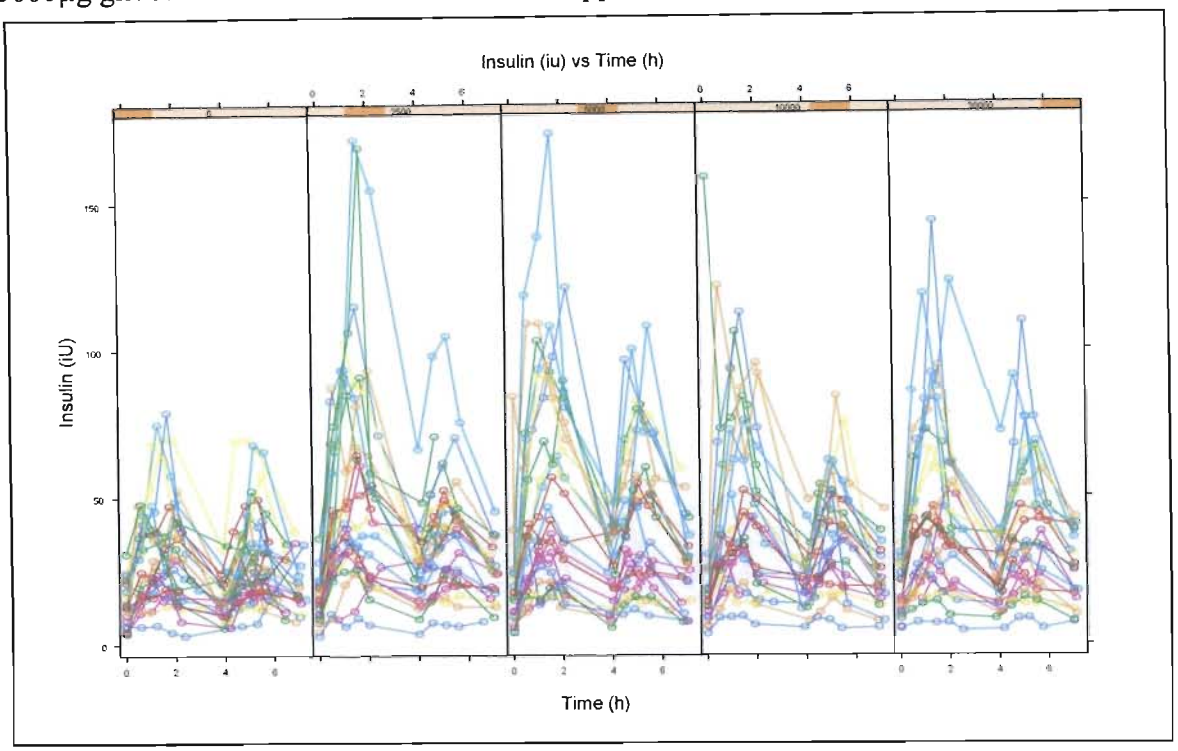


Figure 26: Spaghetti plots of insulin profiles for each dose

Mean Insulin levels

Table 44: Change in mean blood insulin levels with escalating drug dose

Dose range (µg)	*Change in mean plasma insulin levels* (µU/mL)	*p Value*
0-2500	11.38	≤0.001*
2500-5000	4.15	0.113
5000-10000	- 7.11	0.05*
10000-20000	0.82	0.721

Key: * *p* Value < 0.05 statistically significant

All doses of glibenclamide from 2500 to 20000 µg stimulated insulin secretion compared to the zero dose. The percentage increase of insulin from zero dose was 51.38; 58.34, 44.41 and 33.54 % for 2500, 5000, 10000, and 20000µg respectively. The 5000µg dose stimulated maximal insulin secretion at time 2.020 hrs. Mean blood insulin increased significantly from dose 0-2500 µg ($p \leq 0.001$). However, the increase from 2500-5000µg is not significant (p=0.113). Thereafter, insulin decreased significantly from dose 5000 to 10000 µg (p=0.05).

Finally, the change in insulin from dose 10000-20000 µg was not statistically significant (p=0.721). In summary, mean insulin levels increased from dose 0-5000µg, decreased steadily to 10000µg and thereafter paralleled the x-axis to dose 20000µg as graphically represented in figure 29 below and tabulated in table 43 above.

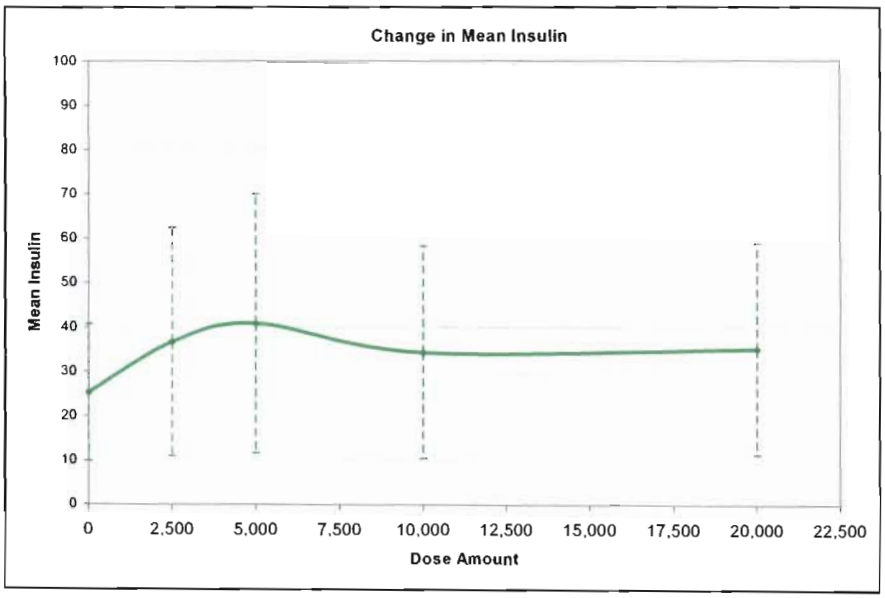


Figure 27: Change in mean insulin levels with dose escalation

Mean Fasting Blood Insulin

Table 45: Dose vs mean fasting blood insulin

Dose of Drug (µg)	Mean Fasting Insulin (µmol/L)	(±SD)
0	13.92	6.87
2500	14.35	6.04
5000	15.66	8.29
10000	14.99	6.86
20000	17.95	8.70

The change in mean fasting insulin is statistically not significant at all doses (p> 0.05). Furthermore there is no statistically significant difference between doses (p>0.05).

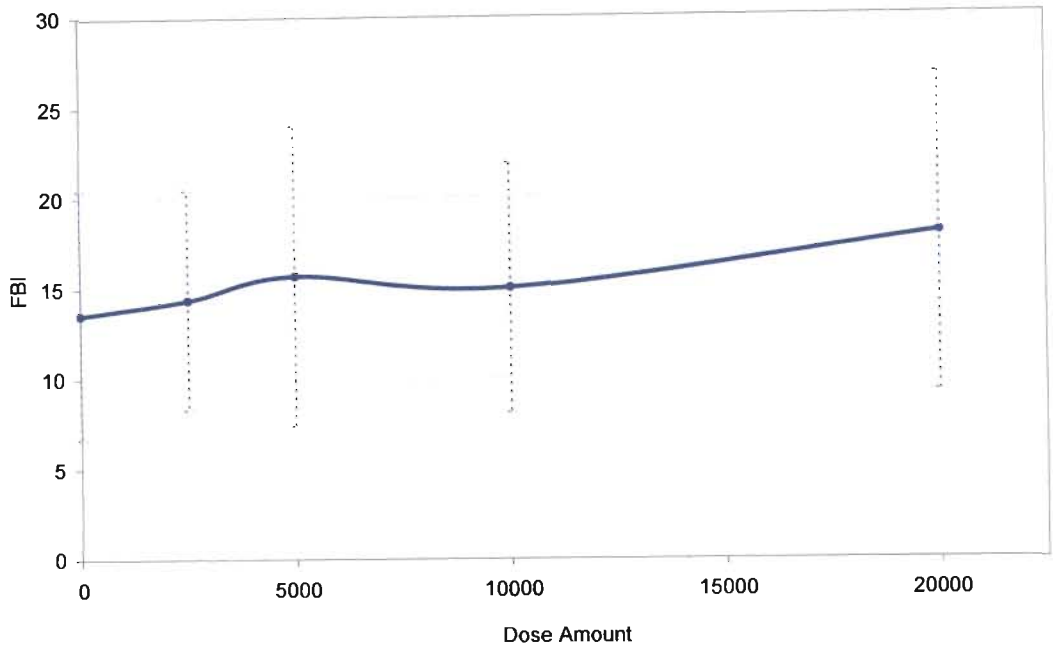


Figure 28: Fasting blood insulin vs dose amount

Post-prandial insulin

Table 46: Dose vs mean single point insulin determination (µmol/L)

Dose of Drug (µg)	Mean Single Point* Postprandial insulin			
	2hr	(±SD)	6hr	(±SD)
0	30.997	17.976	26.433	14.513
2500	52.221	35.819	35.433	18.993
5000	56.848	36.448	41.253	20.977
10000	43.006	23.089	38.006	17.075
20000	43.035	26.304	37.016	17.132

*The single point determination is derived from the highest value between 1.6-2.0 hours for 2 hour and 5.6-6.0 hours for 6 hour.

The change in mean 2 hour single point insulin determination is statistically significant between doses 0-2500 µg, ($p=0.002$) and 5000-10000 µg ($p=0.015$). There is no statistically significant difference between doses 2500-5000 µg ($p=0.640$) and 10000-20000 µg ($p=0.537$). The change in mean 6 hour single point determination of postprandial blood insulin is not statistically significant at all doses.

Comparison between 2 and 6 hour single point determination shows no statistically significant difference except at dose 2500 µg (p=0.066). The figures below represent the effect of glibenclamide dose on 2 and 6 hr mean insulin levels (postprandial).

Effect of AUC glibenclamide on FBI, PPI and insulin AUC

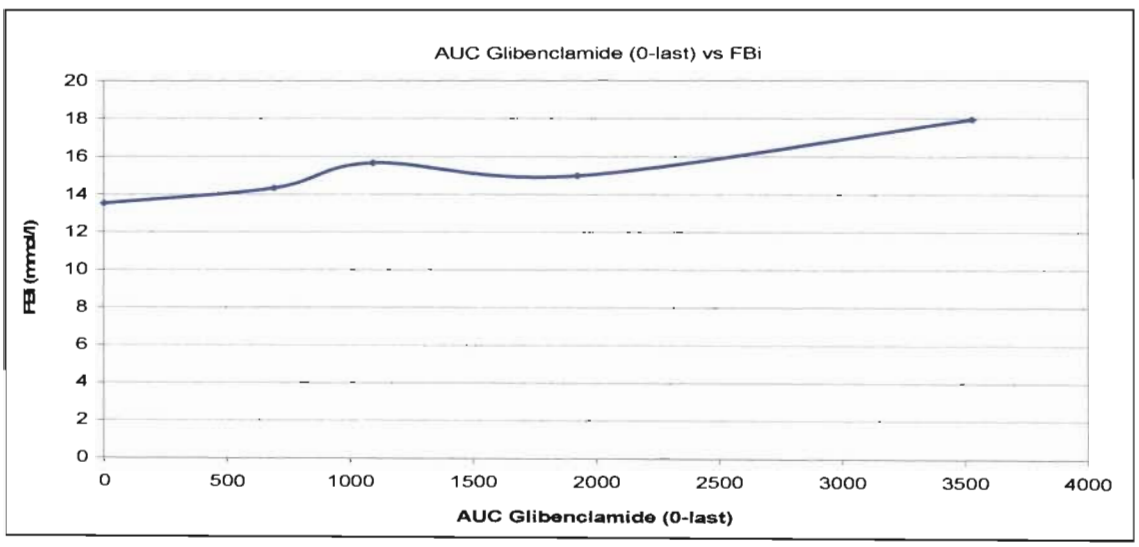


Figure 29: Effect of glibenclamide AUC on fasting blood insulin

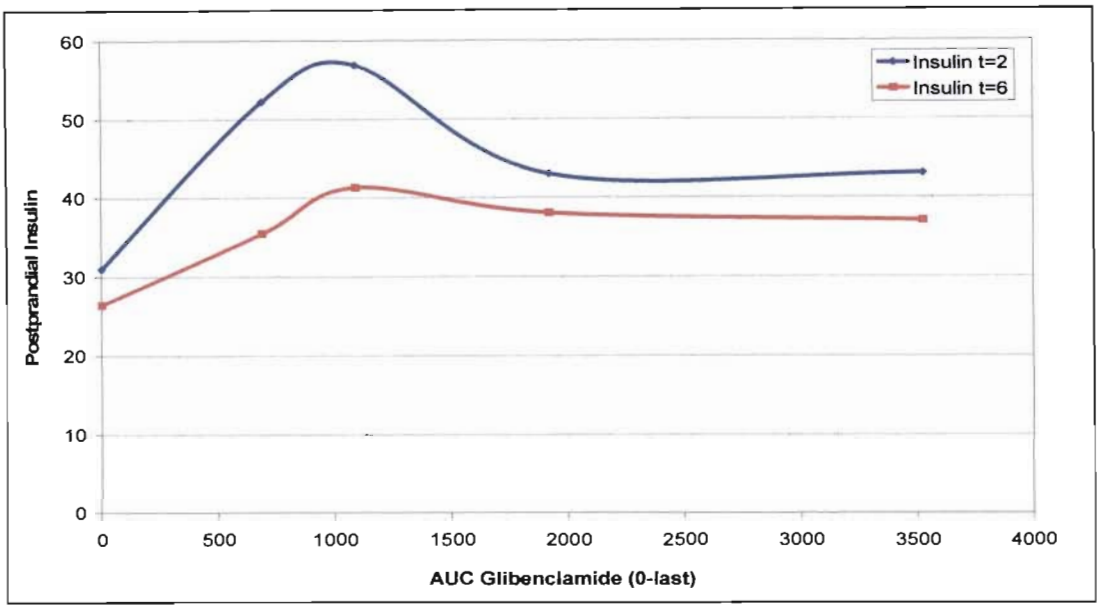


Figure 30: Effect of glibenclamide AUC on post-prandial insulin

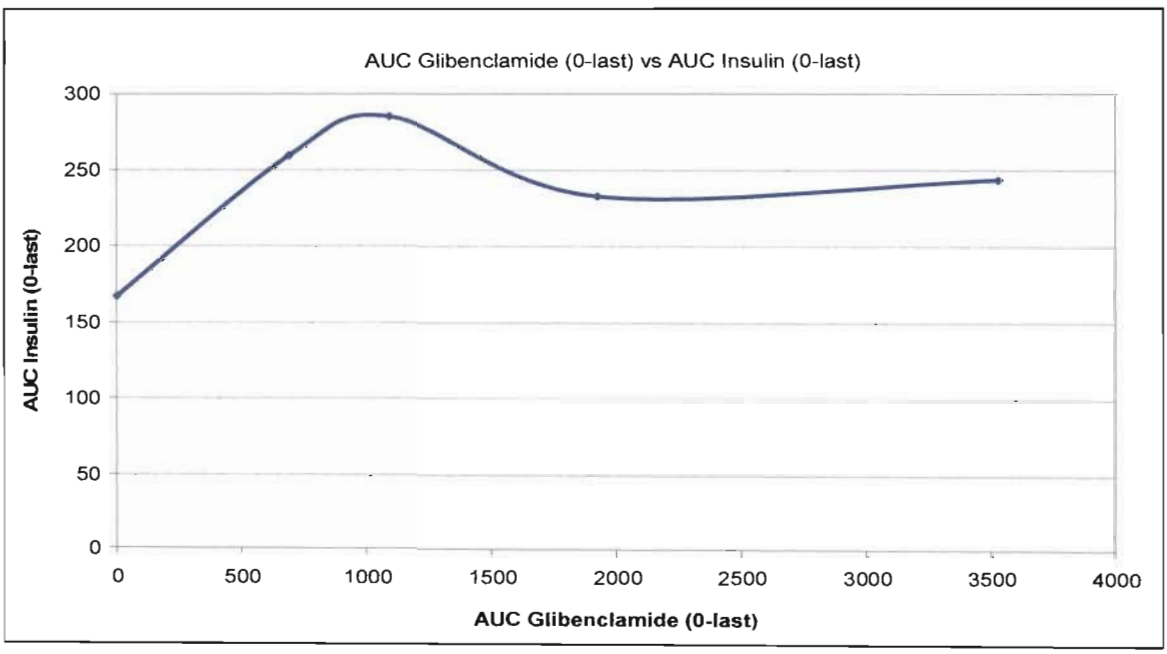


Figure 31: Effect of glibenclamide AUC on insulin AUC

The effect of AUC glibenclamide on FBI, PPI, and AUC insulin is presented above in figures 29, 30 and 31 respectively. There is a statistically significant difference between AUC glibenclamide on AUC insulin corresponding to dose 0 and 2500µg (p=0.01) and a statistically significant difference on PPI at the corresponding dose of 0-2500µg (p=0.002) and 5000 and 10000 (0.015) µg.

There are no differences at all other AUC's for the corresponding doses for FBI, AUCinsulin and PPI.

4 Evaluation of clinical benefit due to glibenclamide

Table 47 shows the clinical benefit attributable to glibenclamide based on the SEMDSA criteria viz.

- FBG of 4-6 mmol/L (Optimal) and >6-8 mmol/L (Acceptable) control
- PPG of 5-8 mmol/L (Optimal) and >8-10 (Acceptable)

The maximum number of subjects who achieved an 'acceptable' level of glycaemic control i.e. according to the less conservative definition when using either FBG or PPG was 13 (57%).

Table 47: Summary of clinical control achieved

	SEMDSA Criteria	*2500µg*	*5000µg*	*10000µg*	*20000µg*	*Total (n=23)*
Fasting blood glucose	Optimal 4-6 mMol/L	1	0	2	2	5 (22%)
	Acceptable 6-8 mMol/L	2	4	3	4	13 (57%)
Post-prandial blood glucose	Optimal 5-8 mMol/L	1	4	2	2	9 (39%)
	Acceptable 8-10 mMol/L	3	5	2	3	13 (57%)

Table 48: Identities of patients achieving acceptable FBG and PPG at each dose level

Dose (µg)	*ID (FBG 6-8mmol/L)*	*ID (PPG 8-10mmol/L)*
2500	10,18	
5000	17, 18	
10000	9,15,18, 21	10,9,8,6,21,14,15,20,11
20000	10,15,17,18,20,21	9,10,17,15,6,18,8,4,14, 19,11,20,3,22

ID=Patient identity

Table 48 above identifies the individual subjects who achieved acceptable FBG and acceptable PPG at the individual doses based on SEMDSA criteria. Four of the 23 subjects (17%) at 20000µg and 3 of the 23 subjects (13%) at 10000µg reached the acceptable value for both FBG and PPG (these numbers are exclusive).

4.1 Discussion

Glucose and Insulin Exposure

In type 2 DM patients, the combination of IR and inadequate basal insulin secretion may account for excessive endogenous glucose production (EGP). Therefore restoration of basal insulin levels will improve FBG and the daily blood glucose profile.

The reduction of **FBG** from zero dose (15.54mmol/L) for the 2500µg (12.8mmol/L), 5000µg (12.7mmol/L), 10000µg (11.5mmol/L) and 20000µg (11.2 mmol/L) glibenclamide was 17, 18, 25 and 27 % respectively. There was a statistically significant reduction in fasting blood glucose when the dose 2500 µg of glibenclamide was compared to zero dose. However, there was no statistically significant difference on FBG between doses (2500, 5000, 10000 and 20000 µg) of glibenclamide.

Endogenous glucose production (EGP) is a function of insulin resistance and basal insulin secretion. Insulin resistance increases EGP which results in increased FBG. Decreased basal insulin secretion increases EGP which results in increased FBG. FBG serves as a measure of the degree of EGP (De Fronzo et al., 1992). All doses of glibenclamide reduce FBG and thus must also be reducing EGP. Since EGP is related to basal insulin secretion and IR, then the effect of glibenclamide must be on either basal insulin release or IR, or a combination of both, or extrapancreatic effects. SUs increase both basal and glucose stimulated insulin secretion. However, after several weeks of continued therapy, insulin secretion decreases to near pre-treatment levels. The failure of SUs to sustain the initial increase in insulin secretion suggests extrapancreatic mechanisms in regulating blood glucose levels (Feldman, 1971).

The percentage increase in **FBI** from zero dose to 2500µg, 5000µg, 10000µg and 20000µg glibenclamide was 6, 14, 10 and 25%, respectively, although not statistically significant. However, the 20000 µg dose resulted in a 25 % increase in FBI without producing a corresponding reduction of FBG. Doses of glibenclamide above 2500 µg did not significantly decrease fasting glucose levels but increased insulin levels, which in turn, worsened the metabolic state of the type 2 diabetic patient. This finding reinforces the use of low dose glibenclamide in type 2 DM.

The difference in mean FBG from 0 (15.4mmol/L) to 2500µg (12.8 mmol/L) is 2.6 mmol/L and would translate to a change of 1.6 % in HbA1c if this difference is maintained over a three month period (Nathan et al., 1984). Similarly the decrease in mean FBG from 2500µg to 5000µg, 5000µg to 10000 µg and 10000µg to 20000 µg was 0.1 mmol/L, 0.8 mmol/L, and 0.3mmol/L respectively. This decrease is not statistically significant and does not bring the FBG to normoglycaemia or even near normoglycaemia.

The side-effects of life threatening, protracted hypoglycaemia (Krentz, 1994) and the cardiovascular adverse effects (O'Keefe et al., 1999) that accompany the use of high doses of glibenclamide do not warrant the use of such high dosage. On the basis of this finding, the maximal dose of glibenclamide should not exceed 5000 µg per day.

The change in **mean postprandial blood glucose** (both at 2 and 6 hours post breakfast and post lunch, respectively) is statistically not significant at all doses (p> 0.05). Furthermore, there is no statistically significant difference between doses (p>0.05). However when individual response are evaluated 9 (39%) of subjects achieved optimal control. This number increases to 13 (57%) when the less stringent 'acceptable' definition of glcaemic control is applied. The addition of insulin sensitizers or insulin to glibenclamide may decrease PPG.

These findings are similar to the study by Cozma et al. (2002) which compared the effect of repaglinide, glipizide and glibenclamide on insulin secretion and postprandial glucose after a standardised meal. Glibenclamide had no significant effect in lowering postmeal glucose peaks (glucose Cmax) when compared to glipizide and repaglinide. Acute glucose exposure (Cmax) may be as important as total postprandial exposure with respect to long term complications.

Prevention of postprandial hyperglycaemia is important as it is implicated in the development of macro - and microvascular complications associated with diabetes (Baron, 1998). Postprandial hyperglycaemia, rather than fasting blood glucose levels, is related to the risk of cardiovascular disease (Hanefield and Temelkova-Kurktschier, 1997). This finding was confirmed by the National Health and Examination Survey (NHAES) in the USA which reported an association with post-load hyperglycaemia and an increase in all-cause and cardiovascular mortality (Saydah et al., 2001).

Glibenclamide as monotherapy has no clinically significant reduction in postprandial blood glucose levels and therefore will confer no long term beneficial effects in preventing macro- and microvascular complications. However, it does provide benefit when combined with metformin or acarbose as discussed in combination therapy below.

The change in **mean insulin** was significantly higher with all doses when compared to placebo (0-dose). The change in mean 2 hour postprandial blood insulin is statistically significant between doses 0-2500 μg, (p=0.002) and 5000-10000 μg (p=0.015). There is no statistically significant difference between doses 2500-5000 μg (p=0.640) and 10000-20000 μg (p=0.537). On the other hand, the change in mean 6 hour single point determination of postprandial blood insulin is not statistically significant at all doses. These results indicate that the stimulation of insulin secretion occurs during the first 2 hours for low dose glibenclamide (2500μg-5000μg) i.e., an acute insulin response to glibenclamide. However, at 6 hours glibencalmide at all doses does not augment food stimulated insulin secretion.

Comparison between 2 and 6 hour single point determination shows no statistically significant difference. Since there is no difference between AUC and single point glucose measurement for determining postprandial glycaemia, the single point PPG determination (as for FBG) is adequate. This has practical clinical relevance in making decisions on dose adjustment or level of control.

The effect of **glibenclamide AUC on FBG** is similar to that found for glibenclamide dose on FBG. This result is expected because AUC glibenclamide is a function of dose. Similarly, the effect of **glibenclamide AUC on PPG** is the same as reported for effect of **glibenclamide dose on PPG** i.e., there is no statistically significant difference between all doses of glibenclamide. It is thus not necessary to determine AUC of glibenclamide since dose is proportional to AUC, assuming patients are compliant with medication. AUC, which is a pharmacokinetic parameter, does not show a direct relationship with changes in AUC glucose (a pharmacodynamic response).

There is a significant difference in the $AUC_{0\text{-}2h}$, $AUC_{4\text{-}6h}$, and $AUC_{0\text{-}8h}$ for insulin from dose 0-2500 μg (p=0.007), 5000-10000 μg (p=0.033) and 5000-10000 μg (p=0.026) respectively.

All doses of glibenclamide stimulate insulin secretion as measured by the $AUC_{0\text{-}2h}$ insulin. However, there is no statistically sgnificnt corresponding decrease in blood glucose levels as measured by the $AUC_{0\text{-}2h}$.

At 4-6 hours, glibenclamide increases insulin secretion at dose 5000-10000μg. At 0-8 hours insulin secretion increases significantly at doses 0-2500μg and decreases significantly at doses 5000-10000μg (as expected since $AUC_{0\text{-}8h}$ incorporates $AUC_{0\text{-}2h}$ and $AUC_{4\text{-}6h}$).

However, at none of these corresponding time intervals is there a significant reduction in blood glucose. This sustained high insulin levels without corresponding decreases in blood glucose levels is indicative of prevailing insulin resistance in this study population (see below). This suggests a lack of relationship between insulin release and blood glucose reduction.

$AUC_{0\text{-}2h,\ 4\text{-}6h,\ 0\text{-}8h}$ for glucose did not significantly decrease after administration of glibenclamide at all doses. This suggests that glibenclamide therapy in this population does not significantly decrease glucose exposure (glucose toxicity).

Glucose toxicity may be defined as a glucose-induced reduction in insulin secretion and action and it has been shown in animal models of diabetes to contribute to the development of insulin resistance and impaired insulin secretion.

In type 2 diabetes in humans, there is considerable evidence indicating that a chronic physiological increment in the blood glucose concentration leads to progressive impairment in insulin secretion and may also contribute to insulin resistance (Rossetti et al., 1990). Glucose toxicity also contributes to the reduction in the bioavailability of SUs, probably via impairment of gastric motility and emptying (Luzi and Pozza., 1997).

In this study, glibenclamide throughout the dose range, does not decrease glucose exposure and hence is not likely to contribute to the amelioration of micro- and macrovascular complications. In addition, while glibenclamide is producing little effect on glucose exposure, its side effects, in particular cardiovascular risk, are nevertheless not diminished.

The effect of **Glibenclamide AUC on FBI** is similar to that found for glibenclamide dose on FBI. This result is expected because AUC glibenclamide is a function of dose. Similarly, the effect of glibenclamide AUC on PPI is the same as reported for effect of glibenclamide dose on PPI i.e., no statistically difference between doses. It is thus not necessary to measure AUC of glibenclamide since dose is proportional to AUC, assuming patients are compliant with medication. AUC, which is a pharmacokinetic parameter, does not show a direct relationship with changes in AUC insulin (a pharmacodynamic response).

Since the increase in Cmax of glibenclamide does not produce a proportionate increase in the Cmax of insulin (except at doses less than 5000μg), doubling the glibenclamide dose will not increase insulin secretion proportionately. This non-linearity is clinically significant in that high doses of glibenclamide (> 5000μg) are not likely to achieve a pharmacodynamic response i.e., increase in blood insulin. This conclusion is reinforced by the lack of change in the Cmax of glucose at a dose greater than 5000μg. Since insulin is required to decrease the blood glucose levels, and because high doses of glibenclamide (>5000μg) do not proportionately increase insulin, there is very little advantage in trying to decrease blood glucose with doses of glibenclamide greater than 5000μg if glibenclamide exerts its effect via insulin release. The possible role of glibenclamide in reducing blood glucose through extrapancreatic effects has not been exploited.

The Tmax of glibenclamide does not coincide with the Tmax of insulin and the Tmax of glucose. This is an example of a lack of coincidence of pharmacokinetics with pharmacodynamics (glucose decrease and insulin secretion).

This lack of coincidence of pharmacokinetics of glibenclamide with pharmacodynamics (blood insulin and glucose levels), means that drug blood levels do not correlate with a pharmacodynamic response. Therefore in the case of glibenclamide, (and possibly other sulphonylureas), therapeutic drug monitoring is not feasible.

One of the objectives of this study was to determine the number of subjects who achieved **glycaemic control with escalating doses of glibenclamide.**

SEMDSA criteria for FBG of 4-6 mmol/L (optimal) and 6-8 mmol/L (acceptable) control and PPG of 5-8mmol/L (optimal) and 8-10 (acceptable) were used. FBG was and is still used as a determinant of glycaemic control. The Expert Committee of the ADA (2003) encourages the use of FBG for diagnosing DM. The number of subjects who achieved glycaemic control in total was 13 (57%) based on SEMDSA criteria of 'acceptable' glycaemic control. The dose at which this occurred ranged from 2500μg to 20000μg. Therefore the majority of subjects were uncontrolled with increasing doses of glibenclamide. Dose escalation in these subjects did not produce decrease in FBG.

The total number of subjects who achieved glycaemic control was 13 (57%) when PPG was used as the measure of glycaemic control, based on SEMDSA criteria of 'acceptable' glycaemic control. The dose at which this occurred ranged from 2500μg to 20000μg. Similarly, 43% of subjects were uncontrolled with increasing doses of glibenclamide. Dose escalation in these subjects did not improve PPG levels.

When both FBG, and PPG, are evaluated at the same time, then 6 subjects (26%) achieve 'acceptable' glycaemic control. Both PPG and FBG provide important information regarding the risk for both microvascular and macrovascular disease. According this stringent measure (FBG and PPG simultaneously) the response to glibenclamide monotherapy is poor. But with a less stringent criteria i.e., FBG or PPG the response rate was 57%. The possible reasons for this poor response in this study are diabetes of long standing, insulin resistance, monotherapy and poorly controlled diabetes. These patients are likely to benefit from the addition of insulin sensitizers or insulin. Since only 26% of the subjects achieved 'acceptable' glycaemic control when bothe FBG and PPG are evaluated siumultaneously, it may be concluded that glibenclamide in this study population is not beneficial in protecting the subjects from micro – and macrovascular complications. Furthermore, there is little benefit in prescribing high dose glibenclamide as monotherapy.

4.2 Non compartmental pharmacokinetics analysis

4.2.1 Introduction

NCA provided exploratory data for initial estimates and guides for population PK analysis. This analysis included all those subjects (n22) who completed the full dose escalation study and from whom sufficient data was available to characterize concentration vs time profiles.

4.2.2 Results

Non-compartmental pharmacokinetic parameters for glibenclamide are recorded in Table 49.

Table 49 Non-compartmental pharmacokinetic parameters for glibenclamide

	Dose (µg)	*N*	*Mean*	*SD*	*Median*	*Min*	*Max*	*CV%*
AUC_{inf} (ng.h/ml)	2500	20.00	1376.58	1339.70	1281.84	435.73	5066.15	88.21
	5000	22.00	1861.22	1700.44	1732.55	580.29	7038.55	78.51
	10000	21.00	4276.17	1785.81	4334.46	2270.53	7945.56	40.86
	20000	22.00	7513.63	5632.41	7952.93	3216.50	30514.49	54.52
AUC_{last} (ng.h/ml)	2500	21.00	906.77	1036.30	629.48	243.57	3482.59	98.56
	5000	22.00	1416.84	1547.24	1216.97	441.96	6511.81	84.14
	10000	21.00	3410.48	1550.99	3496.84	1407.75	7435.80	45.16
	20000	22.00	6333.86	3678.42	6109.36	2958.56	19332.44	50.18
CL/f (L/h)	2500	21.00	1.94	2.46	1.71	0.72	9.49	82.71
	5000	22.00	2.66	2.20	2.80	0.77	10.58	70.17
	10000	21.00	2.78	1.03	2.86	1.34	5.17	37.34
	20000	22.00	3.09	1.62	2.96	1.03	6.76	51.00
Cmax (ng/ml)	2500	21.00	157.97	64.28	164.52	61.83	338.27	40.19
	5000	22.00	242.22	101.84	250.35	74.06	490.36	45.14
	10000	21.00	422.66	119.49	424.86	278.10	693.81	27.39
	20000	22.00	773.61	391.64	671.62	384.74	1787.62	45.92
t-half (h)	2500	20.00	5.09	3.62	6.25	0.94	12.50	81.05
	5000	22.00	4.42	3.09	4.11	1.60	11.87	63.82
	10000	21.00	8.08	2.25	7.91	3.87	12.13	29.91
	20000	22.00	6.56	3.02	7.32	2.58	14.88	51.19
Tmax (h)	2500	21.00	2.05	1.77	1.68	0.93	6.90	74.14
	5000	22.00	2.09	2.01	1.90	0.78	7.00	76.58
	10000	21.00	1.62	1.16	1.50	0.83	5.52	51.21
	20000	22.00	2.04	1.69	1.60	1.00	7.00	63.61
Vz/f (L)	2500	20.00	14.63	17.98	14.61	3.75	88.14	76.07
	5000	22.00	16.96	8.91	16.64	5.73	41.17	51.74
	10000	21.00	32.48	14.46	32.95	11.01	64.93	45.99
	20000	22.00	29.24	21.80	34.29	7.84	74.87	86.58

This study shows that there is linearity between AUC of glibenclamide with increasing doses using AUC_{last} and AUC_{inf}, using NCA. The corresponding values of Cmax also increased linearly.The Tmax ranged from 1.62-2.09 hours. Clearance (Cl/f) ranges from 1.94 to 3.09 L/h while the half life ranges from 4.42-8.08 hours. The volume of distribution (V_z/f) ranges from 14.63 to 32.48L.

In this dose-escalation study, multiple doses of glibenclaimde were administered to type 2 diabetic patients, at 14 day intervals. Based on half-lives between 8-12 hours [Marble et al., (1987) and White and Cambell., (1986); (Coppack, 1990); (Fleishaker and Phillips, 1991); Jaber., (1994); Jonsonn et al., (2000a) 7.09; Jonsonn (2000b) 2.0-4.5 hrs for Caucasian and Chinese; Courtois et al., (1999)], steady state was assumed to be achieved at approximately 2-3 days.

4.2.3 Discussion of NCA

NCA provided exploratory data for initial estimates and guides for population PK analysis. This discussion provides a comparison of results obtained from NCA in this study to that reported in the literature.

The **clearance** of glibenclamide in this study population ranges from 1.94 to 3.09 L/h. The mean age of this population is 54.1 years, ranging from 39-73 years. The mean creatinine of the study population is 64.1μmol/L which is within the normal range and therefore the study subjects are assumed to have normal renal function. The clearance of glibenclamide in this study population is within the range reported by other researchers who investigated type 2 diabetics with normal renal function and of the same age group [Jaber et al. (1994): 3.2L/h; Jonsonn et al., (2000b): 4.41 L/h (Caucasians) and 4.1 L/h (Chinese); Jonsson et al., (1998) 3.7 L/h]. In patients with impaired renal function (IRF) Jonsson et al. (1998) reported a clearance of 6.3L/h. None of the subjects in this study presented with clearances in this range as described for IRF

The **Vz/f** (L) of this study population ranged from 14.63 to 32.48L and is consistent with the literature. Jaber et al. (1994) reported values of 20, 41, 51L, after 0, 6 and 12 weeks respectively of glibenclamide therapy. Jonsonn et al. (2000b) in his comparison of Caucasian and Chinese subjects reported Vss of 6.31 and 5.49l respectively.

The **half-life ($t_{1/2}$)** of glibenclamide in this study ranged from 4.42-8.08 hours. Marble et al. (1987) and White and Cambell (1986) reported half-lifes of 6-10 hours. This half-life is within the range as reported for type 2 diabetic patients [the half-life for micronised glibenclamide ranged from 2.1 hours (Coppack, 1990) to 8.3 hours (Fleishaker and Phillips, 1991). Jaber et al. (1994) reported a half-life of 12.2 hrs, Jonsonn et al., (2000a) 7.09 hrs, Jonsonn (2000b) 2.0-4.5 hrs for Caucasian and Chinese. Courtois et al. (1999) 2.63 hrs (42-59 years) and 2.78 hrs (71-75 years)]. Frequency of dosage can be determined by the half-life of the drug. In the case of glibenclamide the recommended frequency of administration is once daily. From this study there is little correlation between half-life and frequency of dosage. This implies that blood concentration of glibenclamide (pharmacokinetics) does not correlate with the measured effect on glucose and insulin (pharmacodynamics).

There is a linear increase in the **Cmax** of glibenclamide as the dose is increased from 2500µg to 20000µg viz., 157.97 to 773.61 respectively.

The Cmax reported in this study is approximately twice that reported for corresponding doses by other researchers, namely for dose 5 mg, Fleishaker and Phillips, (1991) reported a Cmax of 179 ng/ml; Coppack (1990) reported a Cmax of 241ng/ml and 354 ng/ml for 10 mg (fasting) and 20mg (fasting) of glibenclamide respectively; while Jaber et al. (1994) reported a Cmax of 278ng/mL for 2.5 mg (in solution) of glibenclamide in solution and Jonsonn reported a low max of 69 (Caucasians) and 82 (Chinese) for 2.5mg of glibenclamide. However, when given paraenterally the Cmax was appreciably higher (Jonsonn (2000b) 1.25 mg IVI 376 (Caucasian) and 368 (Chinese)). This wide variation in Cmax is indicative of the variable bioavailabity of glibenclamide. This is confirmed by the very high Cmax obtained by Jonsonn et al. (1998). It is this variation in biovailabilty that lead to the reformulation of glibenclamide (micronisation). However, there is no correlation between the Cmax of glibenclamide, glucose and insulin as shown in this study.

The **Tmax** of 1.62-2.09 hours is comparable to the values reported in the literature review [Jonsonn et al. (1998), Courtois et al. (1999), Jonsonn et al. (2000a and b). Jaber et al. (1994), Coppack et al.(1990)]. However, as shown in this study there is no coincidence in the Tmax of glibenclamide, glucose and insulin.

4.2.4 Conclusion

The PK parameters of glibenclamide obtained from the NCA are consistent with those obtained from the literature.

5 Dose-exposure-response relationships evaluated using mathematical modeling methodologies

5.1 Population pharmacokinetic modeling

5.1.1 Introduction

The population approach using nonlinear mixed effects modeling as implemented in the software NONMEM (Globomax LLC, USA and NONMEM Project Group, University of California, San Francisco) was used in this analysis.

The primary purpose of this analysis was to describe the population PK of glibenclamide in a concise mathametical manner so that the estimated PK parameters might be used in the subsequent PKPD modeling. For the purposes of these analyses, dose amount of glibenclamide is presented in mg. Appendix 6 contains the NONMEM data file.

5.1.2 Data used for Population PK modeling

There were 24 individuals in the dataset who contributed a total of 841 observation records. Subjects 14, 16, 20 and 24 did not have complete data sets. Appendix 5 contains the glibenclamide, glucose and insulin concentrations.

In the case of subject 14 all 10 samples at dose 2.5 mg and samples at time 0 and 0.5h at dose 5 mg were lost in transit between the clinical centre and the analytical laboratory. Similary in subject 20 who completed all doses, 10 samples at dose 10mg were lost in transit.

Subject 16 could not proceed with dose escalation beyond 5 mg since blood glucose levels were 3.6 mmol/L after 7 hours. Therefore there were no concentration versus time profiles sets at dose 10 and 20 mg.

Subject 20 completed all doses. Subject 24 absconded from the study after dose 2.5 mg. All attempts to contact him were unsuccessful.

5.1.3 Pharmacostatistical Model development

The structural pharmacokinetic model selected was based on the NONMEM objective function value (OF) and diagnostic plots.

The structural pharmacokinetic model was implemented in NONMEM by selecting the appropriate ADVAN and TRANS subroutine from the PREDPP library of models. The first order conditional estimation method with interaction was used throughout this analysis.

Unexplained inter-subject variability in structural model parameters was estimated using the following model with the random effect ηj (Equation 5) and is contained in Appendix 8.

Equation 5

$$Pj = TVP*\exp(\eta j)$$

where TVP is the typical value of the pharmacokinetic parameter P (e.g., CL/f) in the population, Pj is the individual value for P in the jth individual and ηj is a random variable with mean of zero and variance ωP^2. This model assumes a lognormal distribution for the Pj values. Estimates of inter-subject variability in P are presented as the square root of ωP^2, which is an approximation of the coefficient of variation of P for a log-normally distributed quantity.

The glibenclamide concentration data was log transformed prior to fitting. The residual error model of this log-transformed data comprised of an additive model as shown in equation 6 below.

Equation 6

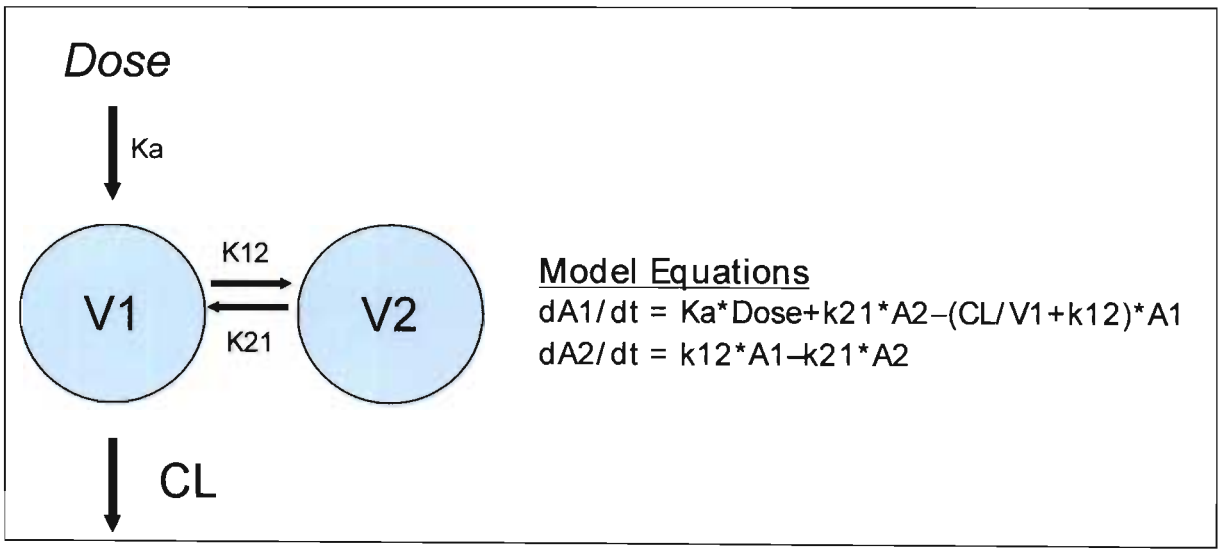
$$C\,ij = C*ij\,(1+\varepsilon ij)$$

where Cij is the ith concentration measured at time ti in the jth individual. C*ij is the respective model predicted concentration and the εij is a normally distributed error term with mean of zero and variances σ^2. Examples of potential sources of residual variability include assay error, deviations from the model specification, and intra-subject variability.

5.1.4 Results from population pharmacokinetic modeling

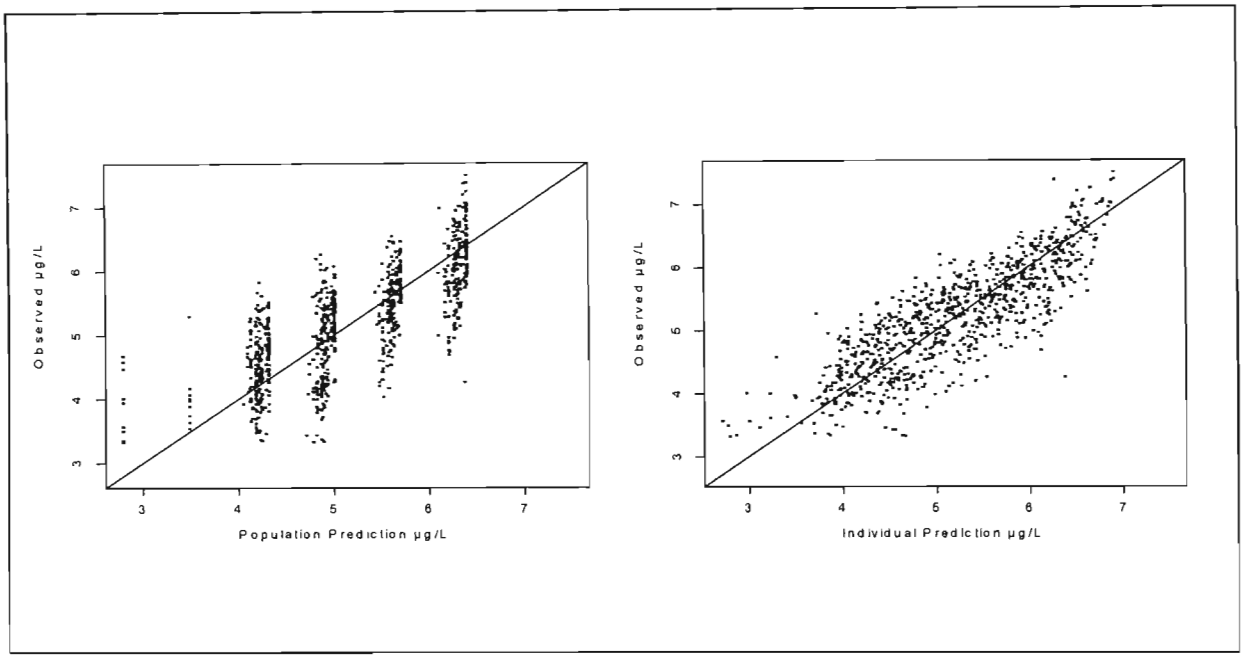
A two-compartmental disposition model Figure 32 was selected after evaluating 1, 2 and 3 compartmental models to describe the time course of glibenclamide plasma concentration data. The 3 compartment model failed to achieve successful convergence as the intercompartmental transfer rates went to infinity suggesting that the third compartment was poorly identified.

Figure 32: Schematic representation of the 2-compartmental pharmacokinetic model and model equations



Note: C1(t)= A1(t)/V1

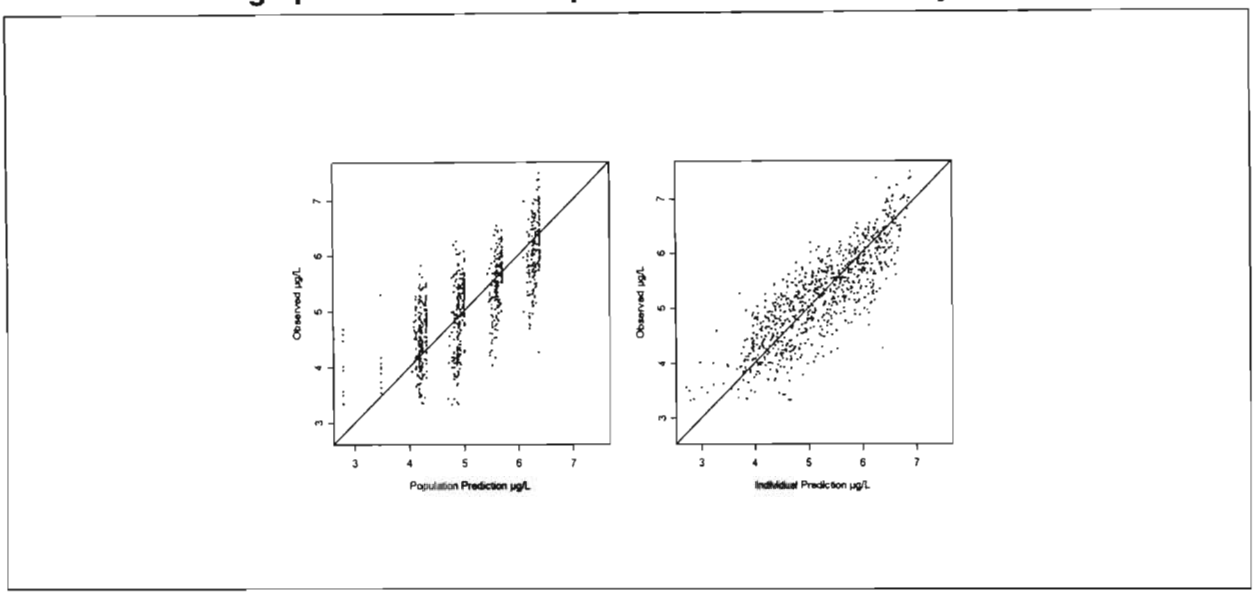
Figure 33: **Model diagnostics – observed versus model predicted concentrations for the one-compartment model are shown as points on the graph. The solid line represents the line of identity.**



As depicted in the model diagnostic plots Figure 33, the 1 compartment model (Appendix 9) gives an adequate description of the data – however the 2-compartment model (Appendix 10) provided a better fit as judged by a drop in objective function value of 188 (-243 versus -431). All attempts to model the entero-hepatic recirculation (EHR) that was observed in the data were unsuccessful. The model that was used for EHR is shown in Appendix 11.

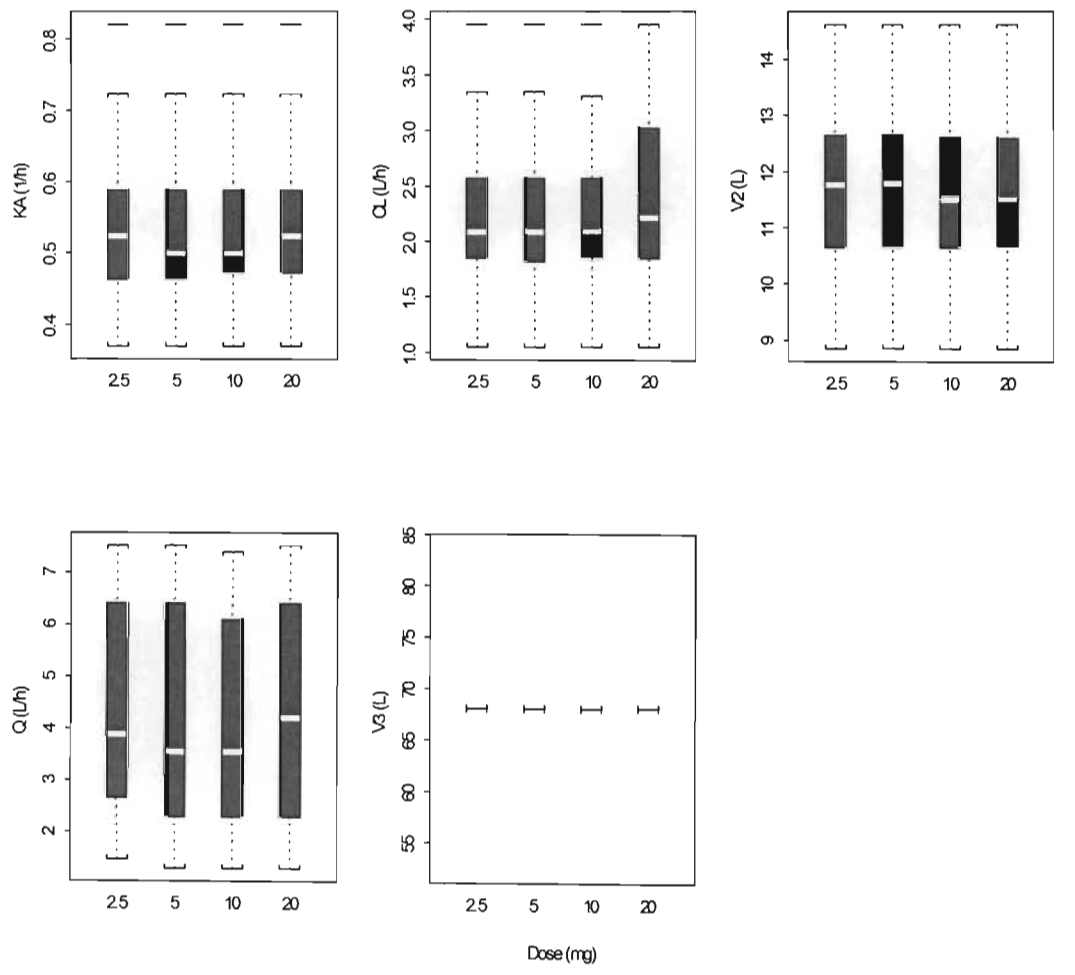
Visual inspection of figure 37 below indicates that subjects 4, 7, 9, 10 and 14 all showed EHC at 20.0mg and subjects 4, 9 and 10 at 10mg of glibenclamide. The majority of subjects (19) did not show EHC. Furthermore there were not enough data points to fully characterize the EHC profile.

Figure 34: **Model diagnostics – observed versus model predicted concentrations from the final two-compartment model are shown as points on the graph. The solid line represents the line of identity.**



The model was implemented in NONMEM as follows. The subroutine ADVAN4, which implements an explicit solution for the two-compartment linear model with first order absorption, was chosen. Using the subroutine TRANS4, the estimated NONMEM/PREDPP model parameters comprised, first order absorption rate constant (Ka); V2/f and V3/f (apparent volume of distribution for the central and peripheral compartments); CL/f and Q/f (apparent clearance from the central compartment and inter-compartmental clearance between the central and peripheral compartments respectively).

Figure 35: **Model diagnostics - boxplots of population pharmacokinetic parameters versus dose. The clear horizontal bar in the centre of the box shows the median; the box encloses the inter-quartile range i.e. 50% of the data. The whiskers show the interval of values outside the box and values far outside are represented by horizontal dashes**



The final model was subjected to a posterior predictive check (Yano et al. 2001). In this procedure, 500 replications were run using the fixed and random effects from the final population pharmacokinetic model and using a study design identical to that used in this study. The median AUC for each dose from each replicate was calculated and the distribution of AUC values was compared with the median for each dose level calculated using NCA methods. Figure 36 shows that the observed (NCA) median AUC falls within the distribution of simulated AUC values confirming the good predictive ability of the model.

Figure 36: **Posterior predictive check of final 2-compartment model. Bars show the distribution of simulated median AUC calculated from 500 replications of a dose escalation PK study with 24 subjects (design identical to current study). The dashed vertical line shows the median AUC observed in the current study**

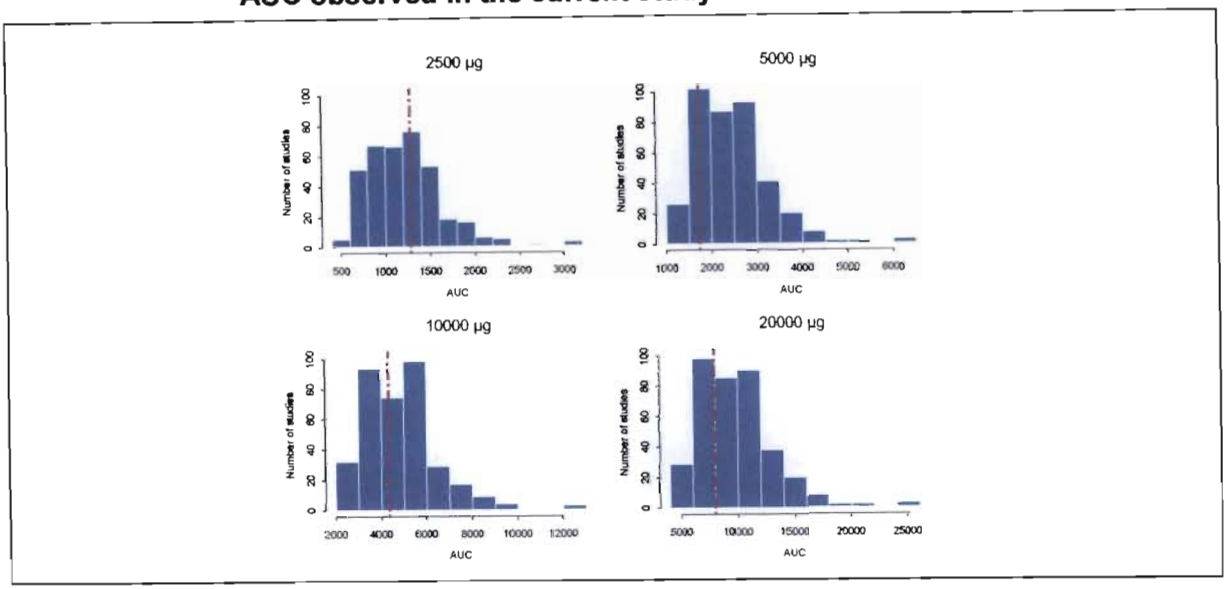


Table 50: **Population pharmacokinetic parameters from the 1-compartment and the final 2-compartment model**

	One Compartment Model				*Final two compartment model*			
	Estimate	*SE*	*RSE (%)*	*BSV (%CV)*	*Estimate*	*SE*	*RSE (%)*	*BSV (%CV)*
KA (1/h)	2.39	0.34	14.39	51.77	0.53	0.04	8.33	28.57
CL/f (L/h)	1.52	0.12	7.63	34.50	2.16	0.16	7.41	33.91
V/2f (L)	38.90	2.70	6.94	25.77	11.70	1.11	9.49	23.04
Q/f (L/h)	-	-	-	-	3.84	0.58	14.97	65.35
V3/f (L)	-	-	-	-	68.10	6.00	8.81	0.02
Residual variability variance (%CV)	0.244 (49.4%)				0.189 (43.5%)			

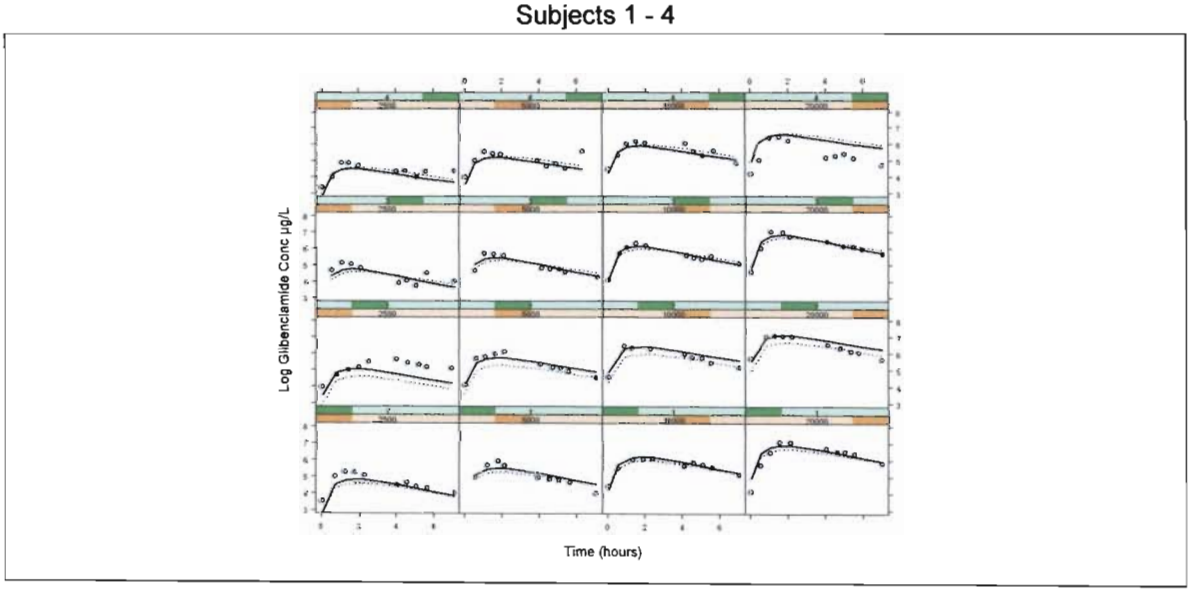
Ka = first order absorption rate; CL/f: apparent clearance; V2/f: apparent volume of the central compartment; Q/f: apparent inter-compartmental clearance; V3/f: apparent volume of the peripheral compartment; SE: standard error of the estimate; RSE: relative standard error of the estimate; BSV: between subject variability; CV: coefficient of variation

The pharmacokinetic parameters of glibenclamide from the 1-compartment and the final 2-compartment model are presented in Table 50. The pharmacokinetics of glibenclamide is linear after multiple dose administration in the dose range 2.5 to 20 mg as suggested by the model diagnostic box plots of population pharmacokinetic parameters versus dose (Figure 35). The clear horizontal bar in the centre of the box shows the median; the box encloses the inter-quartile range i.e. 50% of the data. The whiskers show the interval of values outside the box and values far outside are represented by horizontal dashes. Figure 35 shows the absence of any trend in the distribution of the pharmacokinetic parameters with dose.

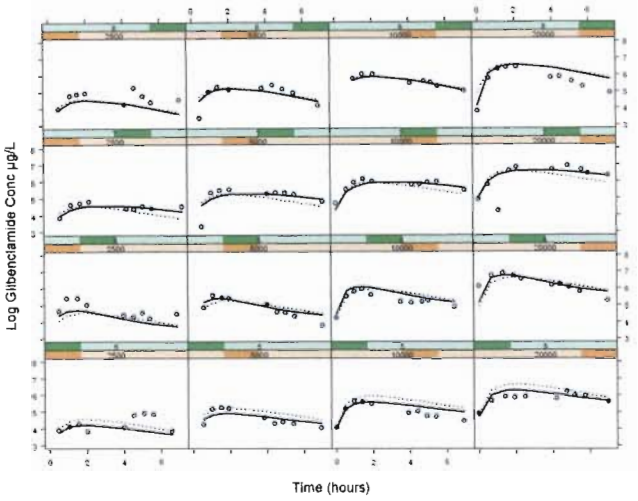
Table 51 **Individual pharmacokinetic parameter estimates from the final 2 compartment model**

ID	Ka (1/h)	CL/f (L/h)	V2/f (L)	Q/f (L/h)	V3/f (L)
1	0.63	2.10	10.03	2.77	68.10
2	0.70	1.46	9.26	2.92	68.10
3	0.67	2.30	9.94	3.56	68.10
4	0.57	2.23	11.42	6.05	68.10
5	0.43	2.45	12.85	6.88	68.10
6	0.72	1.90	9.88	5.77	68.10
7	0.40	1.90	13.41	1.88	68.10
8	0.50	3.03	11.80	1.94	68.10
9	0.37	1.90	14.61	2.32	68.10
10	0.49	1.86	12.28	2.19	68.10
11	0.55	2.01	11.31	2.68	68.10
12	0.48	3.95	12.66	6.41	68.10
13	0.46	2.58	12.61	7.17	68.10
14	0.50	1.83	11.32	1.30	68.10
15	0.55	3.27	11.26	7.30	68.10
16	0.42	1.23	13.41	2.93	68.10
17	0.47	1.81	13.07	7.40	68.10
18	0.59	1.06	10.68	3.42	68.10
19	0.82	3.18	8.87	4.22	68.10
20	0.52	3.35	11.97	7.54	68.10
21	0.48	2.23	11.94	6.11	68.10
22	0.43	1.75	12.49	1.46	68.10
23	0.56	3.31	11.53	5.38	68.10
24	0.53	2.31	11.65	4.48	68.10

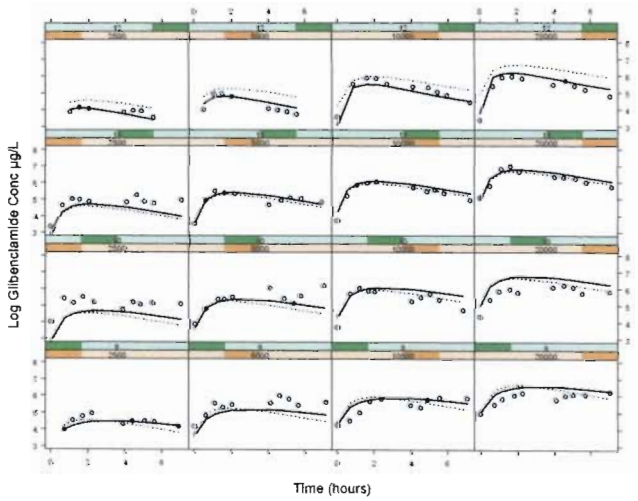
Figure 37: **Plots of observed log glibenclamide concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) from the final 2 compartment model. Each row in the plot represents data for a single subject while each column represents data from a specific dose level. Blank cells indicate the subjects who did not receive that particular dose.**



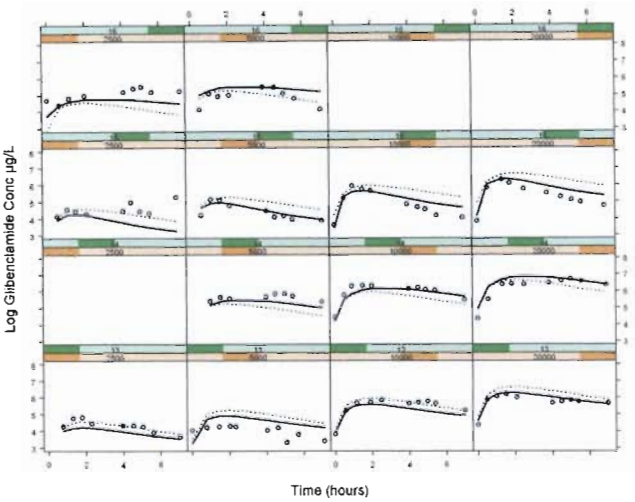
Subjects 5 - 8



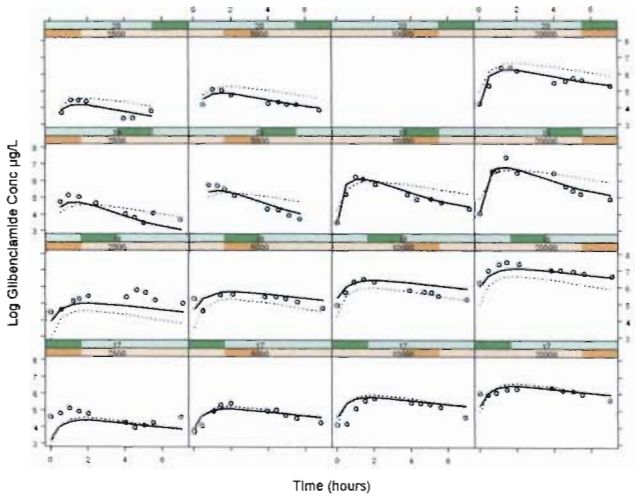
Subjects 9 - 12



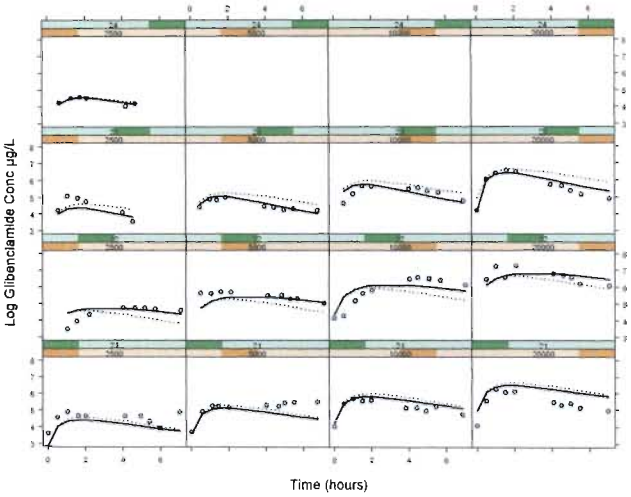
Subjects 13 - 16



Subjects 17 - 20



Subjects 21 - 24



5.1.5 Discussion of Population PK modeling

Three population pharmacokinetic models (Appendix 9-11) were fitted to the glibenclamide PK data using non-linear mixed effects modeling. While both the one and two compartment models terminated successfully, and produced similar graphical model diagnostic plots, the 2-compartment model provided a better comparative fit due to its significantly lower OBJ function (MVOF -243.409 vs MVOF -431.164). A 3-compartment model was also attempted but was considered over parameterized as the model failed to achieve successful convergence due to the inter-compartment transfer rate constants being estimated as infinate. This suggested that the third compartment was poorly defined.

Despite extensive attempts at model refinement, the enterohepatic recycling (EHC) model did not converge successfully. This is possibly due to wide between subject variability (BSV) as well as within subject variability (WSV) in EHC i.e. some subjects show evidence of EHC at some doses but not at other dose levels. Furthermore there were not enough data points to fully characterize the EHC profile in all subjects. This model was eventually abandoned and the 2-compartment model was accepted as the final model. It was acknowledged that the poorly fitted EHC component of the profile in the final model would contribute to an inflated residual variability due to model mis-specification. Despite this drawback however, the 2-compartment model provided a good fit of the model to the data as confirmed by the PPC. In particular, the model was able to provide an estimate of exposure (AUC) to glibenclamide that was consistent with the NCA estimates. This was especially important since one use of the PK model was to provide an estimate of average glibenclamide concentration (Cpss) for use as the driving force in a PKPD model. PPC is a method for the objective assessment of the predictive ability of a model. PPC is a robust method for model evaluation. Yano et al. (2001) concluded that 'if a PPC invalidates a model, one can be reasonably certain that the model has serious deficiencies.'

The low RSE's indicate the good precision of the estimated parameters and the anticipated relatively high residual variability was also noted (43.5% CV). The residual variability comprises of assay error, deviations from the model specification, and intra-subject variability. In the final 2-compartment model selected, one source of model misspecification is the inability to characterise the EHC of glibenclamide that was noted in several subjects. In addition, in some subjects the pre-dose glibenclamide concentration was also not well fitted by the model. This might reflect the lower degree of confidence in the dosing history (compliance with regard to timing and size of dose or even administration) for the un-supervised doses that contribute to the pre-dose concentration.

Individual plots of the glibenclamide concentration versus time profiles from the final 2-compartment model show a very close agreement between the observed and model-predictions. The relatively low BSV in CL (~34% CV) and V (~23% CV), suggested that covariates might not significantly improve the population fit. In addition, graphical examination of the PK parameters vs covariates did not reveal any obvious relationships. Consequently, no formal covariate analysis was conducted.

The pharmacokinetic parameters derived from the 2 compartment model are discussed in relation to published data.

Glibenclamide is completely absorbed after oral administration and the rate and extent of absorption is not affected by food (Jaber et al., 1993). Therefore, estimates for clearance and volume of distribution after oral administration are considered to be close approximations of those after intravenous administration. In this study, the dose of glibenclamide was taken on an empty stomach, 10 minutes before breakfast. Glycomin® (glibeclamide) is bioequivalent to the innovator product registered in South Africa i.e. Daonil®.

Using a 1 compartment model, the **Vd** was 38.90 L (0.55 L/kg) which approximates that reported by Tracewell et al. (1998) [43.7 L; 0.509 L/kg] in their study of glibenclamide pharmacokinetics using a one compartment model with first order absorption and first order elimination. Other studies quoted by Tracewell et al. (1998) reports Vd values of 0.735 ± 0.0150; 0.19 ± 0.01; 0.125 ± 0.008, 0.017 ± 0.00714; 0.200 ± 0.032; 0.144 ± 0.0156 ;0.0413 ± 0.000975 and 0.57 ± 0.57 L/kg. Jaber et al. (1994) reported a Vd of 51 ± 51 L/h using a 1 compartment model after a 12 week study. The Vd of this population is within the range reported in the literature.

The volume of distribution ± SE of glibenclamide for the 2 compartment model is 11.70 ± 1.11 L and 68.1 ± 6.0 L for the central and peripheral compartments respectively. This difference may be due to the separation of the compartments during modeling.

The average **clearance** for glibenclamide is 1.52 ± 0.12 L/h (0.02 L/h/kg) for the 1 compartment and 2.16±0.16 for the 2 compartment model. The intercompartmental clearance (Q/f) is 3.84±0.58L/h. Tracewell et al. (1998) reported average values for glibenclamide clearance of 0.0387 ± 0.00642 L/h/kg in younger diabetics (< 60 years) and 0.0525 ± 0.00349 L/h/kg in older subjects (> 60 years). Other studies quoted by Tracewell et al. (1998) reported clearance values of 0.107 ± 0.051; 0.078 ± 0.00516; 0.0634 ± 0.00803; 0.09 ± 0.03; 0.078 ± 0.029 and 0.0394 ± 0.00891 L/hr/kg. In addition, clearance values ± SE of 3.2 ± 2.1, 4.41 L/h (range 3.38-8.11 for Caucasians) and 4.10 L/h (range 2.91-5.98 for Chinese); and 3.70 L/h (1.15) were reported by Jaber et al. (1994); Jonsson et al., (2000) and Jonsson et al., (1998) respectively. The clearance value obtained for this study [mean age 54.1 ± 9.2 years] fall within the range of other reported studies and that of Tracewell et al. (1998) for their study population aged < 60 years.

The average **Ka** (±SE) is 2.39 ± 0.34 h^{-1} (1 compartment model) and 0.53 ± 0.04 h^{-1} (2 compartment). Jonsson (1998), Rydberg (1997) and Tracewell et al. (1998) reported Ka's of 2.68 ± 1.50; 0.756 and 0.057± 0.244 h^{-1} respectively.The variation in Ka's "*may be due to various problems related to kinetic sensitivity (in reflecting a given change of ka), linearity (when considered versus ka), specificity (they depend on other parameters except the ka) and/or their precision of estimation of the system. With the currently available methodologies and with no ideal absorption rate constant metric available, absorption rate cannot be accurately estimated.*" (Reppas, 2003). Furthermore the sampling interval in the absorptive phase coupled with variations in physiological factors may also contribute to this wide variation.

5.1.6 Conclusions from PK modeling

Using the population approach and the software NONMEM, the pharmacokinetics of glibenclamide in this study population was described by a two compartmental disposition model with first order absorption. The pharmacokinetics of glibenclamide in this study population is comparable to that reported in the literature, save for Ka. The model was subjected to internal validation using the PPC approach and shown to provide acceptable model predictions of AUC and hence average glibenclamide concentration. This estimate of average concentration will be used to further investigate the pharmacokinetics and pharmacodynamics of glibenclamide.

5.2 Population pharmacokinetic-pharmacodynamic modeling

5.2.1 Introduction

In this study, drug concentration and the corresponding blood glucose and blood insulin concentrations were measured. While it is possible to model the full glibenclamide-insulin-glucose system, the glucose-glibenclamide system was used in this study, in keeping with the work of other researchers (Rydberg et al., 1997 and Tracewell et al., 1998). In clinical practice the effect of glibenclamide on glucose requires less technical skill and is less costly, than with insulin. The clinical effectiveness of antidiabetic treatment and hence decisions on management of diabetes, is determined by fasting and/or postprandial blood glucose levels. Hence, this study utilizes the glibenclamide-glucose system for PK/PD analysis.

The advantage of using the model-based analysis, is that the dose is treated as a continuous variable and the breaking point can be identified by interpolation.

In this dose escalation study a zero dose (absence of glibenclamide) was employed to characterize the insulin and glucose profiles. Insulin and glucose (for the purposes of this analysis) are regarded as physiological substances whose production and synthesis are interdependent. Hence, the methodology of Kryzyzanski et al. (2000) using Fourier analysis was adopted. Kryzyzanski et al. (2000) described a mathemathical basis and an algorithm for application of Fourier analysis to quantify variable, but biorhythmic, physiologic substances in order to generate input functions for use in pharmacodynamic indirect response models. This method does not require trial and error non-linear regression analysis to identify the optimal number of harmonics that describe the response pattern (Kryzyzanski et al., 2000).

The population approach using nonlinear mixed effects modeling as implemented in the software NONMEM (Globomax LLC, USA and NONMEM Project Group, University of California, San Francisco) was used in this analysis. Appendix 7 contains the NONMEM data file for the pharmacodynamic analysis. This model-based analysis was conducted to fully characterize the dose-exposure-response relationship as a monotonic function and thereby facilitate interpolation and prediction of response for doses and exposures not formally studied or observed.

In modeling the PKPD relationship, 2 broad categories of models were examined – models with glibenclamide **dose** as the driving force for the PD response variable and models with glibenclamide **concentration** as the driving force. In this way, the role of PK variability on the overall variability was examined. The PD response metric that was tested included FBG, mean glucose concentration and the full glucose concentration profile. Thus during PKPD modeling the following models were tested:

- Dose as driving force on FBG
- Cpss as driving force on FBG
- Dose as driving force on mean glucose concentration
- Cpss as driving force on mean glucose concentration
- Dose as driving force on full glucose profile
- Cpss as driving force on full glucose profile

5.2.2 Data for population PKPD modeling

There were 24 individuals who contributed to the PKPD dataset. All except 2 subjects contributed data on all dose escalation steps. Subject 16 could not proceed to a dose escalation beyond 5 mg/day due to the presence of symptoms of hypoglycaemia and subject 24 did not have complete data sets.

5.2.3 Models with fasting glucose concentration as PD end-point

5.2.3.1 Dose as driving force on fasting glucose concentration

Model description

The effect of glibenclamide on glucose response was modeled with an inhibitory Emax model as shown in Equation 7.

Equation 7

$$\text{Effect} = E_0 * [(1 - (\text{Dose} * \text{Emax})/(\text{Dose} + \text{ED50})]$$

Where:

E_0 is the baseline glucose concentration

ED50 is the dose that leads to 50% inhibition of the glucose concentration

Emax is the maximum response to glibenclamide

Effect is the fasting blood glucose concentration

This Emax model has properties that account for the hyperbolic shape of the dose-response relationship. The parameters are maximum response (Emax) and potency. The model itself is limited in explaining differences between regimens (i.e., different PK inputs) or understanding the sources of variability. Differences in ED50 could be due to differences in drug exposure (PK), or differences in PD sensitivity. Hence, to elucidate these questions regarding regimen and the underlying variability, PK and PD data need to be modeled.

Results from model for dose as driving force on fasting glucose

The observed vs population predicted glucose concentrations shows even distribution around the line of best fit, as does the observed vs individual predicted glucose concentrations. While these graphical representations suggest that the model is suitable in describing the effect of dose on fasting glucose concentrations, it does not consider the changing glucose profile throughout the 8 hour study period, nor does it take into consideration the pharmacokinetics of glibenclamide. This is cofirmed by the coincidence of individual plots of observed glucose concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) from the Dose-Fasting Glucose plots (figure 39). Subject 4 the observed and model prediction profiles are superimposable. This suggests that the model is describing the observed data.

Figure 38: **Model diagnostics – observed versus model predicted glucose concentrations for the Dose-Fasting Glucose Concentration pharmacokinetic-pharmacodynamic model are shown as points on the graph. The solid line represents the line of identity.**

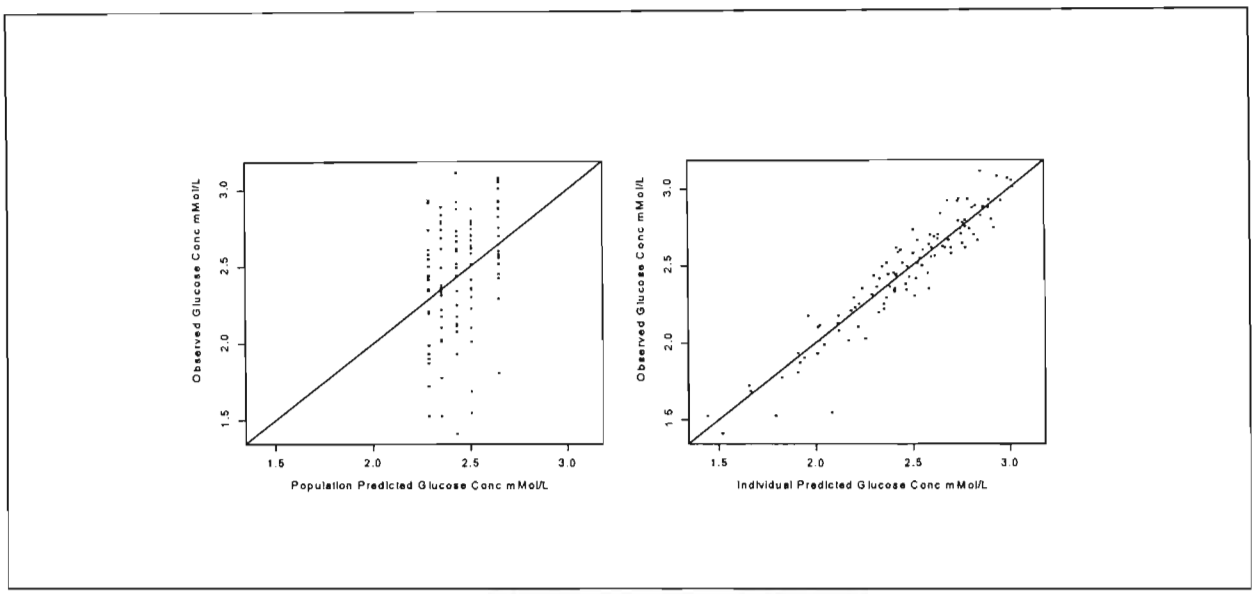
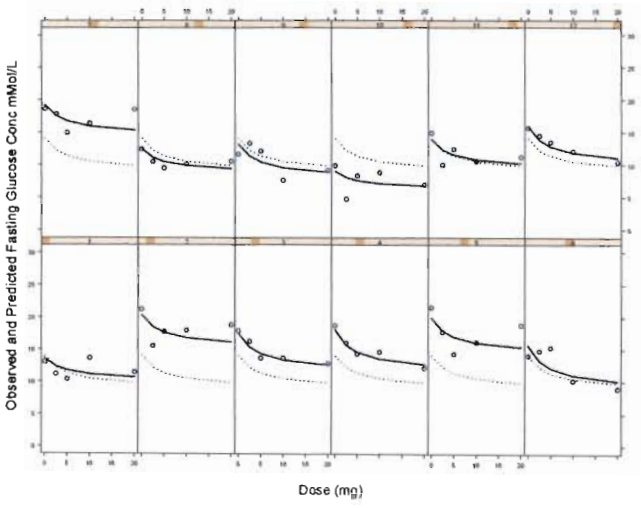


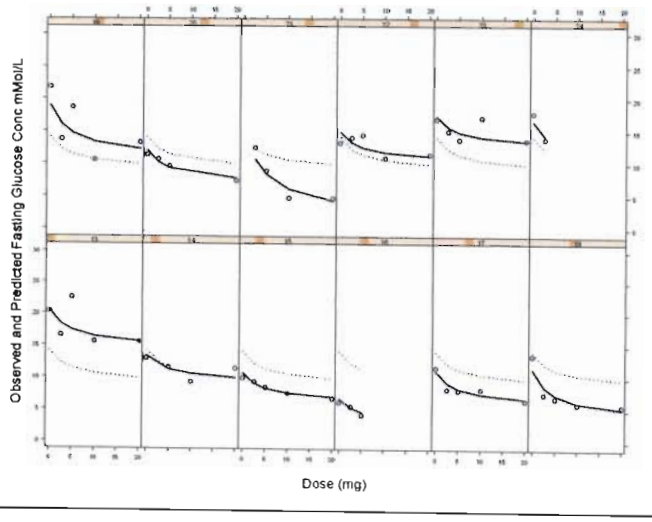
Figure 38 is a model diagnostic plot of observed vs predicted FBG concentrations (population and individual) and shows an even distribution around the line of identity. These global graphical representations suggest that the model provides a satisfactory description of the data. In figure 39, however, the model fit at the individual level is shown. While the model predictions show a generally good correspondence with the observed data, there are several data points in individual subjects that are poorly fitted.

Figure 39: **Plots of observed glucose concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) from the Dose-Fasting Glucose Concentration PKPD model for glucose response to glibenclamide. Each cell represents the data for an individual subject shown as a dose-response plot – i.e. y-axis shows fasting glucose concentration in mmol/L; x-axis shows dose in mg.**

Subjects 1 - 12



Subjects 13 - 24



5.2.3.2 Cpss as driving force on fasting glucose concentration

Model description

This Emax model has properties that account for the hyperbolic shape of the **concentration** response relationships (equation 4 and 5) rather than the **dose** response relationship. In contrast to the previous dose response model it is now possible to differentiate between PK variability (in CL/f) and PD variability.

Equation 8

$$Effect = E_0 * (1 - (Cpss * Emax)/(Cpss + EC50))$$

Where Cpss is calculated as per Equation 9 using the CL/f estimated from the Population PK model (Table 51).

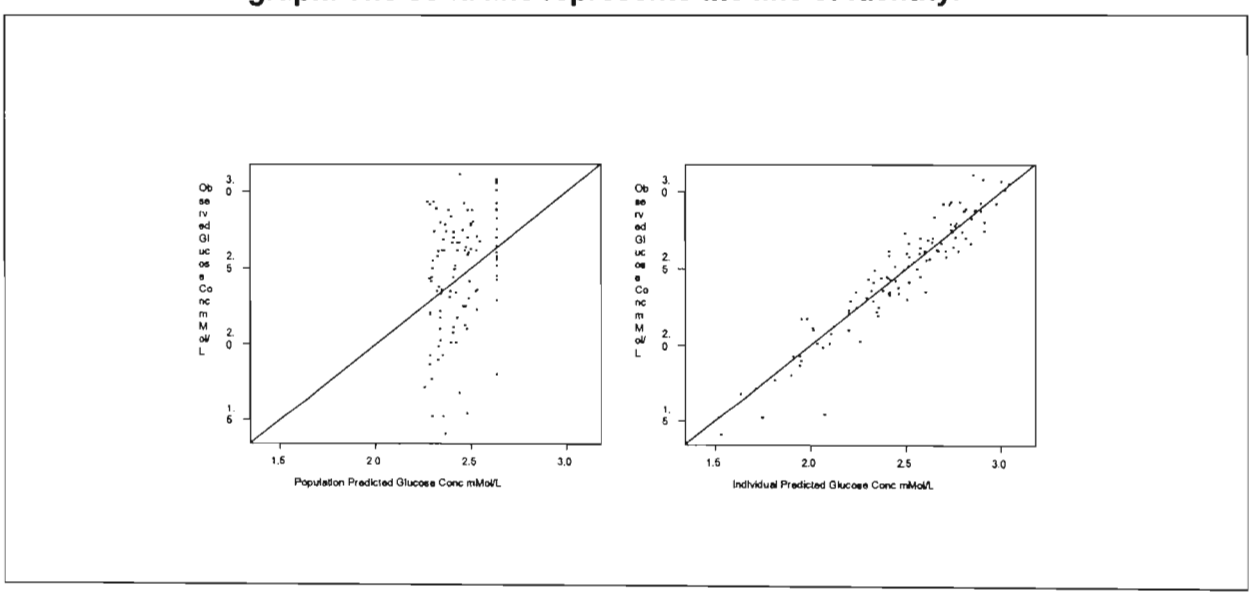
Equation 9

$$Cpss = Dose/(CL/f * 24)$$

This model is not very sensitive for understanding differences between regimens (i.e., different PK inputs) but may be satisfactory for this data set because this is a steady state response.

Results from model for Cpss as driving force on fasting glucose concentration

Figure 40: **Model diagnostics – observed versus model predicted glucose concentrations for the Cpss-Fasting Glucose Concentration pharmacokinetic-pharmacodynamic model are shown as points on the graph. The solid line represents the line of identity.**



In Figure 40 the observed versus model predicted FBG concentrations is shown for the model that now includes PK variability. The individual predictions from this model are shown in figure 41. This model using Cpss as the driving force for the PD response incorporates the individual subject's clearance in determining the FBG concentrations i.e., it accounts for the individual PK variation which might contribute to the overall PD response. When comparing the diagnostic plots for these 2 models (Figure 39 versus Figure 41 and Figure 38 versus Figure 40), there does not appear to be any striking difference in the predictions. The poor fit of some data points in individual subjects that was observed with the Dose model has not been improved by the Cpss model.

The population model predictions are overestimated in subjects 10, 15, 17, 18, 20 and 21 and underestimated in subjects 2, 3, 4, 5, 7, 13, 19 and 23. There does not appear to be any striking differences in the observed vs predicted or individual subject fits of the model to the data when compared to the validity of the dose as driving force on fasting glucose model.

Figure 41: **Plots of observed glucose concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) for the Cpss-Fasting Glucose Concentration PKPD model for glucose response to glibenclamide. Each cell represents the data for an individual subject shown as a dose-response plot – i.e. y-axis shows fasting glucose concentration in mMol/L; x-axis shows dose in mg.**

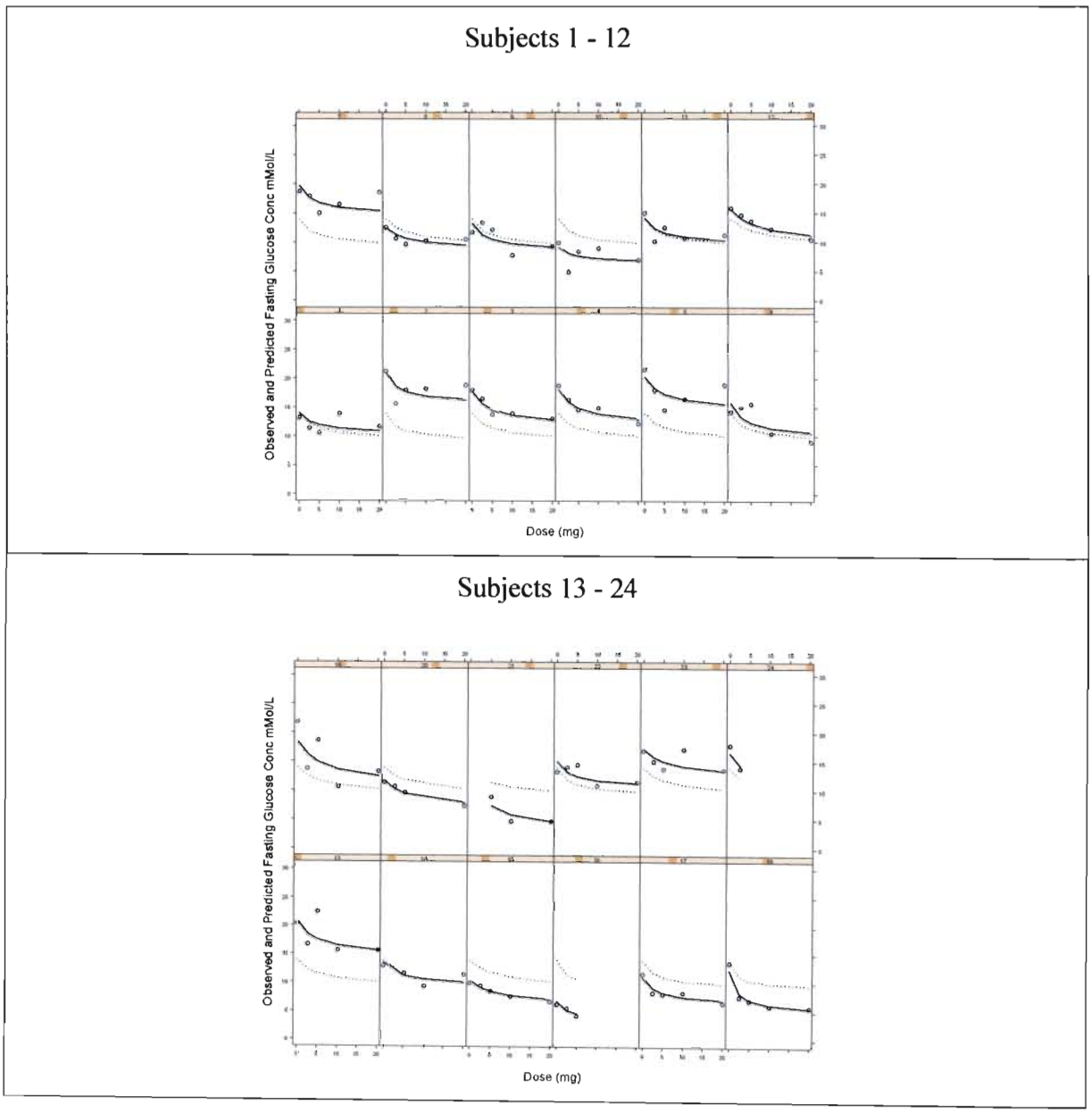


Table 52: Population pharmacokinetic-pharmacodynamic parameters for the models with fasting blood glucose as the PD response

	Dose – Fasting Glucose Model			Cpss – Fasting Glucose Model		
	Estimate	RSE (%)	BSV (%CV)	Estimate	RSE (%)	BSV (%CV)
E_0 (mMol/L)	14.10	6.35	29.05	14.30	6.04	27.40
Emax	0.37	37.47	48.68	0.39	22.64	55.50
ED50 (mg)	4.56	77.19	0.01	Derived ED50 = 4.41*		
EC50 (ug/L)				85.20	26.29	0.04
Residual variability						
variance (%CV)	0.02 (15%)			0.02 (14%)		

E_0 = Baseline glucose concentration; Emax = maximum inhibition of glucose concentration; EC50 is the glibenclamide concentration producing 50% inhibition of glucose concentration; ED50 is the glibenclamide dose producing 50% inhibition of glucose concentration; RSE: relative standard error of the estimate; BSV: between subject variability; CV: coefficient of variation
* Derived ED50 = Cpss * Cl/f * 24 where Cpss = 85.20 ug/L and Cl/f = 2.16 L/h

Comparing Dose vs FBG or Cpss vs FBG as the driving force for the model, the parameters are estimated with better precision i.e., lower RSE (6.35% vs 6.04%) for the model with Cpss as the driving force. Apart from this, there is little difference in the estimated model parameters. There is a low variability in the estimate of potency for dose-fasting glucose model (ED50= 4.56mg) as compared to Cpss-fasting glucose model (ED50 derived= 4.41mg).

The parameters from the Dose and the Cpss models for FBG as the PD response are compared in Table 52. The point estimates for the fixed effects model parameters are essentially the same for the 2 models - however the parameters are estimated with better precision i.e., lower RSE with the Cpss model. The estimates for BSV are different for all parameters - essentially larger for the Cpss model. Of particular interest however, is that the estimate of potency (ED50 and EC50) of between 4 and 5mg/day does not reflect the expectation from Figure 21 that the potency should lie between 0 and 2.5mg. In addition, it is disconcerting that there is remarkably low variability in the estimate of potency with both models.

5.2.4 Models with mean glucose concentration as PD end-point

5.2.4.1 Dose as driving force on mean glucose concentration

Model description

The model is as described in Equation 7. However, response is now the mean glucose concentration rather than FBG. The mean glucose concentration is calculated as the area under the glucose concentration versus time curve divided by the time over which the glucose concentrations were measured. The limitations of this model are similar to those mentioned for the model described above. Appendix 12 contains the NONMEM code for the Dose/Cpss-Mean glucose profile.

Results from model with dose as driving force on mean glucose concentration

Figure 42 is a model diagnostic plot of observed vs predicted mean glucose concentration (population and individual) and shows an even distribution around the line of identity. These global graphical representations suggest that the model provides a satisfactory description of the data. In Figure 43, the model fit for each individual is shown. These model predictions show a generally good correspondence with the observed data.

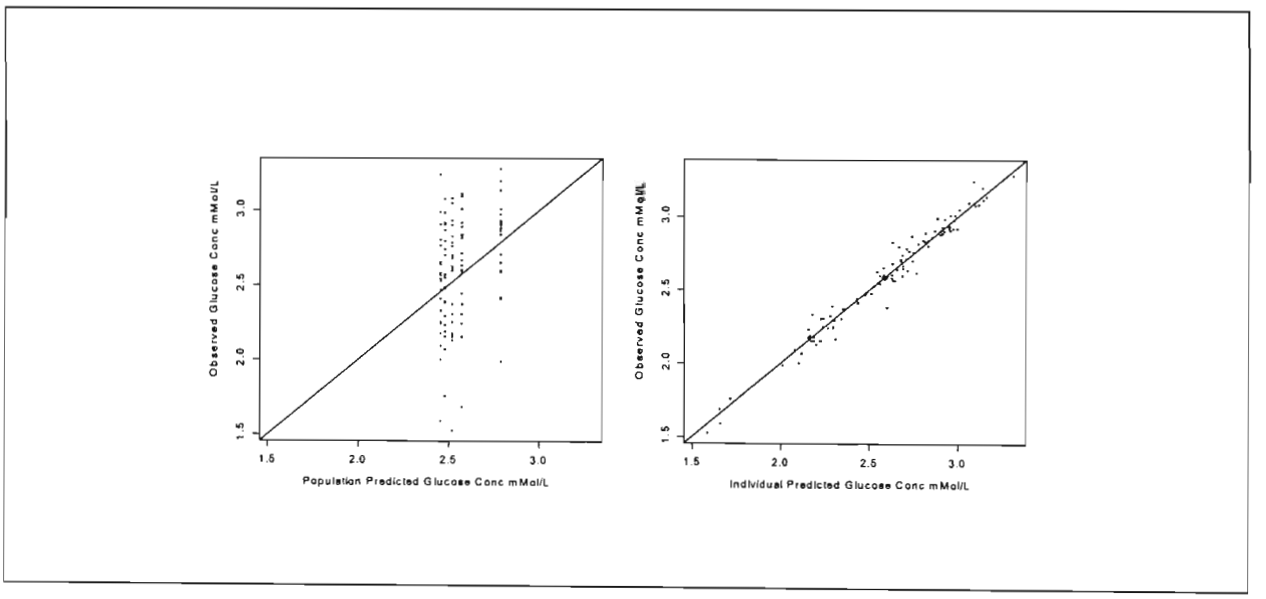
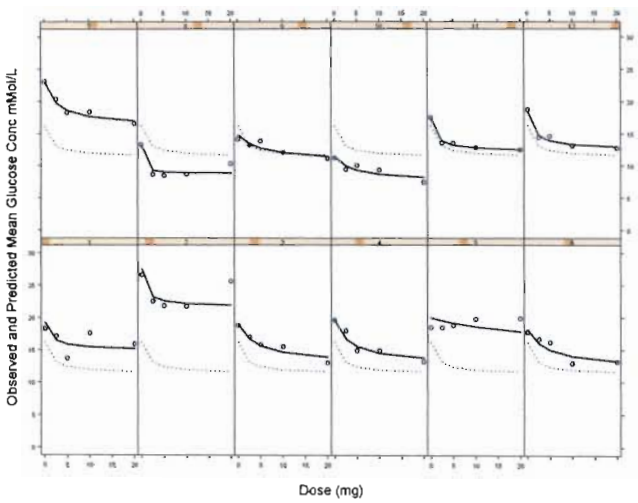


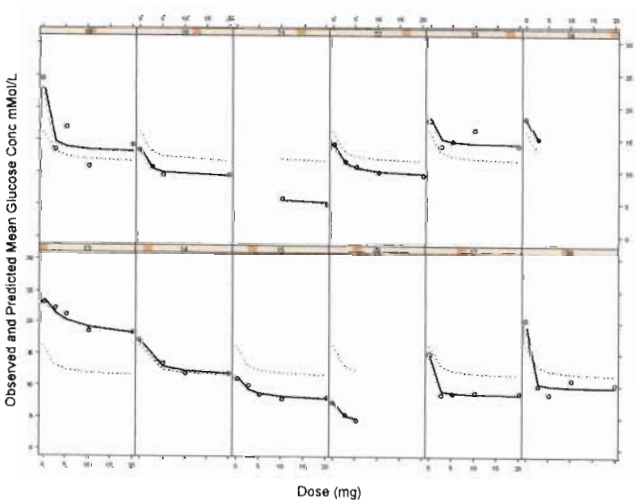
Figure 42: **Model diagnostics – observed versus model predicted glucose concentrations for the Dose-Mean Glucose Concentration pharmacokinetic-pharmacodynamic model are shown as points on the graph. The solid line represents the line of identity.**

Figure 43: Plots of observed mean glucose concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) for the dose-mean glucose Concentration PKPD model for glucose response to glibenclamide. Each cell represents the data for an individual subject shown as a dose-response plot – i.e. y-axis shows mean fasting glucose concentration in mMol/L; x-axis shows dose in mg.

Subjects 1 – 12



Subjects 13 – 24



5.2.4.2 Cpss as driving force on mean glucose concentration

Model development

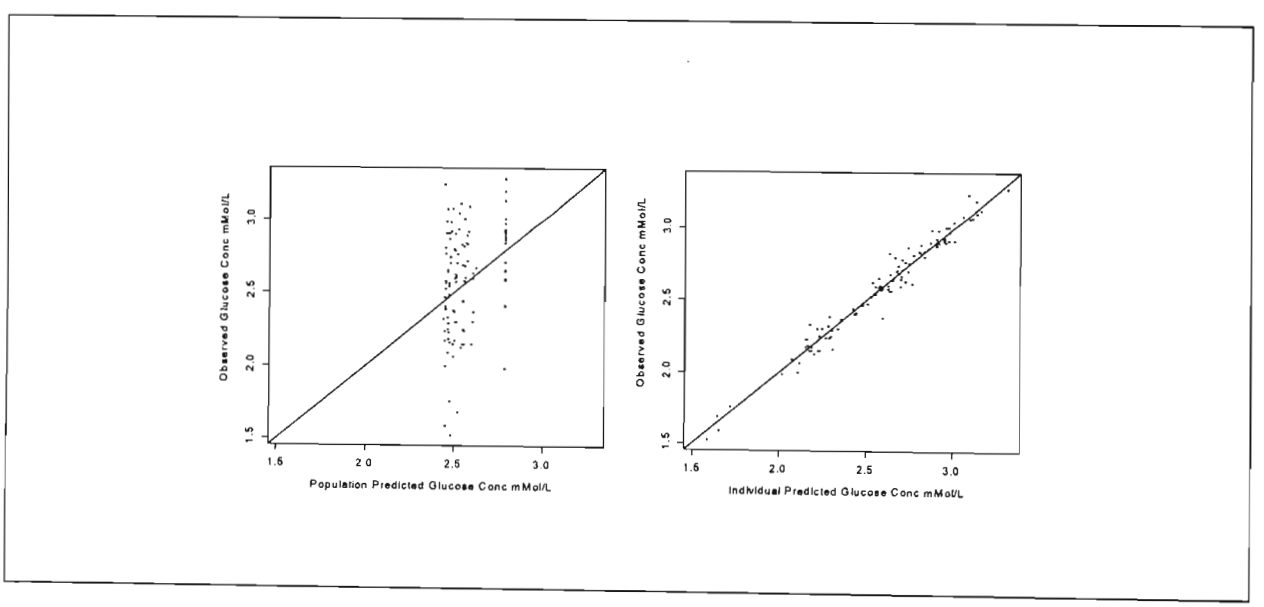
The model is as described in Equation 8. However, response is now the mean glucose concentration calculated as the area under the glucose concentration versus time curve divided by the time over which the glucose concentrations were measured.

Results from model with Cpss as driving force on mean glucose concentration

In this model both the pharmacokinetics of glibeclamide (Cpss) and pharmacodynamics (mean glucose concentration) are incorporated. There is minimal difference between the models: dose vs average glucose concentration and the Cpss vs average glucose concentration. In figure 44 the observed vs individual model predicted glucose concentrations are symmetrically distributed along the line of best fit. This suggests that the model is predictve for individual subjects. In the case of population model predicted glucose concentrations there is equal distribution along the line of best fit, depicting the over and underestimation of the model.

However, the pharmacodynamic variability overshadows the pharmacokinetic variability in this model (Fig 44). Inspection of figure 45 shows that the model describes the individual observed glucose, individual predicted and population predicted glucose concentrations for subjects 9, 11, 12 and 14. It equally underestimates and overestimates the remaining subjects, however the model describes the observed mean glucose and individual predicted average glucose concentrations.

Figure 44: **Model diagnostics – observed versus model predicted glucose concentrations for the Cpss - Mean Glucose Concentration pharmacokinetic-pharmacodynamic model are shown as points on the graph. The solid line represents the line of identity.**



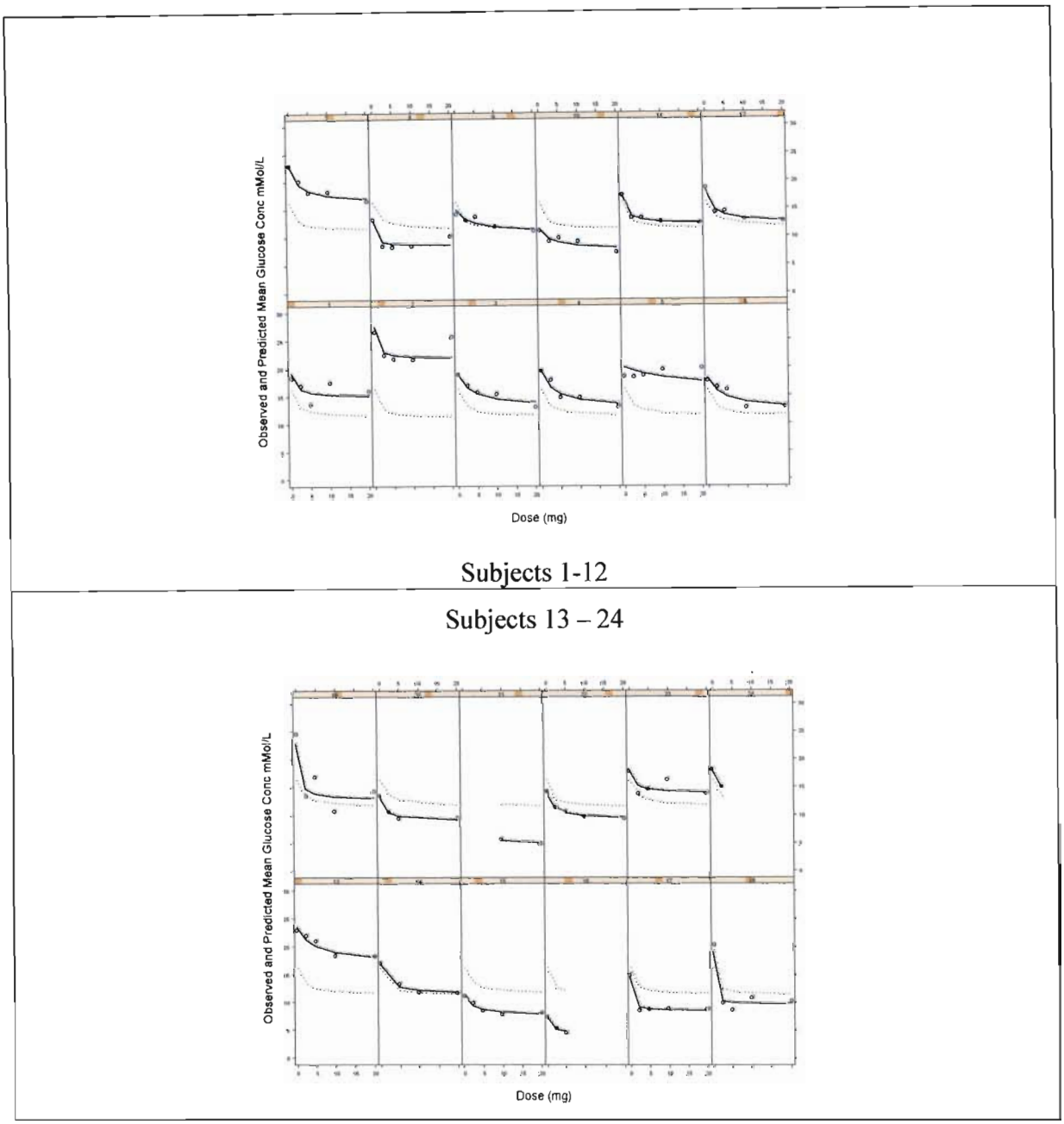


Figure 45: **Plots of observed glucose concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) for the Cpss - Mean Glucose Concentration PKPD model for glucose response to glibenclamide. Each cell represents the data for an individual subject shown as a dose-response plot – i.e. y-axis shows fasting glucose concentration in mMol/L; x-axis shows dose in mg.**

Table 53: Population pharmacokinetic-pharmacodynamic parameters for the models with mean blood glucose as the PD response

	Dose – Mean Glucose Model			Cpss – Mean Glucose Model		
	Estimate	RSE (%)	BSV (%CV)	Estimate	RSE (%)	BSV (%CV)
E_0 (mMol/L)	16.70	5.74	27.07	16.70	5.74	26.94
Emax	0.34	14.32	50.99	0.34	14.49	50.89
ED50 (mg)	1.85	40.49	120.42	Derived ED50 = 1.87*		
EC50 (ug/L)				36.00	42.78	108.17
Residual variability						
Variance	0.008			0.008		
(%CV)	(8.75%)			(8.92%)		

E_0 = Baseline glucose concentration; Emax = maximum inhibition of glucose concentration; EC50 is the glibenclamide concentration producing 50% inhibition of glucose concentration; ED50 is the glibenclamide dose producing 50% inhibition of glucose concentration; RSE: relative standard error of the estimate; BSV: between subject variability; CV: coefficient of variation
* Derived ED50 = Cpss * Cl/f * 24 where Cpss = 36.00 ug/L and Cl/f = 2.16 L/h

Table 53 shows virtually no difference in the fixed effect parameter estimates from the Dose or the Cpss models when mean glucose is the PD response. The random effects parameters are also similar – with the exception of the estimate of potency where a decrease in BSV is noted i.e. inclusion of PK variabilility in the model by using Cpss as the driving force resulted in a decrease in the BSV from 120 % CV for ED50 to 108 % CV for EC50.

When one compares these models where the PD response is mean glucose concentration with the models where FBG was the PD response (Table 52), a higher estimate for the modeled baseline glucose concentration (16.70 vs 14.10 mMol/L) is noted. This is to be expected with these different PD metrics: FBG versus mean glucose concentration. The maximum effect seen with both groups of models is very similar. A more realistic estimate of potency (EC50 or ED50) is noted in the current model viz. approximately 1850-1870 µg. In addition, a much larger variability is noted in the potency parameters relative to the model where the pharmacodynamic response was FBG. By calculating the mean from several glucose concentrations, one more closely approaches the subject's 'true' glucose concentration. It might be argued that a fasting measurement i.e., one taken when the biological system is not being subjected to the known factors that can influence glucose response (e.g. food) would be subject to lower variability. This is likely to be true if several FBG measurements were drawn rather than a single value i.e. the observed better performance of the current model is more likely to be a reflection of the fact that the mean of multiple glucose measurements were used. One striking piece of evidence for this is the dramatic decrease in the residual variance from 0.02 to 0.008 i.e., a decrease of 60%.

While this model provides a good description of the data and is considered adequate for the purposes of this analysis, one further attempt at building a model for the PD effect was considered viz. modeling the full glucose concentration versus time profile.

5.2.5 Models with full glucose profile as PD end-point

Description of model for placebo response

Graphical exploration of the glucose time course data during the placebo phase of the study revealed a consistent harmonic pattern. This data was modeled using a combination of sine and cosine functions as shown in **Equation 10**. This placebo model was used to describe the full glucose versus time profile. The placebo model response was used to ensure that changes in glucose profiles were due to the drug and not to glucose homeostasis.

Equation 10

$$Eo = A_0 + A_1*COS(2*\pi*Time/Period) + B_1*SIN(2*\pi*Time/ Period) + A_2*COS(4*\pi*Time/Period) + B_2*SIN(4*\pi*Time/ Period)$$

A_0 Represents the baseline glucose concentration (measurement at time 0)

A_1 , A_2 , B_1 and B_2 are the coefficients for the harmonic function

Period was 8 hours viz. the time during which glucose concentrations were measured

Unexplained inter-subject variability in the baseline glucose concentration (A_0) was estimated using the following model (**Equation 11**) with the random effect η_j.

Equation 11

$$A_{0j} = TVP*\exp(\eta_j)$$

where TVP is the typical value of Ao in the population, A_0j is the individual value for A_0 in the jth individual and η_j is a random variable with mean of zero and variance ωA_0^2. This model assumes an exponential distribution for the A_0j values so as to constrain the estimate of the baseline glucose concentration to positive values.

On the other hand, the coefficients of the harmonic function could take on both positive and negative values and were therefore modeled with an additive error distribution as shown in **Equation 12**.

Equation 12

$$P_{nj} = TVP_n + \eta_j$$

where TVP_n is the typical value for the coefficient of interest (A_1 , A_2 , B_1 and B_2) in the population, P_{nj} is the individual value for the relevant coefficient in the jth individual and η_j is a random variable with mean of zero and variance ωP_n^2. The omega matrix for all the coefficients was constrained to the same value i.e., the ηvalues were drawn from the same distribution. The glucose concentration data was log transformed prior to fitting.

The residual error model of this log-transformed data comprised of an additive model as shown in

Equation 13.

Equation 13

$$C_{ij} = C^*_{ij} (1+\varepsilon_{ij})$$

where Cij is the ith glucose concentration measured at time ti in the jth individual. C*ij is the respective model predicted concentration and the εij is a normally distributed error term with mean of zero and variances σ^2. Examples of potential sources of residual variability include assay error, deviations from the model specification, and intra-subject variability.

Appendix 13 contains the NONMEM code for the Dose/Cpss-Full Glucose Profile.

Results of model for placebo response

The fluctuations in glucose concentrations over the observation period were due primarily to the response to food. In this study, subjects were given breakfast, followed by lunch approximately 4 hours later. The initial peak in glucose corresponds to ingestion of breakfast and the subsequent peak 4 hours later, to lunch. Sampling to characterize these two events showed two blood glucose peaks at approximately 2 and 6 hours, temporally related to the ingestion of food. The model selected to describe this data is empirical and has no physiological meaning – the data could also have been fit with a series of spline functions or a polynomial to the placebo data.

The empirical placebo model was used to describe the time course of glucose concentration data over the ~8 hour study observation period. It satisfactorily described the data save for subjects 2, 13, 16 and 19. In all other subjects, the observed, individual predicted and population predicted curves are all almost superimposable, strengthening the validity of the model.

5.2.5.1 Dose as driving force on full glucose profile

Model description

The model is as described in Equation 7. However, response is now the full glucose concentration versus time profile modeled using the placebo model shown in Equation **10**.

Results from model with dose as driving force on full glucose profile

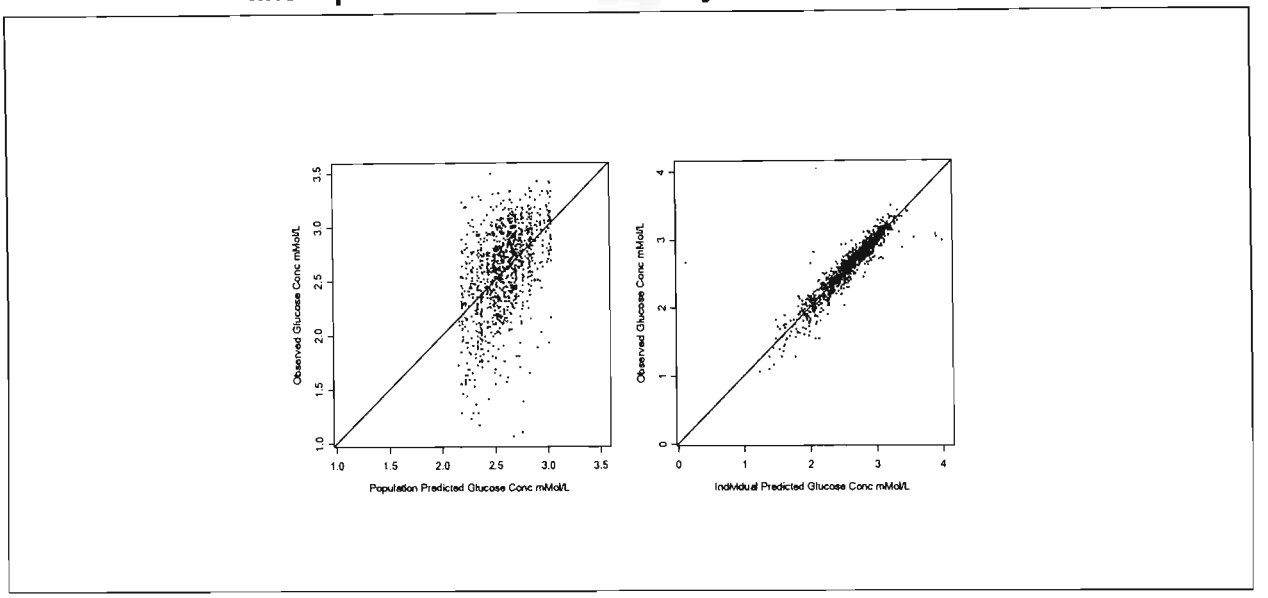
Figure 48 shows that the full glucose profile model adequately describes the data as there is a good correspondence between the observed data and the model predictions. The empirical placebo model used to describe the time course of glucose concentration data over the ~8 hour study observation period was able to satisfactorily characterize the data.

The concentration and dose response is shown by the shift in the entire glucose concentration versus time profile downwards as one progresses from left to right within a row i.e. from placebo on the left to the highest dose administered on the right.

The parameters from this complex full model are shown in Table 54.

As shown for the previous models, Figure 46 provides the global graphical model diagnostic plot to indicate a satisfactory description of the observed vs predicted glucose concentrations (population and individual). In this model there are more data points because the full time course of glucose is being modeled and not a single PD metric such as FBG or the mean glucose concentration. In the interests of brevity, the individual subject fits are not shown for the dose-full glucose profile model.

Figure 46: **Model diagnostics – observed versus model predicted glucose concentrations for the Dose-Full Glucose Profile pharmacokinetic-pharmacodynamic model are shown as points on the graph. The solid line represents the line of identity.**



5.2.5.2 Cpss as driving force on full glucose profile

Model description

The model is as described in Equation **8**. However, response is now the full glucose concentration versus time profile modeled using the placebo model shown in Equation 10.

Figure 47: **Model diagnostics – observed versus model predicted glucose concentrations for the Cpss-Full Glucose Profile pharmacokinetic-pharmacodynamic model are shown as points on the graph. The solid line represents the line of identity.**

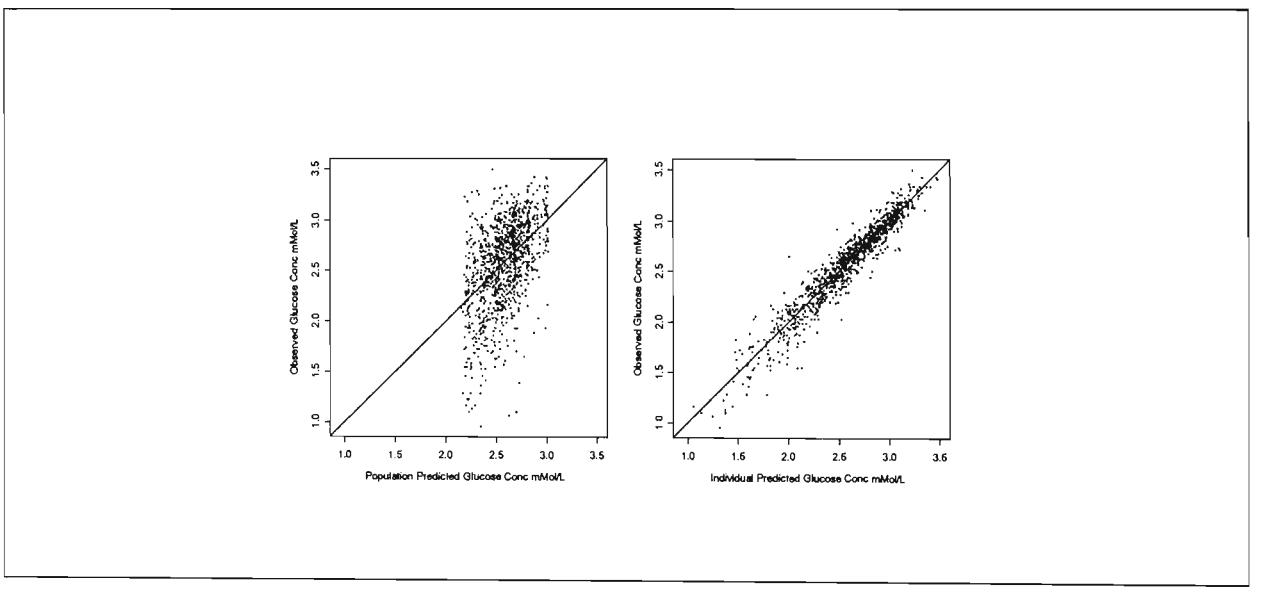
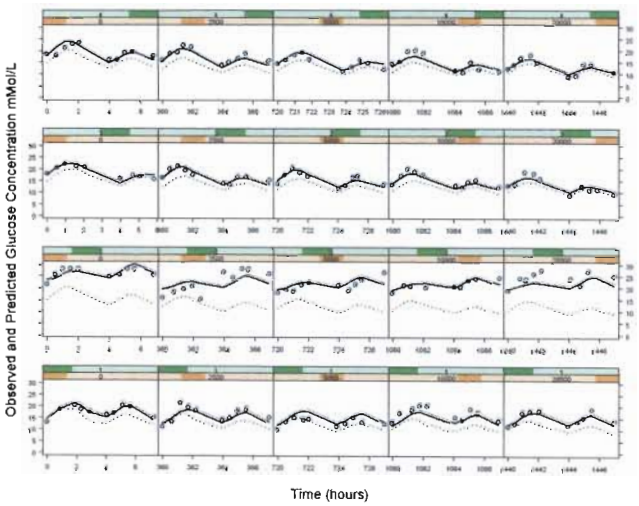
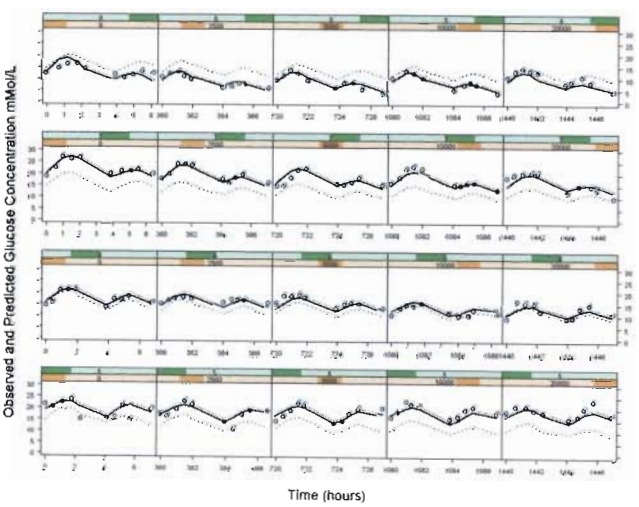


Figure 48: **Plots of observed glucose concentrations (open circles), population model predictions (dotted line) and individual model predictions (solid line) for the Cpss-Full Glucose Profile pharmacokinetic-pharmacodynamic model. Each row represents the data for an individual subject; each column represents a different dose level – with placebo on the extreme left and increasing doses of 2.5, 5, 10 and 20 mg in the subsequent columns.**

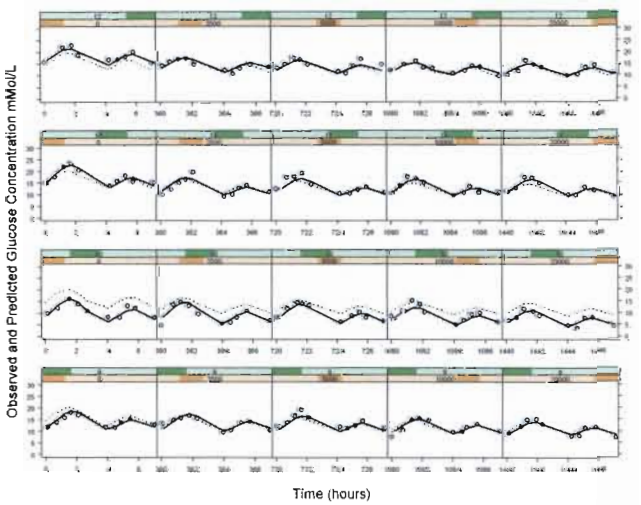
Subjects 1 - 4



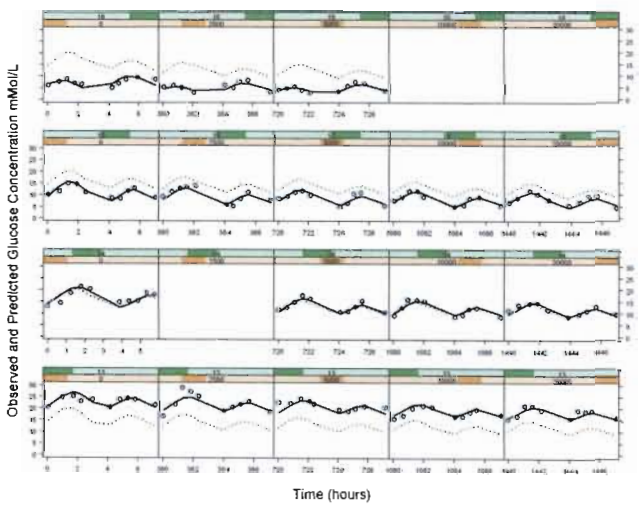
Subjects 5 - 8



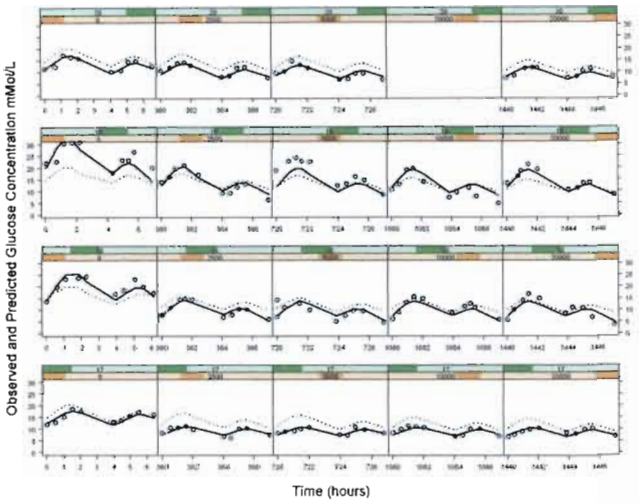
Subjects 9 - 12



Subjects 13 - 16



Subjects 17 - 20



Subjects 21 - 24

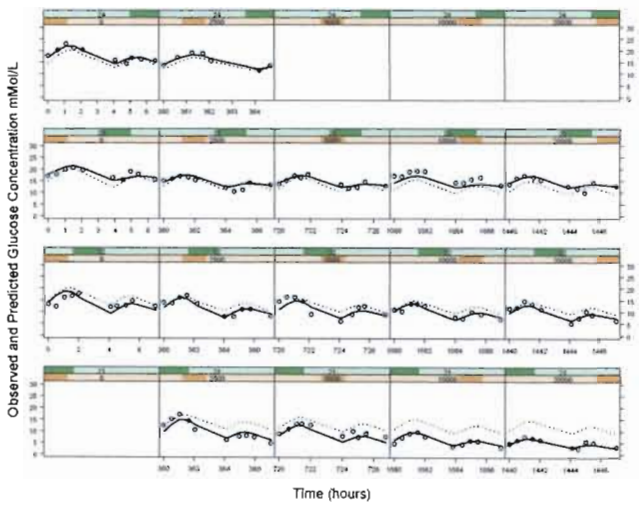


Table 54: Population pharmacokinetic-pharmacodynamic parameters for the models with full glucose profile as the PD response

	Dose – Full Glucose Profile Model			Cpss – Full Glucose Profile Model		
	Estimate	RSE (%)	BSV (%CV)	Estimate	RSE (%)	BSV (%CV)
A_0 (mMol/L)	15.90	4.71	28.71	15.40	5.40	32.71
A_1	0.95	17.44	120.83	0.97	16.36	7.05
B_1	1.45	36.34	120.83	1.45	34.21	7.05
A_2	-1.95	-14.26	120.83	-1.86	-15.38	7.05
B_2	2.63	8.75	120.83	2.57	8.05	7.05
Emax	0.34	14.97	5.75	0.31	9.20	45.39
ED50 (mg)	2.21	29.46	15.13	Derived ED50 = 2.26*		
EC50 (ug/L)				43.60	21.06	220.91
Residual variability variance (%CV)	0.02 (13%)			0.02 (13%)		

A_0 = Baseline glucose concentration; A_1, B_1, A_2, B_2 = coefficients of the harmonic function; Emax = maximum inhibition of glucose concentration; ED50 is the glibenclamide dose producing 50% inhibition of glucose concentration; EC50 is the glibenclamide concentration producing 50% inhibition of glucose concentration; RSE: relative standard error of the estimate; BSV: between subject variability; CV: coefficient of variation

* Derived ED50 = Cpss * Cl/f * 24 where Cpss = 43.60 ug/L and Cl/f = 2.16 L/h

The point estimates of the fixed effects PD model parameters (baseline glucose concentration (A_0), potency (EC50 and ED50) and Emax are essentially not different in the Dose and the Cpss models (table 54). However, there is a difference in the random effects parameters, in particular the Fourier coefficients have higher variability while the primary PD model parameters have lower variability in the dose model compared to the Cpss model. A likely explanation for this is that PK variability is being confounded with PD variability – the dose model can only allocate the PK variability into the Fourier coefficients. The variability in potency is also much larger in this model than that seen with the model where the PD response was the mean glucose concentration (221 % CV versus 108 % CV) [table 53].

Thus despite the very good fit of the model to the data, this is likely to be due to the large amount of flexibility that is allowed by the large number of model parameters. Consequently, the models where mean glucose concentration is the PD response was selected as being adequate for this data.

5.2.6 Discussion of PKPD modeling

PK/PD modeling assists in characterizing and predicting the time course of drug effects (both intensity and duration) in healthy and diseased subjects (Breimer and Danhof, 1997). The PK/PD model helps with dose interpolation. For example, in a clinical trial which utilizes conventional methodologies, comment can only be made on the doses tested. It is not possible to consider doses other than that actually employed in the study. However, with modeling it is possible to interpolate between doses (for example, in this study it is possible to interpolate at dose 1.25mg or 7.5 mg, although these doses were not used in the study).

Graphical exploration of the within-subject drug response shows that there does not appear to be any direct acute response to glibenclamide. An examination of crude data of glucose, insulin and glibenclamide profiles with increasing doses of glibenclamide shows that there is no direct relationship between drug administration and glucose or insulin response i.e., there is no acute effect of drug on these parameters. This deduction is in keeping with that made by Gin et al. (1985) that ordinary doses of sulphonyureas will potentiate insulin mediated glucose uptake after a latent period of 12 hours (Gin et al., 1985). Further, Rydberg et al. (1997) observed that at a "..given concentration of glibenclamide, a more intense effect is observed at the later sample time due to additional hypoglycaemic effect of *in vivo* formed metabolites."

NONMEM was used to fit the following models:

- Dose as driving force on fasting glucose
- Cpss as driving force on fasting glucose concentration
- Dose as driving force on mean glucose concentration
- Cpss as driving force on mean glucose concentration
- Dose as driving force on full glucose profile
- Cpss as the driving force on full glucose profile

In comparing **Dose vs FBG** or **Cpss vs FBG** as the driving force for the model, the parameters are estimated with better precision i.e., lower RSE (6.35% vs 6.04%) for the model with Cpss as the driving force. Apart from this, there is little difference in the estimated model parameters. There is a low variability in the estimate of potency for dose-fasting glucose model (ED50= 4.56mg) as compared to Cpss-fasting glucose model (ED50 derived= 4.41mg).

There is no difference in the parameter estimates from the **Dose** or the **Cpss** models, where the pharmacodynamic (PD) response is **mean glucose concentration**. There is a higher estimate for the modeled baseline glucose concentration for the dose vs mean glucose concentration model as compared to the dose vs FBG model (16.70 vs 14.10 mmol/L). The ED50 (1.85 mg) and the derived ED50 (1.87mg) for the dose vs mean glucose concentration model are almost identical with a between subject variability (BSV) of approximately 12% (120.42-108.17).

In addition, a much larger variability is noted in the potency parameters relative to the model where the pharmacodynamic response was FBG. By calculating a mean glucose concentration, the variability in concentrations during the course of the observation period is being brought into the model. Intuitively, one would expect a fasting measurement i.e. one taken when the biological system is not being subjected to the known factors that can influence glucose response (e.g., food) to be subject to lower variability. FBG is also the pharmacodynamic marker directly influenced by the sulphonylureas and is probably the best PD response variable to monitor. This accounts for the differences in the estimates of random effects parameter estimates. The maximum effect seen with both groups of models is very similar.

The point estimates of the primary PD model parameters for the **Dose vs full glucose profile** and the **Cpss vs full glucose profile** are not essentially different i.e., baseline glucose concentration, (A_0) [15.9 and 15.4 respectively], potency (ED50=2.21 and ED50 derived=2.26 respectively) and Emax (0.34 and 0.31 respectively). However, there is a difference in the random elements, in particular, the Fourier coefficients have higher variability while the primary PD model parameters have lower variability in the dose model compared to the Cpss model. It seems therefore that the greater freedom allowed to the model with the large number of extra parameters results in 'inappropriate' apportioning of relative contributions of variability. An attempt was made to resolve this by estimating the Fourier coefficients from the placebo data alone and then subsequently fixing these in the estimation step for the full data set. However, the model with the full data set failed to converge.

The **Emax** of for the 6 models tested varies from 0.37 for the dose vs FBG to 0.31 for the Cpss vs full glucose profile models. This means that the maximal benefit of glibeclamide therapy in this population would vary from 31 to 37 percent. This translates to a reduction of the FBG from 15.4mmol/L to 10.6mmol/L and 9.7mmol/L respectively. This reduction in FBG, while statistically significant, is clinically inadequate for effective control of diabetes because this value is higher than that accepted by ADA (4.4-6.6 mmol/L) and SEMDSA (4-6 mmol/L).

The **ED50** and the **derived ED50** for the 6 models described are 4.56 mg, 4.41mg, 1.85 mg, 1.87 mg, 2.21 mg and 2.26 mg respectively. Analysis of our data which describes the relationship between dose, glucose, insulin and glibenclamide concentrations in diabetic patients supports a maximum effective dose of 5 mg. In addition, an evaluation of glycaemic control in individual patients showed that only 7 (30%) and 4 (17%) subjects achieved acceptable and optimal control respectively at doses greater than 5 mg per day. Both the observed and the modeled data suggest a maximal dose not exceeding 5 mg in the study population. This conclusion is supported by the findings of Rydberg et al. (1997) and Groop et al. (1991) that "the maximum effect of glibenclamide would be obtained by 5 mg or less." Further increase in glibenclamide dose (as is the current clinical practice in SA, See chapter 1) are not likely to produce significant reductions in blood glucose. High dose glibeclamide is associated with various side effects two of which are: increased cardiovascular risk and masking of the severity of a myocardial infarction (Huizar, 2003).

In South Africa, the case against the use of high dose glibenclamide was first presented by Robertson and Jackson (1989) who showed that a reduction in the dose of glibenclamide from 15 mg/day to 2.5 mg/day in 15 type 2 DM subjects resulted in 12 (80 %) subjects achieving a reduction in fasting blood glucose.

High dose glibenclamide cannot be advocated because it is therapeutically ineffective, cost ineffective and potentially hazardous and subjects on high doses are often eating to 'keep up with their glibenclamide'.

The PK of glibenclamide does not enhance predictability of glibenclamide pharmacodynamics. This reaffirms the findings of Tracewell et al. (1998) who concluded that, "interpatient variability in response to glyburide is not primarily due to intersubject differences in the agents pharmacokinetics." A similar conclusion was reached by Rydberg et al. (1997), "there is no simple direct relationship between sulphonlyurea concentrations and hypoglycaemic effect."

This finding vindicates the use of low dose glibenclamide.

While all models presented adequately describe the experimental data, the dose on FBG model is the preffered model for the following reasons:

- In clinical practice in South Africa, dose adjustment of glibencamide is based on FBG.
- FBG is a better measure of response to glibenclamide in type 2 diabetics because glibenclamide decreases FBG with very little effect on PPG.
- FBG is also the pharmacodynamic marker directly influenced by the sulphonylureas and is probably the best PD response variable to monitor.
- Cpss of glibenclamide is not a useful determinant of response (blood glucose levels) as reported by Rydberg et al. (1997) that there "is no simple direct relationship between sulphonlyurea concentrations and hypoglycaemic effect." Furthermore, assays of glibenclamide are expensive and not readily available.
- Insulin determinations in clinical practice are not only expensive but do not contribute to dosage adjustment, unlike FBG.

Application of this model i.e., Dose as a driving force on FBG, can be used for dosage optimization in clinical practice.

Conclusion

In this thesis, six PK/PD models were developed to characterize dose-effect relationships of glibenclamide. These models were developed from NCA which provided exploratory data for initial estimates and guides for population PK analysis (using NONMEM). The population approach was the primary data analytical method used in this study.

A 2 compartment model was developed using parameters derived from population PK. The 2-compartment model provided a good fit of the model to the data as confirmed by the PPC (Figure 36). In particular, the model was able to provide an estimate of exposure (AUC) to glibenclamide that was consistent with the NCA estimates. This was especially important since one use of the PK model was to provide an estimate of average glibenclamide concentration (Cpss) for use as the driving force in a PKPD model.

The model most suitable to describe the data and clinically relevant and practical is the Dose on FBG model. The dose derived from this model suggests a maximum of 5 mg of glibenclamide per day which coincides with the dose derived from the exploratory analysis.

6 Limitations

- Serial insulin and glucose evaluations were only performed during the study period of approximately 8 hours. Ideally, a 24 hour study would have been preferred to full characterize these parameters (terminal phase of glibenclamide elimination) but was viewed as a major inconvenience by subjects
- Earlier and more frequent sampling for insulin and glucose would have allowed measurements of acute insulin response (AIR). It is recommended that future studies should include this in the study design
- Patient inclusion criteria should have included insulin resistance status

7 Recommendations

- Dose of glibenclamide should not exceed 5-10 mg
- Patients on high dose glibenclamide should have their dosages reviewed
- Both FBG and PPG be used as markers of glycaemic control i.e., choice of drug and adjustment of dosage
- Patients not responding to the recommended dose of glibenclamide should receive combination therapy, preferably with an insulin sensitizer
- Future studies should profile the PK/PD of glibenclamide and its metabolites
- Future studies should validate the modified insulinogenic index as a measure of acute insulin release
- Future studies should include more data points to characterize EHC and the terminal elimination phase of glibenclamide
- Insulin resistance status should be established in inclusion criteria in future studies

8 Summary of Findings

This dose escalation study evaluated the PK and PD and the clinical benefit of glibenclamide in type 2 DM subjects.

Evaluation of clinical benefit from this dose escalation study of glibenclamide revealed that only 57% were controlled, using SEMDSA's criteria for acceptable control. Only 17% achieved acceptable control when FBG and PPG were evaluated simultaneously.

Using the HOMA-IR and QUICKI methods, all patients were insulin resistant and did not respond effectively to glibenclamide.

While the mean blood concentration of glibenclamide increased linearly with increasing doses, there is no proportional increase in insulin secretion or proportional decrease in blood glucose concentration. Evaluation of the pharmacokinetics of glibenclamide, insulin and glucose shows no coincidence. This implies that glibenclamide blood levels do not correlate with pharmacodynamic response.

The pharmacokinetics of glibenclamide as determined using NCA and compartmental analysis was similar to that reported in the literature. Using the population approach the PK of glibenclamide was described by a two compartment model with first order absorption. The PK reported in this study was comparable to that reported by other researchers. Validation of the model using the PPC approach provided acceptable model predictions of AUC and therefore glibenclamide concentration.

PKPD relationships were modeled using the software NONMEM to fully characterize the dose-exposure-response relationships. Six models using dose as the driving force and concentration as the driving force were implemented. On evaluation of the six models the dose on FBG was preferred because FBG is the parameter directly influenced by sulphonylureas and clinically is the best PD response to monitor.

PK/PD modeling showed that the maximum mean reduction in blood glucose concentrations (E_{max}) was approximately 34% from a baseline of 15mmol/L. In addition, the glibenclamide dose producing 50% inhibition of glucose concentration (ED_{50}) was estimated from the models to be in the region of 2.5 to 5mg. The $\mathbf{E_{max}}$ and $\mathbf{ED_{50}}$ confirm that dose escalation of glibenclamide in these subjects is unlikely to produce substantial clinical benefit.

Since most subjects were insulin resistant and are not likely to benefit from glibenclamide monotherapy, insulin sensitizers should be added to the regimen of those patients not responding to doses greater than 5 mg of glibenclamide.

High doses of glibenclamide i.e., 20 mg/day does not produce proportional decreases in blood glucose but expose the patients to increased incidence of cardiovascular side effects.

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Appendices

1 Packaging records of glibenclamide

Centre A

Glibenclamide	Unit of Issue	2000	2001	2002
	28	4245	4980	5960
	42	723	826	504
	56	6894	6165	7738
	84	2620	2400	3220
	112	4728	4514	5694
TOTAL (%)		19210 (24.6)	18885 (23.9)	23116 (24.6)

Centre B

Pack size	14	28	42	56	70	84	112		TOTAL	Percentage of 20 mg used
2002	4010	12425	1504	11499	343	6628	28334		64743	44
2001	3195	10830	1218	10845	445	5294	27567		60214	46
2000	4133	9333	960	10776	385	5239	26877		57703	47
TO										

Centre C

Pack size	14	28	42	56	84	112	TOTAL	Percentage of 20 mg used	
2002	2499	7600	3175	24800	3258	3083	44415	7	
2001	3650	6042	3426	25360	1950	2120	42548	5	
2000	3383	6336	4202	23800	1702	1312	40 735	3	
1999	3497	9673	10238	25307	2191	2832	53 738	5	
1998	3488	5998	9476	14246	1056	1718	35 982	5	

Centre D

Pack Size	14	28	42	56	84	112	Total Packed	Percentage of 20 mg used	
2002	1104	2391	897	2664	4580	3594	15230	24	
2001	1096	2365	694	2841	4539	2367	13902	17	
2000	397	1418	516	1696	3424	628	8079	8	

Centre E

Pack size	14	28	42	56	70	84	112	TOTAL	Percentage of 20 mg used
2002-2003	851	1400	710	3800	322	1085	6590	14 758	45

Centre F

Pack Size	112		TOTAL	Perecntage of 20 mg used
2002/2003	2621		38750	7%

2 Informed Consent form

INFORMED CONSENT

1. Title of Study

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF GLIBENCLAMIDE IN NON-INSULIN DEPENDENT DIABETES MELLITUS

2. Investigators and Contact People

Dr LI Robertson / Dr L Mayet
Diabetes Clinic
Addington Provincial Hospital
Durban 4000
Telephone : 208-6128

Mr V Rambiritch
Department of Pharmacology
Private Bag X54001
Durban 4000
Telephone : 204-4766(w) or 821158 (h)

Dr G Pillai
Department of Pharmacology
Private Bag X54001
Durban 4000
Telephone : 204-4908 (w)

1. Purpose of the study

Thank you for expressing interest in this study. You have been selected for inclusion because of being a diabetic on treatment with diabetes tablets.

Medicines form an important part of the treatment of your type of diabetes, when other methods of treatment such as diet modification and exercise are not successful.

While the drugs have been widely used, there is little information on the relationship between the dose and its effect on insulin secretion. Insulin is the substance produced by your body that controls blood glucose levels. There is also little agreement among the world authorities as to the maximum dose of the diabetes tablets (glibenclamide) that may be used in patients.

This study will investigate the dose-response relationship of glibenclamide.

The study will have immediate benefits to you in that there will be closer monitoring and control of your diabetes. However, in addition, this study will benefit many other diabetics because we will know how to treat them.

2. Procedure

This study will be conducted at the Addington Hospital Diabetes Clinic. A physical examination, a complete medical history and specific laboratory tests will be determined at certain specified times during the trial.

After recruitment all oral hypoglycaemic drug treatment will be stopped for a 2 week washout period.

During this time, patients will be requested to keep a diabetic diary in which they will record their blood glucose levels.

Thereafter treatment with glibenclamide will be given at doses of 2.5mg; 5mg; 10mg and 20mg daily for periods of 14 days each. At the end of each 14 day period, the patient must report to the clinic **without having taken their morning dose of glibenclamide**. The clinic staff will administer this dose and a series of blood tests will be conducted.

A patient will be not allowed to proceed to the next dose level if

- ☐ *Your doctor advises against it;*
- ☐ *any blood glucose level less than 3.5 mmol/L is recorded; or*
- ☐ *if any symptoms of hypoglycaemia are reported.*

3. Risks

It must be stressed that this study involves the use of procedures and tests that are conducted in the interests of the patient during usual routine care in medical practice. Dosages of glibenclamide used in this study will not exceed the maximum used in Diabetic clinics in South Africa. The only physically invasive procedure used in this study will be venepuncture for venous blood collection.

4. Withdrawal from the Study

Participation in this study is voluntary.
A participant may decide to withdraw from the trial by withdrawing his/her consent at any time. Patients will be allowed to remain in the study only if they do not have hypoglycaemic reactions and if their fasting blood glucose concentrations do not fall below 3.5 mmol/L during the study. In addition, the principal investigator may decide to terminate participation of a subject in the event of logistical difficulties such as it being difficult to obtain blood samples.

5. Confidentiality

All clinical information obtained during this study will be regarded as confidential. In all reports, patients will be identified by code number only - the key to which will be known to the Investigators.

6. Consent Form

I have read the information above and understand the contents thereof. I consent to participating in this study.

PATIENT

Name ....

Signature.

Date .....

WITNESS		**WITNESS**	
Name	.....	**Name**	.....
Signature.	.....	**Signature**	.....
Date	.....	**Date**	.....

3 Indemnity form

University of Durban-Westville

INDEMNITY

I, the undersigned

.....

of .....

.....

born on .....

Identity No. .....

do hereby indemnify and hold blameless the UNIVERSITY OF DURBAN-WESTVILLE, or any FACULTY, DEPARTMENT, INSTITUTE or UNIT of the said University of Durban-Westville or any member or employee of the said University for or in respect of any damages sustained by me as a result of, or following any experiment, procedure and/or test conducted on me with my consent, as set out in Annexure A hereof, by any Faculty, Department, Institute, Unit, member or employee of the University of Durban-Westville.

Signed at ..... on this the ..... day of .....

19..... in the presence of the undersigned witnesses.

.....

AS WITNESSES:

1. .....

Address: .....

2. .....

Address: .....

4 Permission to Conduct Study and Ethical Clearance

DEPARTMENT OF HEALTH
PROVINCE OF KWAZULU-NATAL

Private Bag X9051, Pietermaritzburg 3200 - 330 Longmarket Street, Pietermaritzburg
Tel: 0331-95 2711 Fax: 0331-42 3992
Email:

PHARMACEUTICAL SERVICES

FAX MESSAGE

TO	:	**DEPT. OF HEALTH**
ATTENTION	:	**MR T PILLAY**
FROM	:	**MR N DRUMMOND**
PGS (INCL.)	:	**27 JULY 1999**

RE : SUPPORT DIABETES STUDY IN KZN HOSPITALS

I refer to your memo concerning the proposed investigation by Mr V Rambiritch of the Dept. of Pharmacology at University of Durban Westville in the use of glibenclamide in diabetes.

The Department supports the request but would like to be advised as to which Hospital would be taking part in this exercise.

SECRETARY : DEPARTMENT OF HEALTH
KWAZULU-NATAL

lg/faxstan.diabetes

Loena Coetzer
DOH - Policy, Planning
Procurement
012 - 312 0339

031 3681093

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FAX: (031)820-2883

28 November 1995

Mr V Rambiritch
Department of Pharmacology

Dear Mr Rambiritch

ETHICAL CLEARANCE: NUMBER 95181A

I wish to confirm that ethical clearance has been granted in respect of the following project:

"Pharmacokinetics and Pharmacodynamics of Glibenclamide in non-insulin dependent diabetes mellitus"

Thank you

Yours faithfully

.....
NEMRU NAIDOO
(for) HEAD: RESEARCH ADMINISTRATION

PS: The following general condition is applicable to all projects that have been granted ethical clearance:

THE RELEVANT AUTHORITIES SHOULD BE CONTACTED IN ORDER TO OBTAIN THE NECESSARY APPROVAL SHOULD THE RESEARCH INVOLVE UTILIZATION OF SPACE AND/OR FACILITIES AT OTHER INSTITUTIONS/ORGANISATIONS

CC: THE HEAD: DEPARTMENT OF PHARMACOLOGY

5 Glibenclamide, glucose and insulin concentrations

ID	Time	Glu	Ins	inspmo	Glib
1	0.000	13	13.2	91.674	0
2	0.000	21.2	4.4	30.558	0
3	0.000	17.9	18.4	127.79	0
4	0.000	18.7	4.8	33.336	0
5	0.000	21.5	6.7	46.532	0
6	0.000	14.1	22.8	158.35	0
7	0.000	18.6	13.7	95.147	0
8	0.000	12.4	10.1	70.145	0
9	0.000	11.6	10.1	70.145	0
10	0.000	9.9	22.3	154.87	0
11	0.000	14.9	14.5	100.7	0
12	0.000	15.7	5.9	40.976	0
13	0.000	20.3	11.1	77.09	0
14	0.000	12.9	21.2	147.23	0
15	0.000	9.9	18.5	128.48	0
17	0.000	11.6	3.8	26.391	0
18	0.000	13.5	24.7	171.54	0
19	0.000	21.8	13.3	92.369	0
20	0.000	11.3	17.1	118.76	0
21	0.000	17.8	12.7	88.202	0
22	0.000	13.2	23.9	165.99	0
23	0.000	16.9	4.2	29.169	0
1	0.000	11.2	15.9	110.43	33.15
2	0.000	15.6	7.5	52.088	51.4
3	0.000	16.3	19.3	134.04	0
4	0.000	16.1	4.3	29.864	27.84
5	0.000	17.8	3	20.835	0
6	0.000	14.9	15.5	107.65	0
7	0.000	17.8	15.1	104.87	0
8	0.000	10.5	9.4	65.283	0
9	0.000	13.3	15.4	106.95	0
10	0.000	4.7	12.7	88.202	55.26
11	0.000	10	17.8	123.62	28.37
12	0.000	14.5	10.8	75.006	0
13	0.000	16.5	7.8	54.171	0
14	0.000	11.7	20	138.9	[illegible]
15	0.000	9.3	28	194.46	0
17	0.000	8.2	8.7	60.422	98.43
18	0.000	7.5	15.8	109.73	87.84
19	0.000	13.7	21.3	147.93	0
20	0.000	10.6	15.7	109.04	0
21	0.000	12.4	14	97.23	35.42
22	0.000	14	22.1	153.48	0
23	0.000	15	15.5	107.65	0

1	0.000	10.4	14.9	103.48	0
2	0.000	17.8	6.8	47.226	58.36
3	0.000	13.7	15.9	110.43	0
4	0.000	14.4	6.6	47.226	53.01
5	0.000	14.4	4	27.78	0
6	0.000	15.4	18.2	126.4	0
7	0.000	14.9	17.5	121.54	0
8	0.000	9.5	9.1	63.2	0
9	0.000	12.1	10.9	75.701	64.95
10	0.000	8.3	14.9	103.48	48.84
11	0.000	12.4	38.9	270.16	34.39
12	0.000	13.5	9.9	68.756	0
13	0.000	22.5	9.5	65.978	64.76
14	0.000	11.5	17.6	122.23	0
15	0.000	8.4	19.7	136.82	0
17	0.000	8	4.4	30.558	42.17
18	0.000	6.9	25.5	177.1	0
19	0.000	18.6	24	166.68	0
20	0.000	9.5	22.6	156.96	0
21	0.000	8.8	17.8	122.23	37.91
22	0.000	14.4	24.5	170.15	0
23	0.000	13.7	11.3	78.479	0
1	0.000	13.7	25.8	179.18	82.16
2	0.000	18	6	41.67	93.89
3	0.000	13.7	158.57	1101.3	58.19
4	0.000	14.7	6.3	43.754	84.83
5	0.000	16.2	3.6	25.002	59.29
6	0.000	10.3	18	125.01	69.22
7	0.000	16.4	16.3	113.2	108.89
8	0.000	10.1	12.8	88.898	0
9	0.000	7.6	9.4	65.283	70.33
10	0.000	8.8	18.2	126.4	39.98
11	0.000	10.8	24.8	172.24	40.45
12	0.000	12.1	8.8	61.116	35.08
13	0.000	15.5	12.3	85.424	46.4
14	0.000	9.2	14.1	97.925	82.47
15	0.000	7.5	16.6	116.88	37.07
17	0.000	8.2	12.7	88.202	82.22
18	0.000	5.9	18.7	129.87	133.42
19	0.000	10.5	19	131.96	32.21
20	0.000	8.8	[illegible]	0	[illegible]
21	0.000	4.8	14.5	100.7	55.25
22	0.000	10.8	30.4	211.13	62.28
23	0.000	17.1	11.2	77.784	0
1	0.000	11.5	30.6	212.52	60.59
2	0.000	18.8	5.6	38.892	290.45
2	0.000			0	
3	0.000	12.6	26.3	182.65	95.14
4	0.000	12.2	9.5	65.978	63.54
5	0.000	18.8	5.1	35.42	139.46

6	0.000	9	34.1	236.82	449.22
7	0.000	18.5	23.8	165.29	136.89
8	0.000	10.5	11.3	78.479	46.59
9	0.000	9.1	10.7	74.312	141.64
10	0.000	6.9	15.2	105.56	76.09
11	0.000	11.2	27.4	190.29	161.67
12	0.000	10.4	9.7	67.367	28.95
13	0.000	15.5	13.3	92.369	78.09
14	0.000	11.4	21.3	147.93	76.92
15	0.000	6.7	21.9	152.1	46.24
17	0.000	6.5	8.5	59.033	411.33
18	0.000	5.6	12.1	84.035	520.99
19	0.000	13.2	23.8	165.29	56.47
20	0.000	7.3	26.2	181.96	67.81
21	0.000	4.6	14.3	99.314	58.51
22	0.000	11.4	28.9	200.71	0
23	0.000	13.6	15.2	105.56	65.06
18	0.010	14.1	84.2	584.77	199.88
8	0.417	11.4	27.7	192.38	30.76
4	0.417	14	15	104.18	147.37
17	0.417	7.3	11.3	78.479	388.8
17	0.433	8.5	16.3	113.2	59.09
18	0.433	9.8	16.2	112.51	1074.87
5	0.450	17.1	10.5	72.923	50.36
18	0.450	10.8	59.7	414.62	95.19
23	0.450	15.2	20.4	141.68	80.49
18	0.450	8.8	44.5	309.05	290.45
19	0.467	12.7	66.2	473.65	175.16
6	0.483	15.7	43.1	299.33	0
11	0.500	17.6	47.6	330.58	0
17	0.500	12.4	19.5	135.43	0
22	0.500	12.2	29.4	204.18	0
3	0.500	20.3	68.5	475.73	105.86
6	0.500	16.4	38.3	265.99	108.12
12	0.500	16.1	32.5	225.71	0
17	0.500	10.2	23	159.74	123.34
19	0.500	15.8	82.8	575.05	112.83
21	0.500	15.1	31.8	220.85	91.29
3	0.500	17.8	71.6	497.26	101.9
4	0.500	18.3	11	76.395	146.04
7	0.500	15.3	18	125.01	27.89
12	0.500	15.1	21.4	148.62	54.32
14	0.500	12.5	36.7	254.88	0
20	0.500	10.1	52.3	363.22	64.97
22	0.500	16.3	118.6	823.68	270.69
1	0.500	17.6	36.9	256.27	237.78
4	0.500	15.5	11.7	81.257	196.55
5	0.500	19.2	8.9	61.811	184.96
8	0.500	10.8	[illegible]	0	0
11	0.500	13.8	121.5	843.82	179.86

14	0.500	12.3	25.9	179.88	317.51
15	0.500	8.9	38.6	268.08	179.54
17	0.500	9.6	24.9	172.93	66.92
20	0.500	8.2	[illegible]	0	[illegible]
21	0.500	7.1	27.9	193.77	206.71
22	0.500	10.2	28.6	198.63	71.21
23	0.500	16.8	27	187.52	100.13
1	0.500	13.3	47.3	328.5	294.15
10	0.500	8.3	35.6	247.24	209.98
11	0.500	12.7	72.1	500.73	328
13	0.500	16.8	17.8	122.23	367.48
20	0.500	8.2	45.1	313.22	196.78
21	0.500	8	42	291.69	247.07
22	0.500	11.8	85.6	594.49	615.5
23	0.500	16.1	22.3	154.87	412.09
15	0.517	11.4	58.6	406.98	81.56
3	0.517	13.7	62.8	436.15	394.46
2	0.533	25.9	10.7	74.312	0
3	0.533	20.5	36.6	254.19	0
23	0.533	17.7	13.8	95.841	0
8	0.533	12	21.6	150.01	52.8
18	0.533	10.6	27.1	188.21	103.19
23	0.533	15.9	24.3	168.76	64.8
15	0.533	9.3	69.7	484.07	65.45
7	0.533	20	37.2	258.35	338.75
15	0.533	8.6	48.4	336.14	329.7
4	0.550	19.1	9.3	64.589	52.49
22	0.550	13.7	33.4	231.96	0
5	0.550	17.1	35.3	245.16	69.92
11	0.567	12.3	87.4	606.99	101.91
14	0.567	14.5	45.9	318.78	[illegible]
1	0.567	13.9	36.7	254.88	141.76
5	0.583	21.1	6.3	43.754	0
9	0.583	13.8	17.2	119.45	0
12	0.583	18.5	[illegible]	0	0
18	0.583	19.5	11.9	82.648	0
20	0.583	12.3	31.4	218.07	0
7	0.583	20.3	44	305.58	46.99
20	0.583	11.7	39.6	275.02	40.1
2	0.583	19.3	12.4	86.116	268.23
6	0.583	19	36.2	251.41	130.83
9	0.583	13.7	20.8	144.46	127.59
10	0.583	12.1	55.98	388.84	121.98
11	0.583	17.4	108.9	756.31	138.6
21	0.583	10.8	40.9	284.05	129.97
3	0.583	17.3	72.74	505.18	291
6	0.583	13.9	36.8	255.58	257.58
7	0.583	18.9	36.5	253.49	253.22
13	0.583	16.9	22.7	157.65	190.69
14	0.583	13.7	38.1	250.71	250.17

8	0.600	13.4	23.5	163.21	331.14
4	0.617	18.2	22.5	156.26	0
7	0.617	22.8	24.7	171.54	0
9	0.633	10.5	17	118.07	92.04
10	0.667	12.2	32.7	227.1	0
14	0.667	14.4	38.1	264.6	0
21	0.667	20.8	16.9	117.37	0
1	0.667	13.9	28	194.46	145.6
9	0.667	14.8	32.7	227.1	53.86
10	0.667	13.7	65.9	457.68	225.11
10	0.667	11	60.4	419.48	298.11
5	0.667	21.9	7.2	50.004	299.53
12	0.667	12	26.4	183.35	214.51
19	0.667	16.1	68.6	476.43	687.08
19	0.683	22.4	41.1	285.44	0
6	0.683	16.5	51.8	359.75	871.56
15	0.717	11.4	44.2	306.97	0
8	0.733	14.5	15.4	106.95	0
2	0.750	18.2	8.6	59.727	106.01
9	0.750	11.9	20.3	140.98	242.98
13	0.767	21.8	26.3	182.65	73.34
13	0.783	22.1	16.9	117.37	68.1
19	0.783	22.5	72.8	505.6	307.99
2	0.833	21.4	27.2	188.9	620.23
12	0.833	14.9	21.5	149.32	245.78
2	0.833	24	14.9	103.48	1107.46
17	0.867	8.7	13.4	93.063	434.37
1	0.917	19.2	17.5	121.54	0
13	0.917	24.6	11.1	77.09	0
6	0.917	17.7	46.1	320.16	226.65
8	0.917	13.9	35	243.08	144.15
14	0.917	15.9	33.4	231.96	547.86
18	0.917	12.9	59.4	412.53	540.98
19	0.933	19.4	93.3	647.97	169.15
4	0.950	17.1	22.4	155.57	579.94
19	0.950	18.4	71.8	498.65	738.85
4	0.967	19.6	18.2	126.4	392.89
5	0.967	23.2	9	62.505	294.27
15	0.967	11.5	72.3	502.12	376.73
2	1.000	28	11.7	81.257	0
3	1.000	22.4	37.8	262.52	0
6	1.000	21.1	52.7	368	0
12	1.000	17.2	41.3	286.83	47.91
17	1.000	10.1	24.9	172.93	169.29
3	1.000	20.8	103	715.34	289.07
4	1.000	16.2	21.3	147.93	245.05
7	1.000	18.7	33.2	230.57	206.27
14	1.000	14.8	43.3	300.72	222.59
20	1.000	14.6	91.3	634.08	161.24
23	1.000	17.1	31.3	217.38	126.8

3	1.000	20.3	76.27	529.7	420.57
6	1.000	15.4	38.4	266.69	339.16
7	1.000	22.4	32.2	223.63	383.13
8	1.000	13.6	50.3	349.33	325.3
17	1.000	11.2	30.4	211.13	166.25
19	1.000	18.8	93.7	650.78	494.31
20	1.000	12.7	[illegible]		0 [illegible]
21	1.000	8.9	30.8	212.52	279.47
23	1.000	18.9	27.2	188.9	173.19
1	1.000	17.9	82.3	571.57	627.57
10	1.000	12	42.4	294.47	351.85
13	1.000	21.3	15.2	105.56	445.13
21	1.000	7.6	37	256.97	494.01
22	1.000	14.6	118.5	822.98	1328.09
23	1.000	17.2	28.9	200.71	583.44
17	1.017	14.9	22.1	153.48	0
5	1.017	19.8	13.5	93.758	179.65
18	1.017	13.9	29.7	206.27	1566.84
7	1.033	27.4	22.9	159.04	0
23	1.033	19.8	23.2	161.12	0
4	1.033	19.6	20.5	142.37	126.39
21	1.033	17	36.8	255.58	128.54
22	1.033	18	88.8	616.72	31.81
23	1.033	18.9	31	215.3	153.82
17	1.033	8.8	15.3	106.26	141.66
11	1.033	17.7	92.9	645.19	349.51
12	1.050	22.3	17.8	123.62	0
22	1.050	18.1	47.6	330.58	0
3	1.050	21.6	91.1	632.89	164.52
5	1.050	20.2	6.3	43.754	63.15
15	1.050	12.6	93.5	649.36	94.31
2	1.050	18.8	14.3	99.314	318.09
15	1.050	11.1	93.4	648.66	168.42
22	1.050	16.2	138.4	961.19	264.39
3	1.050	18.7	72.2	501.43	1084.61
9	1.083	15.6	21.4	148.62	0
18	1.083	23	28.85	200.36	0
20	1.083	17.2	68.7	477.12	0
21	1.083	19.8	16.1	111.81	0
20	1.083	13.7	102.2	709.78	85.39
6	1.083	18.8	55.7	386.84	267.94
9	1.083	16.6	26.7	199.32	258.91
11	1.083	17.7	108.9	756.31	237.71
12	1.083	17.7	26.5	184.04	137.78
7	1.083	20.8	43	298.84	71.3
11	1.100	15.1	59	409.76	154.23
14	1.100	18.2	46.5	322.94	[illegible]
8	1.100	14.7	36.3	252.1	579.1
11	1.117	22	35.8	248.63	0
9	1.117	15.6	39.6	275.02	94.84

21	1.117	13	34.9	242.38	180.85
22	1.117	13.4	62.1	431.28	170.87
11	1.117	17.5	78.4	544.49	901.32
1	1.150	15.7	53.5	371.56	288.37
4	1.167	21.4	26.7	185.43	0
19	1.167	30.2	45.7	317.39	0
7	1.167	24.5	48.9	339.61	102.16
8	1.167	13.2	35	243.08	117.48
10	1.167	14.8	84.8	588.94	172.61
10	1.167	15.5	105.9	735.48	424.86
13	1.167	20.1	13.8	95.841	300.46
9	1.167	15	27.9	193.77	338.3
12	1.167	16.5	22.7	157.65	363.71
20	1.167	11.4	65.9	457.68	576.31
9	1.183	14.9	30.6	212.52	149
18	1.200	14.2	60.2	418.09	175.34
5	1.217	23.2	6.7	48.532	0
14	1.217	18.6	38.5	267.38	0
1	1.217	22	34.7	240.99	192.47
8	1.250	16.3	21.2	147.23	0
10	1.250	14.6	68.9	478.51	215.67
19	1.250	24	83.7	581.3	296.47
2	1.250	21	16.3	113.2	552
2	1.250	23.8	21.3	147.93	1161.08
15	1.283	14.7	74.9	520.18	0
13	1.300	28.8	31.8	220.85	119.63
1	1.300	19.3	34.2	237.52	403.56
6	1.300	16.2	59.3	411.84	1010.2
14	1.333	14.3	46.3	321.55	612.15
15	1.333	11.7	91.7	636.86	545.39
23	1.367	16.3	27.3	189.6	121.82
8	1.383	12.5	46.6	323.64	197.98
19	1.383	19.4	112.4	780.62	439.21
2	1.400	19.8	11.4	79.173	143.07
10	1.417	16.2	35.1	243.77	0
4	1.417	22.8	24.2	168.07	124.65
18	1.417	12.5	94.6	657	251.46
5	1.417	22.6	9.4	65.283	276.89
6	1.417	14.8	42.9	297.94	398.9
18	1.417	15.4	86.8	602.83	636.86
5	1.417	21.8	7	48.615	383.37
19	1.417	21.2	143.1	993.83	1586.82
17	1.433	9.7	19	131.98	206.34
16	1.433	16.7	45.8	318.08	1787.62
13	1.450	24.1	16	111.12	71.96
21	1.450	13.2	34.7	240.99	179.54
17	1.450	9.6	14.4	100.01	525.55
4	1.467	19.5	21.1	146.54	219.12
7	1.467	22	42.2	293.08	234.45
3	1.467	19.2	83.5	579.91	539.46

4	1.467	20.3	20.5	142.37	462.6
4	1.467	18.2	30.6	212.52	611.6
23	1.483	20.8	15.07	104.66	0
2	1.500	28	17	118.07	0
6	1.500	21.3	69.9	485.46	0
9	1.500	17.9	24	166.68	0
6	1.500	18.2	39.9	277.11	228.86
12	1.500	17.6	33	229.19	61.83
15	1.500	13.3	83.8	581.99	82.07
17	1.500	10.7	25.6	177.79	138.79
19	1.500	20.8	115	798.68	150.94
20	1.500	13.9	88.1	611.85	85.89
3	1.500	18.7	92.5	642.41	279.51
9	1.500	19.2	37.5	260.44	203.61
12	1.500	16.9	24.4	169.46	140.19
20	1.500	12.9	90.3	627.13	153.72
7	1.500	23.6	51.9	360.45	470.92
8	1.500	12.6	28.4	197.24	421.34
14	1.500	15.6	44.4	308.36	564.6
15	1.500	11.5	61.9	429.9	307.95
17	1.500	10.8	35.6	247.24	261.74
20	1.500	12.8	[redacted]	0	[redacted]
21	1.500	9.5	32.7	227.1	245.75
23	1.500	19.2	31.1	215.99	278.1
1	1.500	18.5	82.5	572.96	1159.25
13	1.500	21.1	16.7	115.98	506.25
21	1.500	6.9	40.8	283.38	419.45
22	1.500	13.1	87	604.22	691.76
17	1.517	18.2	31.3	217.38	0
11	1.517	16.8	67.3	467.4	142.19
5	1.517	22.9	15.9	110.43	196.93
8	1.533	11.2	33.7	234.05	130.97
18	1.533	14.5	81.1	563.24	196.68
22	1.533	17	171.7	1192.5	50.63
15	1.533	11.7	108.1	750.75	160.45
22	1.533	13.3	75.4	523.65	264.88
20	1.550	18.3	63.5	441.01	0
22	1.550	16.7	24.1	167.37	0
3	1.550	19.7	63.1	438.23	151.97
5	1.550	23.7	9	62.505	71.4
14	1.550	19	64.5	447.95	[redacted]
14	1.550	17.8	56.7	393.78	281.63
8	1.550	13.7	25.9	179.88	625.96
7	1.567	26.5	32.8	227.8	0
21	1.583	21.8	19	131.96	0
23	1.583	16.5	27	187.52	135.3
6	1.583	19.5	63.4	440.31	231.41
10	1.583	14	61.1	424.34	216.13
11	1.583	19.2	83.7	581.3	213.97
22	1.583	14.4	173.6	1205.7	293.17

11	1.583	16.8	63.2	438.92	392.49
12	1.583	16.3	17	118.07	353.78
10	1.583	10.9	38.3	265.99	397.97
23	1.583	16.6	24	166.68	699.39
11	1.600	23.3	35.6	247.24	0
2	1.600	21.6	20.2	140.29	382.98
3	1.617	21.5	28.6	198.63	0
11	1.617	17.2	94.3	654.91	1051.06
21	1.633	14.3	29.4	204.18	104.75
19	1.633	22.6	97.6	677.83	240.19
12	1.650	23.1	16.5	114.59	0
4	1.667	23.2	24.8	172.24	0
13	1.667	25	13.3	92.369	0
7	1.667	24.3	50.9	353.5	112.21
9	1.667	16.7	60.9	422.95	117.52
2	1.667	22.7		0	
10	1.667	14	80.5	559.07	358.05
2	1.667	25.9	17.2	119.45	1125.37
20	1.667	11.7	57.7	400.73	577.53
19	1.683	30.6	78.9	547.96	0
10	1.683	13.2	90.9	631.3	247.9
3	1.683	18.2	67.7	470.18	1041.31
7	1.700	21.1	36	250.02	776.95
12	1.700	14.8	16.9	117.37	384.74
1	1.733	14.7	63.8	443.09	364.58
9	1.733	15.5	42.9	297.94	292.64
14	1.750	21.3	47.3	328.5	0
1	1.750	20.1	37.1	257.66	187.49
15	1.750	10.4	46.1	320.16	441.41
18	1.800	23.5	29.9	207.66	0
13	1.800	27.1	21.6	150.01	125.89
5	1.833	24.2	4.3	29.864	0
8	1.833	16.5	20	138.9	0
15	1.833	14.5	57.6	400.03	0
23	1.833	17.8	31.7	220.16	142.92
9	1.833	15.2	51.5	357.67	422.49
14	1.833	14.5	32.3	224.32	627.32
1	1.850	20.8	42.9	297.94	416.77
4	1.867	16.3	12.5	86.813	211.97
6	1.883	16	78	541.71	872.33
1	1.917	20.8	25.9	179.88	0
20	1.917	12.7	86	597.27	79.56
5	1.917	22.1	21.17	147.03	186.82
5	1.917	21.7	6.5	45.143	246.4
6	1.917	16	47	326.42	281.38
13	1.917	21.1	13.7	95.147	315.05
5	1.917	20.2	7.1	49.31	366.45
6	1.933	21.3	69.9	485.46	0
4	1.933	21.8	20.4	141.68	106.27
2	1.950	20.9	19.3	134.04	165.59

6	1.950	18.3	57.3	397.95	227.54
15	1.950	9.1	47.2	327.8	276.83
17	1.967	10.3	18.3	127.09	227.38
4	1.967	19.1	13.4	93.063	414.74
21	1.967	7.8	25.7	178.49	254.44
4	1.967	15.1	18.1	125.7	484.07
17	1.967	10.1	9.5	65.978	554.45
8	1.983	10	30.6	213.91	138.24
2	2.000	28	16.2	112.51	0
9	2.000	16.8	28.9	200.71	0
10	2.000	13.9	32.7	227.1	0
20	2.000	15.8	36.7	254.68	0
22	2.000	17.7	48.6	338.92	0
6	2.000	17.6	43.2	300.02	154.44
12	2.000	15	20.7	143.76	57.84
17	2.000	9.4	15.4	106.95	123.35
18	2.000	14.2	93.3	647.97	235.79
7	2.000	22.6	34.4	238.91	248.54
9	2.000	15.9	28.8	200.02	239.1
12	2.000	14.6	13.6	94.452	118.06
20	2.000	11.6	65.7	456.29	116.47
21	2.000	12.6	28.7	199.32	165.54
3	2.000	17.5	51.5	357.67	470.91
7	2.000	22.3	48.9	339.61	405.96
10	2.000	10.6	60.3	418.78	343.45
14	2.000	15.2	39.7	275.72	553.57
17	2.000	10.4	20.2	140.29	302.16
18	2.000	14.7	95.3	661.86	550.76
20	2.000	13.3		0	
23	2.000	19	22.9	159.04	273.69
10	2.000	9	36.3	252.1	326.29
19	2.000	19.1	60.9	422.95	634.66
20	2.000	11.5	43.8	304.19	484.61
21	2.000	6.1	33.5	232.66	438.49
17	2.017	17	14.6	101.4	0
5	2.017	22.4	6.6	47.226	45.23
11	2.017	19.7	64.4	447.26	127.03
8	2.017	9.5	35.7	247.94	171.09
21	2.033	10.5	23.5	163.21	102.73
23	2.033	15.3	22.4	155.57	110.94
3	2.033	17.1	83.5	579.91	257.66
10	2.033	13.6	89.5	621.58	245.49
13	2.033	23.1	16.3	113.2	74.06
22	2.033	12.6	66.5	461.84	326.23
3	2.050	17.9	52.2	362.53	123.5
14	2.050	16.2	51.2	355.58	261.84
19	2.050	13.9	73	506.99	321.2
3	2.050	16	38.7	266.77	818.32
23	2.067	19.6	13.41	93.132	0
1	2.067	15.1	56.5	392.39	260.95

15	2.067	9.8	85.7	595.19	115.78
7	2.067	21	32.5	225.71	947.58
3	2.083	21.1	18.9	131.26	0
4	2.083	23.7	43.5	302.11	0
7	2.083	27	36.3	252.1	0
11	2.083	20.5	52.6	365.31	0
12	2.083	18.9	13.5	93.758	0
21	2.083	20.7	18.2	126.4	0
14	2.083	17.2	46.4	322.25	[illegible]
15	2.083	13.9	53.1	368.78	70.59
2	2.083	22.5	22.3	154.87	434.98
18	2.083	9.7	75.3	522.96	259.22
8	2.083	10.5	23.5	163.21	422.06
11	2.083	15.2	91.7	636.86	426.34
12	2.083	13.6	14.7	102.09	342.53
1	2.083	18.9	61.2	425.03	1138.33
11	2.083	14.9	80.3	418.78	765.48
13	2.083	19.3	15.3	106.26	416.22
22	2.083	11.2	122.9	853.54	1391.59
23	2.083	15.3	18.3	127.09	647.98
8	2.100	13	29.5	204.88	643.84
18	2.100	14.8	54.3	377.11	1600.73
22	2.117	9	80.4	558.38	287.79
7	2.133	24.2	54.2	376.42	122.19
13	2.167	22.9	12.2	84.729	0
9	2.167	15.5	41.6	288.91	141.97
11	2.167	14.4	69.5	482.68	204.77
19	2.167	22.3	121.2	841.73	164.08
2	2.167	27.5	20.7	143.78	1105.15
19	2.183	30.7	40.2	279.19	0
22	2.200	13.3	154.6	1073.7	74.99
8	2.250	14.3	15.4	106.95	0
14	2.250	20.4	38.7	268.77	0
18	2.250	24.1	29.6	205.57	0
1	2.250	18.7	37.1	257.68	155.08
2	2.250	20.3	20.4	141.68	519.62
9	2.250	13.1	50.2	348.64	460.87
12	2.250	13.7	14.4	100.01	340.12
10	2.300	9.7	49.8	345.86	182.58
13	2.300	25.2	19.3	134.04	86.27
6	2.300	12.6	51.9	360.45	719.77
1	2.333	19.1	22.7	157.65	0
13	2.333	21.8	15.3	106.26	71.36
5	2.350	15.1	3.15	21.877	0
1	2.350	20.7	33.6	233.35	436.58
9	2.350	14.6	38.6	268.08	337.65
15	2.417	11.1	31.4	218.07	0
19	2.417	16.7	71.1	493.79	105.87
2	2.483	15.1	26.3	182.65	235.8
13	2.500	20.5	18.1	125.7	350.9

5	2.500	19.1	4.5	31.253	385.46
14	2.500	11.6	30.9	214.6	605.9
15	2.500	7.9	38.1	264.6	309.18
10	2.583	11	22.3	154.87	0
12	2.667	13.3	13.9	96.536	249.21
1	2.917	17.9	18.7	129.87	0
13	2.917	23.7	12.4	86.118	0
6	3.500	11.7	28.7	199.32	183.11
9	3.750	11.6	12.9	89.591	0
15	3.750	5.6	25.6	177.79	244.07
8	3.783	6.3	24.4	169.46	188.04
18	3.800	4.9	34.4	238.91	222.71
19	3.800	7.1	11.9	82.646	173.3
18	3.817	8.4	21.7	150.71	1098.65
14	3.833	14.7	24.3	168.76	0
9	3.833	9.6	40.2	279.19	75.82
10	3.833	5.8	22.5	156.28	118
4	3.833	11.4	9.1	63.2	141.5
5	3.833	14.4	10	69.45	107.15
21	3.833	3.4	22.4	155.57	160.86
14	3.833	8.7	20.2	140.29	661.27
17	3.833	8.1	8.5	59.033	586.17
23	3.867	16.3	10.1	70.145	0
1	3.900	11.9	36.4	252.8	136.69
6	3.917	13.9	32.8	227.8	0
7	3.917	20.3	20.8	144.46	0
8	3.917	11.4	12.6	87.507	0
10	3.917	8.4	13.5	93.758	0
18	3.917	18.7	13.5	93.758	0
12	3.917	12.5	18.5	128.48	46.41
20	3.917	7.6	29.1	202.1	28.36
17	3.917	7.2	5.7	39.587	144.81
23	3.917	13.4	21.4	148.62	81.9
5	3.917	16.3	5.6	38.892	143.7
15	3.917	4.9	16.9	117.37	125.84
18	3.917	8.7	25.46	176.82	330.72
10	3.917	4.7	18.8	130.57	445.26
23	3.917	12.6	13.7	95.147	300.84
17	3.933	12.6	5.7	39.587	0
9	3.933	9.7	17.5	121.54	232.71
12	3.933	10.1	13.2	91.674	232.08
19	3.950	12.2	40.3	279.88	73.08
2	3.967	26.7	28.4	197.24	273.46
4	3.967	14	12.4	86.118	74.78
13	3.967	18.8	14.7	102.09	77.26
8	3.967	9.7	29.4	204.18	493.65
8	3.967	8.2	16.4	113.9	340.44
23	3.983	12.1	15	104.18	57.78
2	4.000	25	15.5	107.85	0
3	4.000	16.1	22.4	155.57	0

4	4.000	16.4	8.3	57.644	0
20	4.000	10.1	17.3	120.15	0
21	4.000	16.7	9.7	67.367	0
1	4.000	14.6	25.5	177.1	87.72
5	4.000	14.8	3.7	25.697	59.38
6	4.000	15.5	30.6	212.52	83.04
17	4.000	6.5	8.7	60.422	70.92
19	4.000	9	39.6	275.02	54.89
22	4.000	7.9	66.4	461.15	112.37
2	4.000	21.5	14.7	102.09	202.1
6	4.000	13.8	37.2	258.35	153.5
11	4.000	10.5	40.3	279.88	105.83
12	4.000	11.9	11	76.395	56.93
14	4.000	10.5	26.7	185.43	286.93
20	4.000	6.7	48.1	334.05	69.13
21	4.000	7.8	19	131.96	190.51
10	4.000	5.1	27.1	188.21	192.69
13	4.000	16.6	11.9	82.646	291.63
14	4.000	8.6	19	131.96	469.03
17	4.000	6.6	12.2	64.729	235.83
20	4.000	7.8	[illegible]	0	[illegible]
23	4.000	14.3	21	145.85	232.19
1	4.000	12.6	38.3	265.99	796.03
4	4.000	9	10	69.45	171.21
11	4.000	9.8	35	243.08	565.53
13	4.000	15.8	14.4	100.01	303.6
19	4.000	10.6	31.1	215.99	613.66
20	4.000	7.3	36.8	255.58	232.79
7	4.017	12.5	30.1	209.04	830.63
7	4.033	16	38.2	265.3	193.89
13	4.033	19.3	11.7	81.257	57.45
15	4.033	4.9	29.8	206.96	86.82
22	4.033	7.7	43.1	299.33	630.68
11	4.050	9.3	29.2	202.79	122.69
15	4.050	5.8	22.2	154.18	84.71
18	4.050	6.5	27.8	193.07	222.1
1	4.050	15.3	25	173.63	280.29
3	4.050	9.1	36.9	256.27	597.67
3	4.067	11.9	40.3	279.88	118.93
1	4.083	16.9	15.2	105.56	0
11	4.083	13.7	17.1	118.76	0
12	4.083	17	10.3	71.534	0
13	4.083	20.3	8.6	59.727	0
22	4.083	12	25	173.83	0
3	4.083	14.1	35.1	243.77	48.34
8	4.083	6.2	17.4	120.84	70.77
14	4.083	11.1	[illegible]	0	[illegible]
9	4.083	11.8	18.7	129.67	267.22
10	4.083	6.2	23.5	163.21	424.77
22	4.083	8	48.2	334.75	228.22

2	4.083	20.9	16.2	112.51	381.63
6	4.083	10.7	25.4	176.4	176.11
8	4.083	5.4	23.9	165.99	250.75
11	4.083	9.7	48.9	339.61	295.58
12	4.083	11.1	13.5	93.758	208.46
2	4.083	24.1	10.5	72.923	664.54
9	4.083	8	20.2	140.29	324.92
21	4.083	3	17.2	119.45	226.88
22	4.083	5.1	71.9	499.35	862.44
15	4.117	8.7	17.3	120.15	0
21	4.117	6.3	19.5	135.43	102.03
4	4.133	11.6	7.8	54.171	409.7
7	4.167	18.1	35.2	244.46	80.64
3	4.167	13.2	33.9	235.44	255.92
7	4.167	15.9	29.4	204.18	338.98
5	4.167	16.6	4.9	34.031	332.72
5	4.200	16.1	6	41.67	0
19	4.300	17.6	14.8	102.79	0
19	4.300	9.3	15.4	106.95	128.44
18	4.317	10.8	31.5	218.77	1111.79
9	4.333	11.5	17	118.07	0
14	4.333	15.1	38.8	269.47	0
9	4.333	10.4	25.9	179.88	89.35
4	4.333	13	13.9	96.536	100.65
8	4.367	8.4	41.5	288.22	223.61
5	4.383	15.4	10.1	70.145	75.35
6	4.383	10	37.1	257.66	520.26
10	4.400	6.1	39.6	275.02	180.6
18	4.400	7.3	53.5	371.56	225.66
18	4.417	18.3	21.5	149.32	0
12	4.417	11.1	29.6	205.57	51.69
20	4.417	8.2	45.9	318.78	28.81
17	4.417	7	13	90.285	153.74
23	4.417	11.9	18.5	128.48	77.54
5	4.417	17.5	8.7	60.422	156.58
21	4.417	4.3	22.1	153.48	165.27
6	4.433	17.5	69.4	481.96	0
17	4.433	13.2	17.7	122.93	0
4	4.433	15.3	14.2	98.619	76.57
8	4.433	9	24.8	172.24	356.49
17	4.450	7.7	12.4	86.118	502.2
23	4.467	15.4	14.7	102.09	0
15	4.467	5.2	27.8	193.07	142.45
1	4.483	13.2	48.7	338.22	123.35
9	4.483	10	19.7	136.82	204.78
22	4.483	7.1	36.5	253.49	693.81
4	4.500	16.4	20.9	145.15	0
8	4.500	10.5	12.2	84.729	0
21	4.500	15.8	14.5	100.7	0
22	4.500	12.4	23.1	160.43	0

1	4.500	15.5	37.6	261.13	100.34
3	4.500	13.5	51.2	355.58	55.5
17	4.500	6	15	104.18	52.43
19	4.500	8.9	50.9	353.5	44.28
7	4.500	15.9	38.8	269.47	205.73
12	4.500	11.1	21.5	149.32	53.08
14	4.500	10.9	36.6	254.19	340.02
15	4.500	6.4	36.1	250.71	59.43
2	4.500	20.7	22.6	156.96	304.36
10	4.500	7.1	50.5	350.72	239.98
13	4.500	16.6	16	111.12	308.51
14	4.500	9.4	30.8	213.91	500.43
15	4.500	5.5	45.1	313.22	105.7
17	4.500	7	21.7	150.71	229.15
23	4.500	14.2	20.9	145.15	250.63
4	4.500	9.4	15	104.18	190.24
10	4.500	3.6	35	243.08	503.14
11	4.500	9.8	51.9	360.45	548.13
13	4.500	19.3	13.7	95.147	329.25
14	4.500	9.9	34.1	236.82	752.56
15	4.500	7	46.2	320.86	194.46
21	4.500	2.6	27.6	191.68	194.56
3	4.517	13.3	69.4	481.98	113.42
5	4.533	11.5	7.4	51.393	122.84
6	4.533	16.9	40.6	281.97	74.79
23	4.533	10.5	16.2	112.51	33.22
19	4.533	13	96.5	670.19	69.23
23	4.533	11.7	16.2	112.51	282.37
13	4.550	20.4	16.1	111.81	76.78
6	4.550	15.4	43.2	300.02	100.32
20	4.550	6.9	65.2	452.81	75.37
1	4.550	14.7	40.4	280.58	337.6
4	4.550	10.8	10.7	74.312	242.87
2	4.567	24.3	26.3	182.65	220.43
1	4.583	17.6	22.7	157.65	0
7	4.583	21.5	31	215.3	0
11	4.583	15.9	37.4	259.74	0
20	4.583	10.6	31	215.3	0
7	4.583	16.3	41.1	285.44	79.75
9	4.583	11.2	30.5	211.82	411.16
3	4.583	12.4	53.8	373.64	222.84
7	4.583	15.7	42.2	293.08	346.48
20	4.583	9	[illegible]	0	[illegible]
1	4.583	13.9	53	368.09	650.27
9	4.583	8.1	22.7	157.65	410.61
12	4.583	10.5	29.4	204.18	296.34
8	4.600	6.5	26.2	181.96	190.9
11	4.600	10.1	35.1	243.77	190.33
18	4.617	7.6	30.8	213.91	338.27
13	4.617	18.7	16.5	114.59	67.04

19	4.617	11.1	67.3	467.4	279.55
20	4.617	7.8	50.9	353.5	260.24
22	4.617	7.4	90.9	631.3	792.03
14	4.633	9.3	43.6	302.82	[illegible]
22	4.633	7.9	98	680.61	108.69
2	4.667	25.9	21.5	149.32	0
5	4.667	16	8.4	44.448	0
10	4.667	8.2	24.5	170.15	0
12	4.667	17	20.1	139.59	0
15	4.667	8.4	31.1	215.99	0
2	4.667	19.1	27	187.52	166.81
11	4.867	10.5	61.3	425.73	136.95
21	4.667	9.9	23.9	165.99	180.44
6	4.667	10.8	40.2	279.19	184.18
3	4.700	17.4	37.9	263.22	0
18	4.700	11.3	53.6	372.25	299.49
9	4.750	13.5	17.1	118.76	0
13	4.750	23.8	13	90.285	0
2	4.750	21.4	22	152.79	542.56
5	4.750	17.6	8.4	58.336	525.69
10	4.800	8.3	32.4	225.02	159.43
14	4.833	15.3	47.4	329.19	0
4	4.833	16.1	16.3	113.2	119.41
5	4.833	18.4	11.8	81.951	82.64
10	4.833	8.8	52.2	362.53	224.64
22	4.833	8.9	100	694.5	239.02
8	4.833	7.6	30	208.35	273.88
11	4.833	10.9	51.8	359.75	232.53
9	4.850	11.8	29	201.41	325.56
7	4.850	15.6	46.6	323.64	1027.69
12	4.867	13.3	33.3	231.27	50.63
6	4.883	14.1	43.5	302.11	437.5
6	4.900	17.2	69.8	484.76	0
19	4.917	23	44.1	306.27	0
20	4.917	11.6	47	326.42	0
8	4.917	8.5	47.2	327.8	175.76
17	4.917	10.5	16	111.12	109.96
18	4.917	9.5	57.1	396.56	196.32
5	4.917	20.3	8	55.58	117.87
12	4.917	12.3	30.6	212.52	198.33
14	4.917	11.9	40.9	284.05	446.11
15	4.917	8.4	82	430.59	93.33
21	4.917	5.8	19.7	136.82	137.5
17	4.933	15	21.1	146.54	0
3	4.933	12.9	57.3	397.95	452.91
23	4.950	19	17	118.07	0
15	4.950	8.4	45	312.53	82.51
21	4.950	7.8	22	152.79	101.23
23	4.950	12.2	18.7	129.87	67.36
19	4.967	11.5	60.3	418.78	32.37

6 Data listing: NONMEM data file contruction - Pharmacokinetics - only subjects 1 and 2 shown

#ID	TIME	AMT	DV	CMT	EVID	MDV	II	ADDL	DAMT
1	0	2500	0	1	1	1	24	12	2500
1	336	0	33.15	2	0	0	0	0	2500
1	336.01	2500	0	1	1	1	0	0	2500
1	336.667	0	145.6	2	0	0	0	0	2500
1	337.217	0	192.47	2	0	0	0	0	2500
1	337.75	0	187.49	2	0	0	0	0	2500
1	338.25	0	155.08	2	0	0	0	0	2500
1	340	0	87.72	2	0	0	0	0	2500
1	340.5	0	100.34	2	0	0	0	0	2500
1	341	0	80.25	2	0	0	0	0	2500
1	341.583	0	71.57	2	0	0	0	0	2500
1	343.083	0	55.31	2	0	0	0	0	2500
1	360	5000	0	1	1	1	24	12	5000
1	696.01	5000	0	1	1	1	0	0	5000
1	696.567	0	141.76	2	0	0	0	0	5000
1	697.15	0	288.37	2	0	0	0	0	5000
1	697.733	0	364.58	2	0	0	0	0	5000
1	698.067	0	280.95	2	0	0	0	0	5000
1	699.9	0	136.69	2	0	0	0	0	5000
1	700.483	0	123.35	2	0	0	0	0	5000
1	700.983	0	118.26	2	0	0	0	0	5000
1	701.567	0	103.35	2	0	0	0	0	5000
1	702.983	0	54.2	2	0	0	0	0	5000
1	720	10000	0	1	1	1	24	12	10000
1	1056	0	82.16	2	0	0	0	0	10000
1	1056.01	10000	0	1	1	1	0	0	10000
1	1056.5	0	237.78	2	0	0	0	0	10000
1	1057.3	0	403.56	2	0	0	0	0	10000
1	1057.85	0	418.77	2	0	0	0	0	10000
1	1058.35	0	436.58	2	0	0	0	0	10000
1	1060.05	0	280.29	2	0	0	0	0	10000
1	1060.55	0	337.6	2	0	0	0	0	10000
1	1061.05	0	301.81	2	0	0	0	0	10000
1	1061.55	0	252.54	2	0	0	0	0	10000
1	1063	0	167.11	2	0	0	0	0	10000
1	1080	20000	0	1	1	1	24	12	20000
1	1416	0	60.59	2	0	0	0	0	20000
1	1416.01	20000	0	1	1	1	0	0	20000
1	1416.5	0	294.15	2	0	0	0	0	20000
1	1417	0	627.57	2	0	0	0	0	20000
1	1417.5	0	1159.25	2	0	0	0	0	20000
1	1418.083	0	1138.33	2	0	0	0	0	20000
1	1420	0	796.03	2	0	0	0	0	20000
1	1420.583	0	650.27	2	0	0	0	0	20000
1	1421	0	653.14	2	0	0	0	0	20000
1	1421.5	0	571.01	2	0	0	0	0	20000
1	1423	0	332.26	2	0	0	0	0	20000
2	0	2500	0	1	1	1	24	12	2500
2	336	0	51.4	2	0	0	0	0	2500
2	336.01	2500	0	1	1	1	0	0	2500
2	336.75	0	106.01	2	0	0	0	0	2500
2	337.4	0	143.07	2	0	0	0	0	2500
2	337.95	0	165.59	2	0	0	0	0	2500
2	338.483	0	235.8	2	0	0	0	0	2500
2	339.967	0	273.46	2	0	0	0	0	2500
2	340.567	0	220.43	2	0	0	0	0	2500
2	341.167	0	196.23	2	0	0	0	0	2500
2	341.583	0	170.92	2	0	0	0	0	2500
2	342.917	0	158.27	2	0	0	0	0	2500
2	360	5000	0	1	1	1	24	12	5000
2	696	0	58.36	2	0	0	0	0	5000
2	696.01	5000	0	1	1	1	0	0	5000

#ID	TIME	AMT	DV	CMT	EVID	MDV	II	ADDL	DAMT
2	696.583	0	288.23	2	0	0	0	0	5000
2	697.05	0	318.09	2	0	0	0	0	5000
2	697.6	0	382.98	2	0	0	0	0	5000
2	698.083	0	434.98	2	0	0	0	0	5000
2	700	0	202.1	2	0	0	0	0	5000
2	700.667	0	166.81	2	0	0	0	0	5000
2	701.083	0	160.49	2	0	0	0	0	5000
2	701.5	0	131.07	2	0	0	0	0	5000
2	702.967	0	90.15	2	0	0	0	0	5000
2	720	10000	0	1	1	1	24	12	10000
2	1056	0	93.89	2	0	0	0	0	10000
2	1056.01	10000	0	1	1	1	0	0	10000
2	1056.833	0	620.23	2	0	0	0	0	10000
2	1057.25	0	552	2	0	0	0	0	10000
2	1058.25	0	519.62	2	0	0	0	0	10000
2	1060.083	0	381.63	2	0	0	0	0	10000
2	1060.5	0	304.36	2	0	0	0	0	10000
2	1061	0	292.37	2	0	0	0	0	10000
2	1061.5	0	219.52	2	0	0	0	0	10000
2	1063	0	167.08	2	0	0	0	0	10000
2	1080	20000	0	1	1	1	24	12	20000
2	1416	0	290.45	2	0	0	0	0	20000
2	1416.01	20000	0	1	1	1	0	0	20000
2	1416.833	0	1107.46	2	0	0	0	0	20000
2	1417.25	0	1161.06	2	0	0	0	0	20000
2	1417.667	0	1125.37	2	0	0	0	0	20000
2	1418.167	0	1105.15	2	0	0	0	0	20000
2	1420.083	0	664.54	2	0	0	0	0	20000
2	1420.75	0	542.56	2	0	0	0	0	20000
2	1421.333	0	441.34	2	0	0	0	0	20000
2	1421.75	0	415.79	2	0	0	0	0	20000
2	1423	0	272.82	2	0	0	0	0	20000

7 Data listing: NONMEM data file contruction - Pharmacodynamics - only subjects 1 and 2 shown

#ID	TIME	AMT	DV	CMT	EVID	MDV	II	ADDL	SS	DAMT
1	0	0	0	2	1	1	0	0	1	0
1	0	0	13	2	0	0	0	0	0	0
1	0.917	0	19.2	2	0	0	0	0	0	0
1	1.917	0	20.8	2	0	0	0	0	0	0
1	2.333	0	19.1	2	0	0	0	0	0	0
1	2.917	0	17.9	2	0	0	0	0	0	0
1	4.083	0	16.9	2	0	0	0	0	0	0
1	4.583	0	17.6	2	0	0	0	0	0	0
1	5.167	0	20.9	2	0	0	0	0	0	0
1	5.667	0	20.4	2	0	0	0	0	0	0
1	7.25	0	15.4	2	0	0	0	0	0	0
1	24	2500	0	1	1	1	24	13	0	2500
1	360	0	11.2	2	0	0	0	0	0	2500
1	360.01	2500	0	1	1	1	0	0	0	2500
1	360.667	0	13.9	2	0	0	0	0	0	2500
1	361.217	0	22	2	0	0	0	0	0	2500
1	361.75	0	20.1	2	0	0	0	0	0	2500
1	362.25	0	18.7	2	0	0	0	0	0	2500
1	364	0	14.6	2	0	0	0	0	0	2500
1	364.5	0	15.5	2	0	0	0	0	0	2500
1	365	0	18.4	2	0	0	0	0	0	2500
1	365.583	0	19.2	2	0	0	0	0	0	2500
1	367.083	0	15.2	2	0	0	0	0	0	2500
1	384	5000	0	1	1	1	24	13	0	5000
1	720	0	10.4	2	0	0	0	0	0	5000
1	720.01	5000	0	1	1	1	0	0	0	5000
1	720.567	0	13.9	2	0	0	0	0	0	5000
1	721.15	0	15.7	2	0	0	0	0	0	5000
1	721.733	0	14.7	2	0	0	0	0	0	5000
1	722.067	0	15.1	2	0	0	0	0	0	5000
1	723.9	0	11.9	2	0	0	0	0	0	5000
1	724.483	0	13.2	2	0	0	0	0	0	5000
1	724.983	0	15.8	2	0	0	0	0	0	5000
1	725.567	0	13.8	2	0	0	0	0	0	5000
1	726.983	0	12.5	2	0	0	0	0	0	5000
1	744	10000	0	1	1	1	24	13	0	10000
1	1080	0	13.7	2	0	0	0	0	0	10000
1	1080.01	10000	0	1	1	1	0	0	0	10000
1	1080.5	0	17.6	2	0	0	0	0	0	10000
1	1081.3	0	19.3	2	0	0	0	0	0	10000
1	1081.85	0	20.8	2	0	0	0	0	0	10000
1	1082.35	0	20.7	2	0	0	0	0	0	10000
1	1084.05	0	15.3	2	0	0	0	0	0	10000
1	1084.55	0	14.7	2	0	0	0	0	0	10000
1	1085.05	0	18.9	2	0	0	0	0	0	10000
1	1085.55	0	17.6	2	0	0	0	0	0	10000
1	1087	0	14.5	2	0	0	0	0	0	10000
1	1104	20000	0	1	1	1	24	13	0	20000
1	1440	0	11.5	2	0	0	0	0	0	20000
1	1440.01	20000	0	1	1	1	0	0	0	20000
1	1440.5	0	13.3	2	0	0	0	0	0	20000
1	1441	0	17.9	2	0	0	0	0	0	20000
1	1441.5	0	18.5	2	0	0	0	0	0	20000
1	1442.083	0	18.9	2	0	0	0	0	0	20000
1	1444	0	12.6	2	0	0	0	0	0	20000
1	1444.583	0	13.9	2	0	0	0	0	0	20000
1	1445	0	15.8	2	0	0	0	0	0	20000
1	1445.5	0	18.8	2	0	0	0	0	0	20000
1	1447	0	14	2	0	0	0	0	0	20000
2	0	0	0	2	1	1	0	0	1	0

#ID	TIME	AMT	DV	CMT	EVID	MDV	II	ADDL	SS	DAMT
2	0	0	21.2	2	0	0	0	0	0	0
2	0.533	0	25.9	2	0	0	0	0	0	0
2	1	0	28	2	0	0	0	0	0	0
2	1.5	0	28	2	0	0	0	0	0	0
2	2	0	28	2	0	0	0	0	0	0
2	4	0	25	2	0	0	0	0	0	0
2	4.667	0	25.9	2	0	0	0	0	0	0
2	5.133	0	28	2	0	0	0	0	0	0
2	5.583	0	28	2	0	0	0	0	0	0
2	6.833	0	25.7	2	0	0	0	0	0	0
2	24	2500	0	1	1	1	24	13	0	2500
2	360	0	15.6	2	0	0	0	0	0	2500
2	360.01	2500	0	1	1	1	0	0	0	2500
2	360.75	0	18.2	2	0	0	0	0	0	2500
2	361.4	0	19.8	2	0	0	0	0	0	2500
2	361.95	0	20.9	2	0	0	0	0	0	2500
2	362.483	0	15.1	2	0	0	0	0	0	2500
2	363.967	0	26.7	2	0	0	0	0	0	2500
2	364.567	0	24.3	2	0	0	0	0	0	2500
2	365.167	0	28	2	0	0	0	0	0	2500
2	365.583	0	28	2	0	0	0	0	0	2500
2	366.917	0	25.9	2	0	0	0	0	0	2500
2	384	5000	0	1	1	1	24	13	0	5000
2	720	0	17.8	2	0	0	0	0	0	5000
2	720.01	5000	0	1	1	1	0	0	0	5000
2	720.583	0	19.3	2	0	0	0	0	0	5000
2	721.05	0	18.8	2	0	0	0	0	0	5000
2	721.6	0	21.6	2	0	0	0	0	0	5000
2	722.083	0	22.5	2	0	0	0	0	0	5000
2	724	0	21.5	2	0	0	0	0	0	5000
2	724.667	0	19.1	2	0	0	0	0	0	5000
2	725.083	0	21.5	2	0	0	0	0	0	5000
2	725.5	0	23.6	2	0	0	0	0	0	5000
2	726.967	0	26.5	2	0	0	0	0	0	5000
2	744	10000	0	1	1	1	24	13	0	10000
2	1080	0	18	2	0	0	0	0	0	10000
2	1080.01	10000	0	1	1	1	0	0	0	10000
2	1080.833	0	21.4	2	0	0	0	0	0	10000
2	1081.25	0	21	2	0	0	0	0	0	10000
2	1082.25	0	20.3	2	0	0	0	0	0	10000
2	1084.083	0	20.9	2	0	0	0	0	0	10000
2	1084.5	0	20.7	2	0	0	0	0	0	10000
2	1085	0	23.6	2	0	0	0	0	0	10000
2	1085.5	0	24.3	2	0	0	0	0	0	10000
2	1087	0	24.2	2	0	0	0	0	0	10000
2	1104	20000	0	1	1	1	24	13	0	20000
2	1440	0	18.8	2	0	0	0	0	0	20000
2	1440.01	20000	0	1	1	1	0	0	0	20000
2	1440.833	0	24	2	0	0	0	0	0	20000
2	1441.25	0	23.8	2	0	0	0	0	0	20000
2	1441.667	0	25.9	2	0	0	0	0	0	20000
2	1442.167	0	27.5	2	0	0	0	0	0	20000
2	1444.083	0	24.1	2	0	0	0	0	0	20000
2	1444.75	0	21.4	2	0	0	0	0	0	20000
2	1445.333	0	27.3	2	0	0	0	0	0	20000
2	1445.75	0	33	2	0	0	0	0	0	20000
2	1447	0	25.3	2	0	0	0	0	0	20000

8 Inter-subject variability - NONMEM approximations

If the model is Pj = TVP*exp(ηj), then the first order approximation (a Taylor series expansion at eta=0) is Pj is approximately TVP * (1+ηj),

where TVP is a fixed parameter and η ~ multivariate N (0, Ω).

Consequently,

E(TVP) is approximately TVP and var(TVP) is approximately var(TVP) + var(TVP*η) = TVP^2 * var(η) = TVP^2 *Ω.

Therefore,

CV(TVP) = sqrt(var(TVP))/E(TVP) is approximately [TVP * Ω^(1/2)] / TVP = Ω^(1/2).

http://www.cognigencorp.com/nonmem/nm/99feb042003.html

http://www.cognigencorp.com/nonmem/nm/98sep261997.html

9 NONMEM code for 1-compartment pharmacokinetic model

```
; 1cmtlog GP 27Nov2003
; 1 Cmt Model
$PROB  Glibenclamide PK
$INPUT ID TIME AMT DVX CMT EVID MDV II ADDL DAMT WT SEX DV TAD
$DATA ..\data\virenehc2.csv
$SUBROUTINE ADVAN2 TRANS2
$PK

; Typical Parameters
      TVKA = THETA(1)
      TVCL = THETA(2)
      TVV  = THETA(3)

; Individual Parameters
      KA   = TVKA*EXP(ETA(1))
      CL   = TVCL*EXP(ETA(2))
      V    = TVV *EXP(ETA(3))

; Scaling AMOUNT to CONCENTRATION
      S2   = V

$ERROR
     DL=0
     IF (F.EQ.0) DL=0.001
     W= DL +F
     Y=LOG(W)+EPS(1)
     IRES = DV-F
     IWRE = (DV-F)/F
     IPRE = LOG(W)

$THETA (0, 1)       ;1:KA
$THETA (0, 5)        ;2:CL
$THETA (0, 15)        ;3:V

$OMEGA
   0.9        ; 1 KA
   0.9        ; 2 CL
   0.9        ; 3 V
$SIGMA 0.9

$COV
$EST   MSFO = 1cmt.msf MAXEVAL=9999 PRINT=10 METH=COND INTER
$TABLE FILE = 1cmt.fit ID AMT TIME CMT DAMT TAD
IPRE ONEHEADER NOPRINT
```

10 NONMEM code for 2-compartment pharmacokinetic model

```
; 2cmtss GP 27Nov2003
; 2 Cmt Model
$PROB  Glibenclamide PK
$INPUT ID TIME AMT DVX CMT EVID MDV II ADDL DAMT WT SEX DV TAD
$DATA ..\data\virenEHC2.csv
$SUBROUTINE ADVAN4 TRANS4
$PK
; Typical Parameters

      TVKA = THETA(1)
      TVCL = THETA(2)
      TVV2 = THETA(3)
      TVQ  = THETA(4)
      TVV3 = THETA(5)

; Individual Parameters

      KA   = TVKA*EXP(ETA(1))
      CL   = TVCL*EXP(ETA(2))
      V2   = TVV2*EXP(ETA(3))
      Q    = TVQ *EXP(ETA(4))
      V3   = TVV3*EXP(ETA(5))


; Scaling AMOUNT to CONCENTRATION

      S2   = V2

$ERROR
     DL=0
     IF (F.EQ.0) DL=0.001
     W= DL +F
     Y=LOG(W)+EPS(1)
     IRES = DV-F
     IWRE = (DV-F)/F
     IPRE = LOG(W)

$THETA (0, 0.5)         ;1:KA
$THETA (0, 3)        ;2:CL
$THETA (0, 10)        ;3:V2
$THETA (0, 2.5)        ;4:Q
$THETA (0, 50)     ;5:V3

$OMEGA 0.9                ; 1 KA
$OMEGA
  0.9                     ; 2 CL
  0.9                ; 3 V2
$OMEGA
  0.9                     ; 4 Q
  0.9                     ; 5 V3

$SIGMA 0.9

$COV
$EST   MSFO = 2cmt.msf MAX=9999 PRI=10 METH=COND INTER NOABORT
$TABLE FILE = 2cmt.fit ID AMT TIME CMT DAMT TAD
IPRE ONEHEADER NOPRINT
```

```
$TABLE FILE = 2cmt.par ID KA CL V2 Q V3 DAMT ONEHEAD FIRST NOPRI
$TABLE FILE = 2cmtall.par ID KA CL V2 Q V3 DAMT ONEHEAD NOPRI
```

11 NONMEM code for Enterohepatic recycling model

```
$PROB ENTEROHEPATIC Circulation for multiple doses - GB Empties after lunch

;Data Description
; Doses of Drug go into CMT=1 with ADDL=12 and II=24 and another dose a day
; later before sampling
; Dummy Dose into CMT=5 with ADDL=13, II=24, and AMT=1
; Dummy Dose into CMT=6 with ADDL=13, II=24, and AMT=1
; Dummy Dose into CMT=7 with ADDL=10, II=24, and AMT=1 if supper as well
; Dummy Dose into CMT=8 with ADDL=10, II=24, and AMT=1 if supper as well

; Structural Model
; 3 compartment model including a depot compartment
; compartment 1 serves as the depot
; compartment 2 and 3 serve as a standard 2 cmt model (central + peri.)
; compartment 4 represents the gall bladder (GB)

; To control the 'on' and 'off' of flow to the GB and from the GB to the
; depot a 'change point' modeling technique will be used. This technique
; uses dummy dose compartments
;  to allow the estimation of lag times which will be used to control the
;  on-off switch for transport to and from the GB.

;   flow into the GB (K24) will be 'on' from 0 to 8 hours after each dose
; and then will be turned 'off' until the following dose
; flow from the GB to the depot compartment (K41) will be turned 'on' at
; the time of lunch and dinner(known times) and turned 'off' at an
; estimated time later



$INPUT ID TIME AMT DVX CMT EVID MDV II ADDL DAMT WT SEX DV TAD
$DATA ..\data\virenEHC.csv

$SUBROUTINES ADVAN6 TRANS1 TOL=5

$MODEL
  COMP=(DEPOT,DEFDOSE,INITIALOFF);1 dosing compartment - also used to turn
                                  ; on GB entry
  COMP=(CENTRAL,NODOSE,DEFOBS)    ;2 central compartment
  COMP=(PERI,NODOSE)              ;3 peripherial compartment
  COMP=(GB,NODOSE)                ;4 gall bladder
  COMP=(GB4ON)                    ;5 dummy cmt to turn on GB exit at lunch
  COMP=(GB4OFF)                   ;6 dummy cmt to turn off GB exit at lunch

$PK

;this allows PK to be called at LAGGED dose times - critical
  CALLFL=-2

  TVKA=THETA(1)
  KA=TVKA*EXP(ETA(1))


  TVCL=THETA(2)
  CL=TVCL*EXP(ETA(2))
```

```
   TVVC=THETA(3)
   V2=TVVC*EXP(ETA(3))

   TVQ  = THETA(4)
   Q    = TVQ*EXP(ETA(4))

   TVV3 = THETA(5)
   V3   = TVV3*EXP(ETA(5))

 ;to prevent drug from accumulating in the gall bladder after the gall
 ; bladder empties
 ; K24 will be turned off from the last modeled time the gall bladder
 ; empties for a day
 ; until the next day
 ;K24= flow from central to the gall bladder (this will be turned on
 ; and off)
 ;K41= flow from gall bladder to depot (this will be turned on and off)
   K24=THETA(6) * EXP(ETA(6))
   K41=THETA(7)


 ;for this model it was assumed that the amount of elapsed time the gall
 ; bladder exit rate will
 ; be on each time it empties will be the same (GBL)

 ;ALAG5 and ALAG7 were set to fixed values based upon the known time of
 ; lunch and dinner
 ; 0.0001 was added in each case to prevent the possibility of an infinite
 ; value of OBJ value
 ; because the dataset had PK samples at time=4 and time=8
 ;ALAG5 AND ALAG7 could be estimated

 GBL=THETA(8)
 ;*EXP(ETA(6))

 ALAG5=4.0001
 ALAG6=ALAG5+GBL
 ;ALAG7=8.0001
 ;ALAG8=ALAG7+GBL

 ;SCALE FACTOR
 S2=V2

 ;the following code sets up indicator variables to control K24 and K41
 ; in the
 ; $DES block- JON/JOFF/Z control K41 and GBON/GBOFF/Z2 control K24

 ;first record in an individual K24 is 'on' and K41 is 'off'
 IF(NEWIND.LT.2)THEN
 JON=0
 JOFF=1
 GBON=1
 GBOFF=0
 ENDIF

 ;set the values of the on'off indicator variables
 IF(JON.EQ.1) Z=1
```

```
IF(JOFF.EQ.1) Z=0
IF(GBON.EQ.1) Z2=1
IF(GBOFF.EQ.1) Z2=0

;reset control switches to zero - note these do not change the value of Z
or Z2
JON=0
JOFF=0
GBON=0
GBOFF=0

; note that DOSREC(variable)=0 except at the time a dose actually enters
; the system
; (doses enter the system at TIME + n*II + ALAG#)

;dose records to cmt 1 should turn on GB entry (K24)
IF(DOSREC(CMT).EQ.1) GBON=1

;dose record to cmt 5 should turn off GB entry (K24)
IF(DOSREC(CMT).EQ.5) GBOFF=1

;dose records to cmt=5 should turn on GB exit (K41)

IF(DOSREC(CMT).EQ.5) JON=1

;dose records to cmt=6 should turn off GB exit (K41)

IF(DOSREC(CMT).EQ.6) JOFF=1

$DES

K10 = CL/V2
K23 = Q/V2
K32 = Q/V3

DADT(1) = -KA*A(1)+K41*Z*A(4)
DADT(2) = KA*A(1) + K32*A(3) - K23*A(2) - K24*Z2*A(2) - K10*A(2)
DADT(3) = K23*A(2) - K32*A(3)
DADT(4) = K24*Z2*A(2) - K41*Z*A(4)
DADT(5) = 0
DADT(6) = 0


$ERROR
     DL=0
     IF (F.EQ.0) DL=0.001
     W= DL +F
     Y=LOG(W)+EPS(1)
     IRES = DV-F
     IWRE = (DV-F)/F
     IPRE = LOG(W)

$THETA
(0, 0.5, 10)     ;Ka :theta 1
(0, 5, )       ;clearance:theta 3
(0,10,)       ;Vc:theta 4
(0, 3,)       ;Q:theta 5
```

```
(0,75,)     ;V3: theta 6
(0, 0.01)     ;K24:theta 7
(0, 2)       ;K41:theta 8
(0, 0.5)     ;elapsed time GB exit is 'on' (Theta9)

$OMEGA 0.9
$OMEGA 0.9
$OMEGA 0.9
$OMEGA 0.9
$OMEGA 0.9
$OMEGA 0.9


$SIGMA 0.9

$EST    MSFO = GLIBEHC6.msf MAX=9999 PRI=10 METH=0 POSTHOC NOABORT
$TABLE FILE = GLIBEHC6.fit ID AMT TIME TAD CMT DAMT
IPRE ONEHEADER NOPRINT
```

12 NONMEM code for Dose/CPss ~ mean glucose pharmacodynamic model

```
$PROB Glucose Dose Model
$INPUT ID TIME AMT DVX CMT EVID MDV II ADDL SS DAMT
$INPUT WT SEX DV KA CL V2 Q V3 TAD
$DATA ..\data\meanglu.csv
$PRED

; PK Driving Force for Glucose Effect
  CPSS     = DAMT/(CL*24)                    ; Average steady State
Glibenclamide conc

; PD parameters
   E0      = THETA(1)      * EXP(ETA(1))    ; Baseline
   EMAX    = THETA(2)      * EXP(ETA(2))    ; Maximum drug effect
   C50     = THETA(3)      * EXP(ETA(3))    ; Average steady StateConc
producing 50% effect
;   GAM     = THETA(4) ;     * EXP(ETA(4))   ; sigmoid shape of Emax model


; Trick to prevent divide by zero errors
   SMALL    = 0.0000001

; Drug Effect and Time Course of Glucose
      F     = E0 * (1 - (EMAX * CPSS)/(C50 + CPSS))

;$ERROR
     DL=0
     IF (F.LE.0) DL=0.001
     W = DL + F
     Y = LOG(W)+ERR(1)
     IPRE = LOG(W)
     IRES = DV-F
     IWRE = (DV-F)/F


$THETA      (0,16  )         ; 1 E0
$THETA      (0,0.3  )        ; 2 EMAX
$THETA      (0,40 )        ; 3 C50
;$THETA      (1 FIX )        ; 4 GAM

$OMEGA     0.9              ; 1 E0
$OMEGA    0.9               ; 2 EMAX
$OMEGA     0.9              ; 3 C50
;$OMEGA     0.9             ; 4 GAM

$SIGMA     0.9
$COVARIANCE
$EST  MAX = 9999 NOABORT METH = 0 POSTHOC PRINT = 1   MSF =
cpssmeanglu00.msf
$TAB  ONEHEA NOPRI ID TIME TAD MDV AMT CMT IPRE DAMT  FILE=
cpssmeanglu00.fit
$TAB  ONEHE NOPRI FIRS ID E0 EMAX C50 FILE=cpssmeanglu00.par
```

```
$OMEGA BLOCK(1) 2        ; 2 A1
$OMEGA BLOCK(1) SAME     ; 3 B1
$OMEGA BLOCK(1) SAME     ; 4 B1
$OMEGA BLOCK(1) SAME     ; 5 B1

$OMEGA    0.9            ; 6 EMAX
$OMEGA    0.9            ; 7 CP50
;$OMEGA    0.9           ; 8 GAM

$SIGMA    0.9
$COVARIANCE
$EST  MAX = 9999 NOABORT METH = 0 POSTHOC PRINT = 1 MSF =
Cpssprofileglu00.msf
$TAB  ONEHEA NOPRI ID TIME TAD MDV AMT CMT IPRE DAMT
FILE=Cpssprofileglu00.fit
$TAB  ONEHE NOPRI FIRS ID A0 A1 B1 A2 B2 EMAX CP50 CL
FILE=Cpssprofileglu00.par
```

13 NONMEM Code for Dose/CPss – Full Glucose Profile model

```
$PROB Glucose Cpss Model
$INPUT ID TIME AMT DVX CMT EVID MDV II ADDL SS DAMT
$INPUT WT SEX DV KA CL V2 Q V3 TAD
$DATA ..\data\kpdglu.csv
$PRED
; Placebo Model
   A0     = THETA(1) *EXP(ETA(1))
   A1     = THETA(2) + ETA(2)
   B1     = THETA(3) + ETA(3)
   A2     = THETA(4) + ETA(4)
   B2     = THETA(5) + ETA(5)

; PK Driving Force for Glucose Effect
  CPSS     = DAMT/(CL*24)                  ; Average steady State
Glibenclamide conc

; PD parameters
   EMAX    = THETA(6)      * EXP(ETA(6))    ; Maximum drug effect
   CP50    = THETA(7)      * EXP(ETA(7))    ; Average Glib conc producing 50%
effect
;   GAM     = THETA(8) ;     * EXP(ETA(8))   ; sigmoid shape of Emax model


; Trick to prevent divide by zero errors
   SMALL    = 0.0000001

; Cyclical changes to baseline
   CIRC1    = A0 + A1*COS(2*3.1416*TIME/8)    + B1*SIN(2*3.1416*TIME/8)
   CIRC2    =      A2*COS(2*3.1416*2*TIME/8) + B2*SIN(2*3.1416*2*TIME/8)
   E0       = CIRC1 + CIRC2
 IF (E0.LE.0) EXIT

; Drug Effect and Time Course of Glucose
      F     = E0 * (1 - (EMAX * CPSS)/(CP50 + CPSS))

;$ERROR
     DL=0
     IF (F.LE.0) DL=0.001
     W = DL + F
     Y = LOG(W)+ERR(1)
     IPRE = LOG(W)
     IRES = DV-F
     IWRE = (DV-F)/F


; Placebo Parameters
$THETA      (15.1    )          ; 1 A0
$THETA      (-3.83   )          ; 2 A1
$THETA      (2.14    )          ; 3 B1
$THETA      (-6.90   )          ; 4 A2
$THETA      (1.86    )          ; 5 B2

$THETA      (0,0.5   )        ; 6 EMAX
$THETA      (0,85    )        ; 7 CP50
;$THETA      (1 FIX )         ; 8 GAM

$OMEGA 2                    ; 1 A0
```

```
$OMEGA BLOCK(1) 2          ; 2 A1
$OMEGA BLOCK(1) SAME       ; 3 B1
$OMEGA BLOCK(1) SAME       ; 4 B1
$OMEGA BLOCK(1) SAME       ; 5 B1

$OMEGA     0.9             ; 6 EMAX
$OMEGA     0.9             ; 7 CP50
;$OMEGA     0.9           ; 8 GAM

$SIGMA     0.9
$COVARIANCE
$EST  MAX = 9999 NOABORT METH = 0 POSTHOC PRINT = 1 MSF =
Cpssprofileglu00.msf
$TAB  ONEHEA NOPRI ID TIME TAD MDV AMT CMT IPRE DAMT
FILE=Cpssprofileglu00.fit
$TAB  ONEHE NOPRI FIRS ID A0 A1 B1 A2 B2 EMAX CP50 CL
FILE=Cpssprofileglu00.par
```