MECHANISMS RESPONSIBLE FOR THE SECOND MEAL RESPONSE TO MAIZE PORRIDGE

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Thesis accepted for the degree Magister Scientiae
in Physiology

by the

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Potchefstroom 1992

ACKNOWLEDGEMENT

This study is what it is - accomplished with the assistance of many people. I herewith would like to thank the following:

* Professor H.H.Vorster - my supervisor for her esteemed and valuable advice and guidance. Her encouragement, support and inspiration at all hours have made this study an enjoyable experience.
* Professor Isidor Segal for acting as co-supervisor, for continuous support, patience and advice offered.
* Dr. Tony Cantrell for all the help, advice and support.
* Ms. Fahmida Cassim whose expertise is evident in the layout and typing of this manuscript; her willingness to assist at all hours is highly appreciated.
* Mrs. B. Daya for her expert technical assistance.
* Mr. S. Bhutt, Mrs. R. Landless and Ms. E. Casimiro for assisting with the computer work at all hours.
* Ms. I. Henseleit for encouragement.
* Professor H. Seftel and Mrs. V. Panz for allowing the use of their laboratory.
* Ms. K. Ambaram and Ms. P. Gcakasi for their interest, encouragement and assistance.
* I wish to record my deep appreciation to the National Centre for Occupational Health for all the support.
* My son Bhiken and daughter Troopti for all their patience and tolerance.
* Above all my husband Ishwar for being at my side at all times.

THIS WORK IS DEDICATED TO:

* THE MEMORY OF MY LATE FATHER,
* MY MOTHER AND
* MY SISTER DAKSHA

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ABBREVIATIONS

BMI	body mass index
CH_4	methane
CHD	coronary heart disease
CHO	carbohydrate
CO_2	carbon dioxide
CV	co-efficient of variance
DF	dietary fibre
DM	diabetes mellitus
FFA	free fatty acids
GI	glycaemic index
GIP	glucose dependent insulinotrophic peptide
GTT	glucose tolerance test
H_2	hydrogen
HDL	high density lipoprotein
IDDM	insulin dependent diabetes mellitus
IGT	impaired glucose tolerance
IRI	immunoreactive insulin
LDL	low density lipoprotein
NIDDM	non-insulin dependent diabetes mellitus
NSP	non starch polysaccharide
PRS	partially resistant starch
RDS	rapidly digestible starch
RIA	radio-immuno-assay
RS	resistant starch
SAIMR	South African Institute for Medical Research

SCFA	short chain fatty acid
SD	standard deviation
SDS	slowly digestible starch
2-SITE IRMA	two site immuno-radio-metric assay
SME	second meal effect
SMR	second meal response
ST	Staub-Traugott
TS	total starch
VLDL	very low density lipoprotein
WHO	World Health Organisation

SUMMARY

The short-term effect of a food on the carbohydrate metabolism is reflected in post-prandial variations of blood glucose levels, expressed as the glycaemic index (GI) of the food, as well as the effect of the food on the glucose response of a subsequent meal. The latter is referred to as the second meal response (SMR).

In South Africa, maize porridge is the staple in the diet of many people from different ethnic groups. There is however, little information available on the GI of maize porridge and none on the SMR to maize porridge. The underlying mechanisms responsible for the SMR are also unknown.

The main objective of this study was to examine the hypothesis that a suppression of circulating free fatty acids, glucagon and growth hormone secretion by a first meal containing carbohydrates which are slowly digested and absorbed, are responsible for the SMR. The GI of maize porridge, with and without added barley fibre, and the effect of this addition on the SMR were also examined.

Six healthy black women voluntarily participated in the study. A Latin square design was used to randomly test three meals as a first meal (glucose, maize porridge and maize porridge with added barley fibre). After four hours, a second meal of glucose was taken by the subjects. All meals contained 50g carbohydrate. Serum levels of glucose, insulin, growth hormone and glucagon,

as well as plasma free fatty acids were measured at specific intervals.

The results showed that the GI of maize porridge when eaten in a precooked, frozen and reheated form, was 57.7 ± 25.6%. The addition of barley fibre to the porridge did not change the GI: 57.5 ± 16.3%. The GI of glucose as second meal was 145.4 ± 33.3% when glucose was the first meal, 74.3 ± 38.1% with maize porridge as first meal, and 77.6 ± 26.4% with maize porridge plus barley fibre as first meal. Therefore, a clear SMR with the two porridges was observed.

The results further indicated that the levels in the blood of glucose, insulin and free fatty acids, four hours after the first meal just before the second meal was taken, had highly significant correlations with the area under the glucose response curve during the second meal (glucose: $r = -0.53$, $p = 0.05$; insulin: $r = -0.70$, $p = 0.005$; free fatty acids: $r = + 0.80$, $p = 0.0005$). Glucagon and growth hormone did not show these correlations. The results therefore support the hypothesis that a first meal with a low GI, which after four hours will be associated with "relative" high levels of glucose and insulin, but low levels of circulating free fatty acids, will elicit a SMR.

Two factors are possibly responsible for the lack of effect of the added barley fibre on the GI. Firstly, the amount of soluble components in this fibre was probably too low to affect digestion and absorption rates of carbohydrate. Secondly, the effect of

retrogradation and consequent development of resistant starch during the cooling and freezing of maize porridge, probably overrided the effect of fibre on the GI.

The results of this study have special applications in the planning of meal frequency - especially in the diet of persons with an abnormal glucose tolerance or with diabetes mellitus.

OPSOMMING

Die korttermyneffekte van 'n voedsel op die koolhidraatmetabolisme word gereflekteer in die skommelinge van bloedglukosevlakke na inname van die voedsel, uitgedruk as die glukemiese indeks (GI) van die voedsel, asook die effek van die voedsel op die glukoserespons tydens 'n opvolgmaaltyd. Laasgenoemde word die tweede-maaltydrespons (TMR) genoem.

In Suid-Afrika is mieliepap die stapelvoedsel van groot gedeeltes van verskillende bevolkingsgroepe. Daar is egter min inligting oor die GI van mieliepap beskikbaar, en geen inligting oor die TMR van mieliepap nie. Verder is die onderliggende meganismes waardeur die TMR ontstaan ook nog onduidelik.

Die hoofdoel van hierdie studie was om die hipotese, dat 'n onderdrukking van sirkulerende vrye vetsure, glukagon en groeihormoonsekresie deur 'n eerste maaltyd wat koolhidrate bevat wat stadig verteer en geabsorbeer word, vir die TMR verantwoordelik is, te ondersoek. Die GI van mieliepap met en sonder bygevoegde garsvesel, en die effek van die byvoeging op die TMR, is ook ondersoek.

Ses gesonde swart vroue het vrywillig aan die studie deelgeneem. 'n Latynse blokontwerp is gebruik om drie maaltye (glukose, mieliepap en mieliepap plus garsvesel) ewekansig as 'n eerste maaltyd te neem. Na vier ure is 'n tweede maaltyd van glukose deur al die proefpersone geneem. Al die maaltye het 50g koolhidrate bevat. Serumkonsentrasies van glukose, insulien,

groeihormoon en glukagon, sowel as vrye vetsure in plasma is met spesifieke intervalle gemeet.

Die resultate het getoon dat die GI van die mieliepap in die vorm waarin dit geeët is (vooraf gaargemaak, bevries en toe verhit) 57.7 ± 25.6% was. Die byvoeging van garsvesel by die mieliepap het nie die GI verander nie: 57.5 ± 16.3%. Die GI van glukose as tweede maaltyd was 145.4 ± 33.6% wanneer glukose die eerste maaltyd was, 74.3 ± 38.1% met mieliepap as eerste maaltyd en 77.6 ± 26.4% met mieliepap plus garsvesel as eerste maaltyd. 'n Duidelike TMR is dus waargeneem.

Die resultate het verder getoon dat die konsentrasies in die bloed van glukose, insulien en vrye vetsure, vier ure na die eerste maaltyd en dus net voor die tweede maaltyd, hoogs betekenisvolle korrrelasies met die oppervlak onder die glukoseresponskromme van die tweede maaltyd gehad het (glukose: $r = -0.53$, $p = 0.05$; insulin: $r = -0.70$, $p = 0.005$; vrye vetsure: $r = +0.80$, $p = 0.0005$). Glukagon en groeihormon het nie hierdie verwantskap getoon nie. Hierdie resultate ondersteun dus die hipotese dat 'n eerste maaltyd met 'n lae glukemiese indeks, wat na vier ure nog in 'relatiewe' hoë bloedglukose-en insulienvlakke, en lae vrye vetsuurvlakke resulteer, 'n TMR ontlok. Die afwesigheid van 'n effek van die bygevoegde garsvesel op die GI van mieliepap kan moontlik aan twee faktore toegeskryf word. Eerstens was die hoeveelheid oplosbare komponente in die vesel waarskynlik nie genoeg om koolhidraatvertering en absorbsie verder te inhibeer nie. Tweedens het die effek van retrogradering en die gevolglike

ontwikkeling van weerstandbiedende stysel tydens die afkoelings en bevriesingsproses van die mieliepap waarskynkik die effek van die vesel oorheers.

Die resultate van hierdie studie het spesiale toepassings in die beplanning van die frekwensie van maaltye - veral in die dieët van persone met 'n abnormale glukoseverdraagsaamheid en met diabetes mellitus .

CHAPTER 1

INTRODUCTION

Urbanization and westernization in the South African black population is accompanied by an increase in the incidence of non-insulin dependent diabetes mellitus (NIDDM) (Silvis *et al.* 1989). The increase in prevalence of diabetes will increase the burden on health care facilities in future. Dietary intervention should be an integral part in the prevention and treatment of NIDDM. One of the major aims of diabetes therapy is to maintain blood glucose levels as close to normal as possible in order to prevent hyperglycaemia and/or hypoglycaemia (American Diabetes Association 1990).

The energy distribution of the current diabetic diet is similar to the energy distribution of the rural African diet, which consists mainly of maize as staple (Silvis *et al.* 1989; American Diabetes Association 1987). Therefore, Silvis (1989) suggested that the rural African diet may possibly be the optimal dietary treatment for westernized black NIDDM patients. Information on both the short and long-term metabolic effects of the staples in the rural African diet is presently not sufficient to make meaningful recommendations to black diabetic patients. Therefore, more research is needed for a better understanding of the biochemical and physiological effects of the staples in the rural African diet.

Many studies have found that different foods produce different glycaemic responses, despite having the same carbohydrate content (Crapo *et al.* 1977; Jenkins *et al.* 1981; Jenkins *et al.* 1983a; Walker & Walker 1984).

The glycaemic index (GI) has been proposed as a method for classifying the blood glucose response to foods (Jenkins *et al.* 1981). Intake of a low GI carbohydrate food will result in a reduced acute post-prandial blood glucose response, and also in a reduced glycaemic response to a subsequent carbohydrate intake. This phenomenon is defined as the second meal response (SMR) to the food (Wolever, 1990). It has also been shown that addition of soluble dietary fibre to a glucose load results in an improved glucose tolerance post-prandially, as well as during a second meal (Jenkins *et al.* 1980a; Pastors *et al.* 1991).

Very little information on the GI of maize porridge and the factors which influence it, and no information on the SMR to maize porridge, is available. Furthermore, the underlying mechanisms responsible for the SMR are unknown. It has been suggested by Wolever (1990), that a reduction of circulating free fatty acids (FFA) after the first meal will reduce the glycaemic response to a second meal.

If the GI of maize porridge, factors which influence it, the SMR and mechanisms responsible for it are better understood, more precise dietary recommendations to black NIDDM patients may be possible. These recommendations could then be based on a physiological basis, rather than on the chemical composition of foods.

HYPOTHESES AND OBJECTIVES OF THE STUDY

The following hypotheses were investigated in this study:

* Barley fibre, which contains appreciable amounts of both soluble and insoluble dietary fibre components, reduce the GI of cooled maize porridge even further. Venter (1990) has shown in white female subjects that cooked and then cooled maize meal porridge had a lower GI than freshly cooked porridge when eaten hot.
* In comparison to glucose as first meal, maize porridge and maize porridge with added barley fibre, improve the SMR of a standard glucose dose four hours later.
* The concentration of circulating FFA and some of the insulin counter-regulatory hormones are responsible for the SMR.

This hypothesis is based on the assumption that the carbohydrate in a low GI food will be slower absorbed than glucose. This will lead to lower blood glucose values and a smaller insulin secretion. Functional hypoglycaemia (reduction of blood glucose levels to levels lower than the fasting level) will therefore not occur. This will lead to lower levels of free fatty acids and of the insulin counter-regulatory hormones such as glucagon, growth hormone and the catecholamines.

These hypotheses were tested on six healthy black female volunteers. The effects of carbohydrate as glucose, maize porridge or maize porridge plus barley fibre on post-prandial blood glucose, insulin, growth hormone and glucagon fluctuations were examined for four hours. Thereafter, a standard glucose meal was given and the subjects monitored for another 3 hours.

In chapter 2, the relevant literature is reviewed. The study design, general methodology, composition of meals and details of experimental methods are discussed in Chapter 3. The results are given in Chapter 4. In Chapter 5 and 6 the observations are discussed, conclusions are drawn and recommendations regarding future studies to examine the underlying mechanism responsible for improved SMR are discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The main potential application of the results of this study, lies in the field of the dietary treatment of diabetes mellitus (DM). Therefore, the literature review will concentrate on DM and related issues, with special emphasis on non-insulin dependent DM (NIDDM).

DM is a common disorder in South African populations, and all population groups are effected (Seedat, 1988). In the rural areas, particularly in the remote parts, diabetes remained very uncommon for decades, but recent studies undertaken by Walker and Walker (1991), indicated an increase in the prevalence of NIDDM. Considerable urbansiation and westernization in the South African black population may be largely responsible for the observed changes in incidence.

Diet remains the cornerstone in the treatment of NIDDM (Nuttal, 1979, 1988). In this chapter current knowledge on DM is reviewed. The definition of DM, classification, epidemiology, pathophysiology and the complications as well as the treatment of NIDDM are discussed (2.2). The carbohydrate metabolism is discussed in Section 2.3. The rural African diet in South Africa consists mainly of maize as staple which has a low glycaemic index (GI). In Section 2.4 various aspects of the GI are

discussed in detail. Dietary fibre, its effect on carbohydrate metabolism and on the GI is reviewed in Section 2.5. Recent studies in man indicated that a considerable amount of dietary starch may escape digestion in the small intestine and pass into the large bowl. The literature on starches and its effect on carbohydrate metabolism is therefore reviewed in Section 2.6. Also, a recent concept is that a low GI meal improves the carbohydrate tolerance of subsequent meals. The underlying mechanism responsible for this effect is still not well understood. Section 2.7 therfore outlines the second meal response to a carbohydrate load.

2.2 DIABETES MELLITUS (DM)

2.2.1 Definitions

DM is a complex syndrome characterized by a relative or absolute deficiency of insulin or its functions, leading to hyperglycaemia (World Health Organization, 1985). The microvascular complications of the disease include thickening of capillary basement membranes, retinopathy and nephropathy. The macrovascular complications are atherosclerosis and coronary heart disease (CHD). Other complications include neuropathy, complications during pregnancy, increased frequency to infections, etc. The disease is also characterized by an increased catabolism of fat and protein (World Health Organization, 1985).

2.2.2 Classification

The World Health Organization (1985) has proposed classification of DM and other states of glucose tolerance, as indicated in Table 2.1.

Table 2.1 Classification of DM

I. Clinical types	
A. Primary	a) Insulin dependent diabetes mellitus (IDDM) b) Non-insulin dependent diabetes mellitus (NIDDM)
B. Secondary:	a) Pancreatic disease e.g. chronic calcific pancreatitis b) Hormonal disorders e.g. Cushing's syndrome glucagonoma, acromegaly, pheochromocytoma c) Drug-induced, e.g. steroid, thiazides d) Insulin receptor abnormalities e) Genetic syndromes f) Abnormal insulin molecule g) Malnutrition and related conditions
C. Gestational:	$\pm$ 50% develop persistant diabetes
D. Impaired glucose tolerance:	a) Non-obese b) Obese c) Impaired glucose tolerance associated with certain conditions
II Increased statistical risk classes:	
	a) Previous abnormality of glucose tolerance b) Potential abnormality of glucose tolerance e.g. diabetic identical twin, family history, large babies

2.2.3 Criteria For Diagnosing DM

The World Health Organization (1985) criteria are based on measurements of glucose in blood specimens by an enzymatic method specific for glucose. The diagnostic criteria for DM, as defined by the World Health Organization (1985) is given in Table 2.2.

TABLE 2:2 World Health Organisation (1985) Diagnostic Criteria for DM

	Glucose concentrations (mmol/L)	
Venous plasma	Fasting	Two hours post 75g glucose load
Diabetes mellitus	$\geq$ 8.0	$\geq$ 11.0
Impaired glucose tolerance	< 8.0	8.0 but $\geq$ 11.0

The majority of diabetics fall into the primary group, with a ratio of NIDDM, to IDDM of 4:1. Primary diabetes profiles are different as can be seen in the Table 2.3.

2.2.4 Impaired Glucose Tolerance (IGT)

The prevalence of IGT in the USA adult population is 11.2% compared to 6.6% for diabetes (Harris 1989). The risk factors for NIDDM e.g. age, blood glucose levels, obesity and family history of diabetes indicate that IGT may be an intermediate condition. It is likely that the events that precede NIDDM and are involved in its pathogenesis occur during the period of IGT (Harris 1989).

Table 2.3 Differences between IDDM and NIDDM Profiles

Characteristic	IDDM (Type 1)	NIDDM(Type 11)
Age of onset	Juvenile (< 40 years)	Middle age (> 40 years)
Aetiology		
Heredity	mild-moderate	strong
HLA related	yes	No
Auto immune manifestation	yes	No
Virus	possibly	Unlikely
Pathology	insulitis, mononuclear cell infiltration in early onset cases, eventual complete beta-cell destruction.	Hypertrophy in early stages, atrophy and hyaline degeneration
Pathogenesis	Beta-cell destruction Insulin deficiency	B-cell preserved Insulin resistance
Phenotype	Thin	Obese
Onset	Rapid	Insidious
Symptoms	Severe	Mild or absent
Urine	Glucose and ketones	Glucose
Ketoacidosis	Prone	Resistant
Treatment	Insulin	Diet and oral agents
Complications		
Retinopathy	After 10 years	After 5 years
Nephropathy	Cause of death	Lesser cause of death
Neuropathy	Common	Common
Hypertension	With nephropathy	Essentially early
Coronary disease	Lesser cause of death	Leading cause of death

Adapted from Wits Diabetes Group 1986

2.2.5 Epidemiology Of NIDDM

Some 60 million people worldwide suffer from DM (Brownlee, 1985) and it is estimated that there are some 25 to 50 million diabetics in the developing nations (Bennet, 1983). NIDDM is a common disease in affluent societies, affecting some five to ten percent of those over the age of 40 years. The high incidence of diabetes among the black Americans is well documented (Cooper *et al.*, 1984; Harris *et al.*, 1987).

Environmental factors inherent in modern western civilization encompassing urbanisation and changes in life style and dietary habits, have been incriminated as largely responsible for the differences observed. Information regarding the occurrence of DM in Africa is scarce (Krolewski and Warram, 1985) but Zimmet (1982) have reported that the incidence of NIDDM are rising among blacks.

DM is a common metabolic disorder in South African populations and all population groups are affected. Table 2.4 indicates the incidence of DM in South Africa as reported by Seedat (1988).

TABLE 2.4 Incidence of DM amongst South Africans (Seedat, 1988)

Population group	Incidence
Indians	10.0 %
Whites	4.0 %
Urban blacks	4.2 %
Coloured	8.0 %

Currently, in the Hillbrow Hospital, Johannesburg, 6% of admissions of adults are due to diabetes (Dean and Gear, 1986). In a study of various South African population groups it was noted that the Indian population had higher fasting immuno-reactive insulin levels than the black and white populations and that the differences were more marked in females (Keller *et al.*, 1972).

Studies on elderly rural blacks undertaken by Walker (Walker and Walker, 1991) during 1965, 1966, and 1990 revealed a rising prevalence of diabetes reaching from 1% to 7,2% and an IGT from 2,6% to 10%. Prevalence of obesity in females were 7.5% in 1965-1966 compared to 19,9% in the 1991 study.

2:2:6 Pathophysiology Of NIDDM

2.2.6..1 Introduction

The pathogenesis of NIDDM is far less clear than that of IDDM. NIDDM is associated with obesity in more than 80% of the patients, suggesting the possibility that it may be due to a disordered mechanism of appetite regulation or energy expenditure (Kahn, 1985).

In obese subjects basal levels of insulin are elevated and increase excessively after a glucose challenge. The insulin resistance in this state has been found to be correlated with a decrease of insulin receptors on the fat cell membrane (Archer *et al.*, 1975). Therefore, in the presence of hyperinsulinaemia,

the number of receptors falls, leading to a state of insulin resistance (Cooppan and Flood, 1985). In both types of diabetes the most striking abnormality is the development of hyperglycaemia, which may be due to a relative deficiency of insulin or resistance to insulin action at the target cell level (Kahn, 1985). Figure 2.1 illustrates the possible pathogenesis of NIDDM.

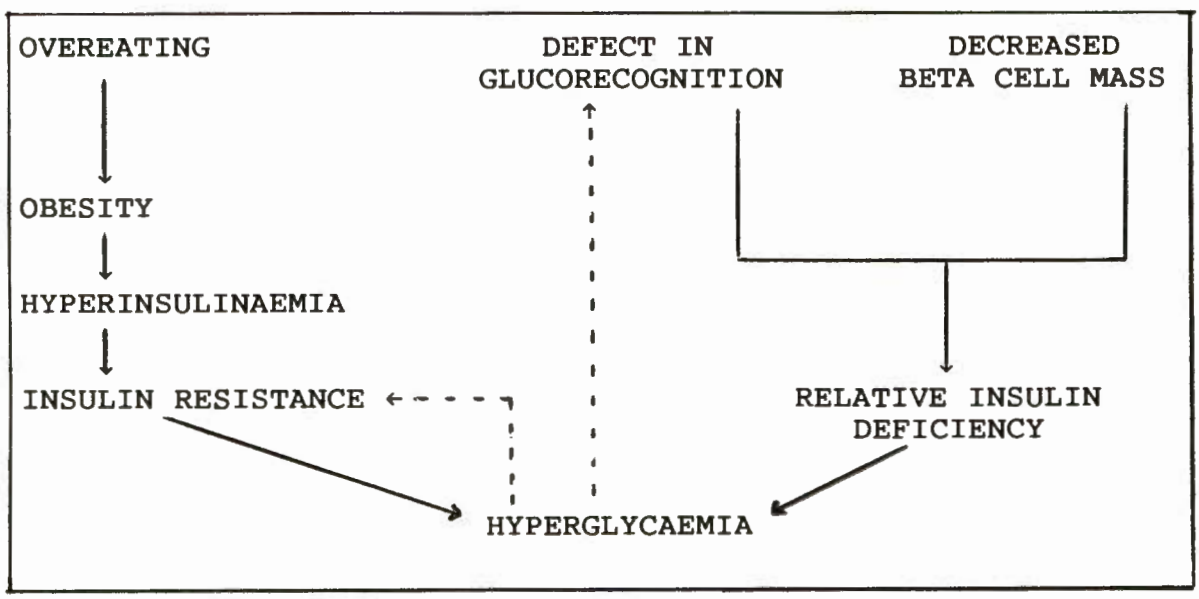

Fig 2.1. Possible pathogenesis of NIDDM
Adapted from Kahn (1985)

2.2.6.2 Insulin Secretion

Basal plasma insulin levels in NIDDM are normal or even elevated. In virtually all NIDDM individuals there is a loss of acute phase insulin release to glucose given intravenously, but not in other stages suggesting a specific defect in glucorecognition. The lack of glucorecognition is linked to the level of hyperglycaemia. This impairment of the beta cell function is reversible in the early phase of the disease. Therefore, with

restricted diet, oral hypoglycaemic drugs or insulin treatment the beta cell function improves and insulin secretion is increased (Kahn, 1985).

2.2.6.3 Insulin Resistance

Insulin resistance is defined as a condition of sub-optimal biological responses to insulin (Del Prato *et al.*, 1990). The normal or elevated plasma insulin levels found in patients with NIDDM suggest a condition of insulin resistance or reduced insulin sensitivity. This can occur as a result of defects at several levels in the action of insulin. There may be a decrease in the number of insulin receptors. There may also be post receptor defects. However, the exact nature of the defects remain unclear. Treatment of the NIDDM patients with diet or insulin will reverse this defect even when receptor abnormality persists (Kahn, 1985).

2.2.6.4 Obesity And Insulin Resistance

Obesity, hypertension, hypertriglyceridaemia and glucose intolerance often co-exist (Kaplan 1989). Current estimates indicate that 35 million people in the United States are obese with body weights more than 20% above the ideal weight (Van Italie,1985) and that 10 million have diabetes (Minaker, 1987). It is now believed that upper body or android obesity is actively involved, *via* hyperinsulinaemia, with the induction of glucose intolerance, hypertension and hypertriglyceridaemia (Kaplan, 1989), as illustrated in Figure 2.2.

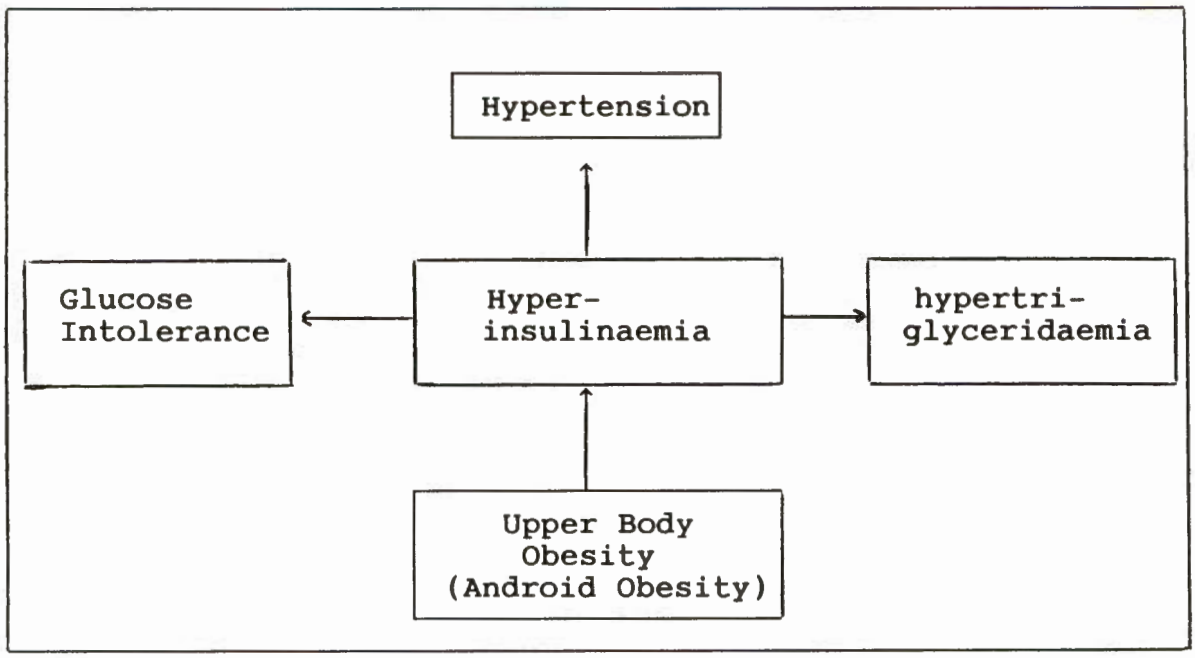

Fig 2.2 Relationship between android obesity and glucose intolerance
Adapted from Kaplan (1989)

2.2.6.5 Android Obesity And Hyperinsulinaemia

Android or upper body obesity is usually accompanied by an increase in pancreatic insulin secretion leading to hyperinsulinaemia. This is thought to reflect peripheral insulin resistance with a secondary increase of insulin secretion to maintain euglycaemia (Kaplan, 1989; Izzo and Swisslocki, 1991). This relationship is schematically presented in Figure 2.3.

Upper body fat is thought to be metabolically more active than lower body fat, and therefore the excess of free fatty acids released during lipolysis of abdominal fat interferes with insulin clearance by the liver (Kaplan , 1989).

Obesity is very common in South African black women. Over 19% of females have a BMI of 30 kg/m^2 or more (Walker *et al.*, 1989). Yet in several studies on obese black women compared to Caucasion women, hyperlipidaemia and hyperglycaemia were uncommon (Joffe *et al.*, 1979; Walker *et al.*, 1989).

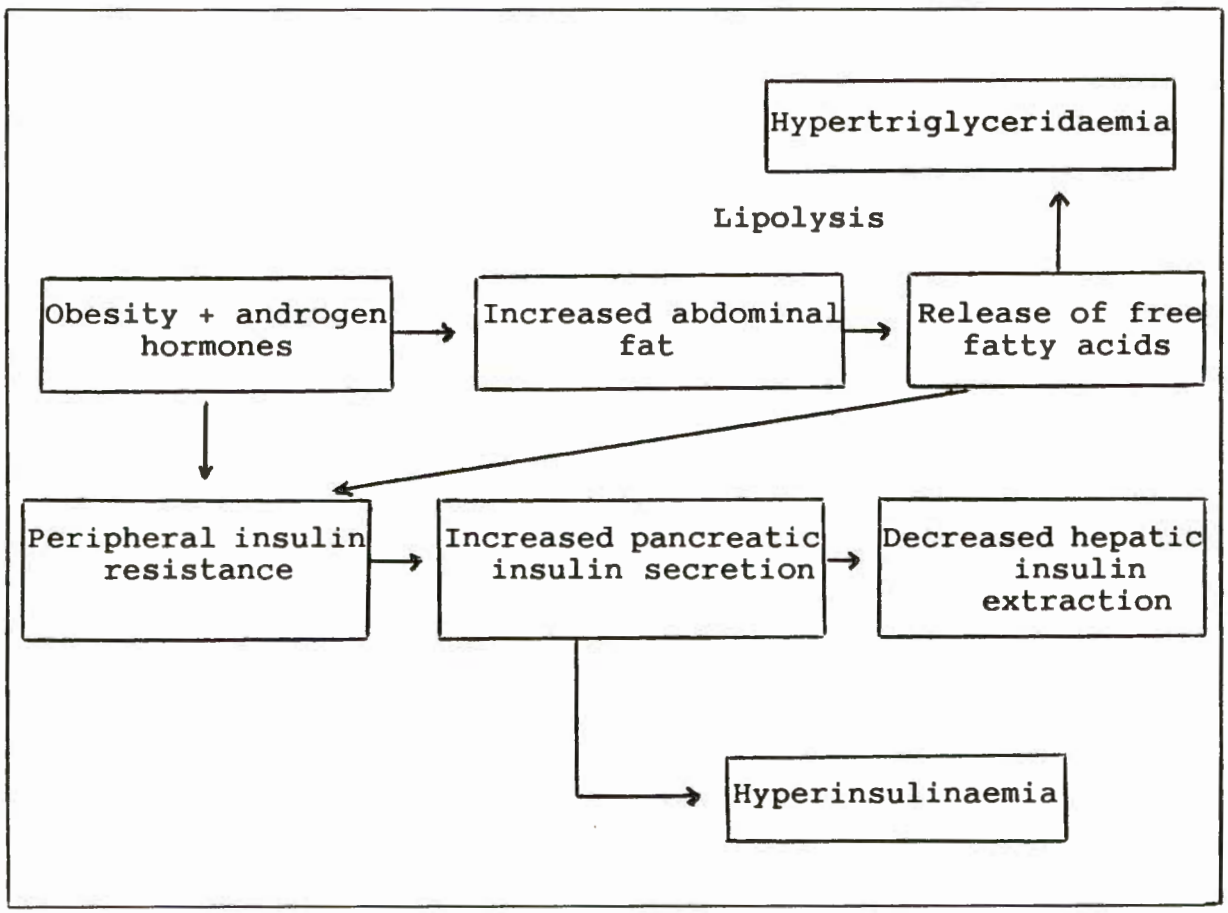

Fig 2.3 The relationship between the development of hyperinsulinaemia and upper body obesity. Adapted from Kaplan, 1989.

2.2.7 Long-term Complications In Untreated DM

Kaplan (1989), Krolewski *et al.* (1985) and Krolewski *et al.* (1991), reviewed the cardiovascular changes associated with poorly controlled DM. These include mobilization of large amounts of fats which causes hyperlipidaemia leading to development of atherosclerosis, arteriosclerosis and eventually gangrene of the toes. However, cardiovascular disease is not the most important cause of death in black South African diabetic patients (Jackson, 1978).

In addition, chronic kidney failure could develop as a result of hypertension, sclerosis, and thickening of the small renal arteries (Zilva and Pannall, 1988). Small haemorrhages and aneurysms may develop in the retina and eventually lead to loss of vision. Cataracts may form as a result of denaturation of the lens proteins by the action of ketoacids (Zilva and Pannall, 1988). Neurological changes are caused inter alia by an accumulation of ketone bodies which are toxic to the nervous system. The peripheral nerves are often affected and reflexes are lost. In the lower limbs muscle wasting, accompanied by pain, may occur. Because glucose is excreted in the sweat, staphylococcal skin infection and candida infection of the vulva and anus are common (Zilva and Pannall, 1988).

2.2.8 Treatment Of Patients With NIDDM

Treatment of diabetes is based on dietary control and administration of insulin or drugs which stimulate the B-cells of the islets of Langerhans (American Diabetes Association, 1987). Oral sulphonylurea agents facilitates insulin secretion in NIDDM patients, increase insulin sensitivity, and could therefore yield effective blood glucose control. Nevertheless, regulation of the type and quantity of food ingested is the basis of treatment for all patients with diabetes (Flood and Cooppan, 1985; Nuttal, 1988; Silvis, 1992). With a low caloric diet the number of insulin receptors on cell membranes increases, and insulin levels decrease, suggesting that overeating is responsible for the hyperinsulinaemia and insulin resistance observed in obese NIDDM subjects (Cooppan and Flood, 1985).

An exercise program should be an integral part of the treatment plan for all obese NIDDM subjects.

Therefore diet alone, or diet plus oral hypoglycaemic agents or insulin, combined with an exercise programme form the corner-stone of treatment of NIDDM.

There has been a virtual revolution in the concepts of what constitutes an appropriate diabetic diet. After decades during which diabetic patients were told to avoid carbohydrates in all forms, the American Diabetic Association, as well as the other major diabetes organisations such as the Canadian Diabetic Association and British Diabetic Association has advocated a high

carbohydrate, low fat diet for all diabetics. Silvis (1992) recommends that :

* A nutritionally adequate, mixed diet is satisfactory for most diabetics and therefore special foods and food supplementation are not required.
* The amount of carbohydrate should be increased in the diet, including a wide variety of fibre-rich complex carbohydrates.
* Foods with lower glycaemic indices should be offered to diabetics on trial basis.
* Total fat intake, especially saturated fat should be restricted.
* Protein intake should be restricted to the Recommended Daily Allowance except in groups at risk of negative nitrogen balance.
* Restriction of salt and alcoholic beverage is also advised.

In South Africa, the Association for Dietetics in Southern Africa in collaboration with the Advisory Committee for Health Services Workgroup for Nutrition Services (1992) recently published nutritional recommendations for diabetic individuals. From these, and the accompanying technical support paper (Silvis, 1992) it is clear that the diabetic diet is fundamentally a high-fibre, low-fat, high-carbohydrate diet. A low fat intake is advised in order to prevent and/or treat the macrovascular complications of the disease, while the complex carbohydrate seems necessary for optimal glucose control. Aspects of the carbohydrate metabolism, relevant to this study, will now be discussed in some detail.

2.3 CARBOHYDRATE METABOLISM

2.3.1 Introduction

In most parts of the world carbohydrate is the major source of energy. Under normal circumstances starch is the main dietary carbohydrate; disaccharides contribute significantly and monosaccharides are a minor component of the diet. The main important monosaccharides are glucose, fructose and galactose. The common disaccharides are sucrose (fructose plus glucose), lactose (galactose plus glucose), and maltose (glucose plus glucose). Naturally occuring polysaccharides are starches found in plants (amylose and amylopectin) and glycogen found in animal tissue (Zilva and Pannall, 1988).

2.3.2 Systemic Effects of a Carbohydrate Load

Dietary carbohydrate is digested or hydrolyzed by pancreatic and small intestinal amylases and disaccharidases to the monosaccharides glucose, fructose and galactose. These monosaccharides are transported to the liver where galactose is converted to glucose and fructose is metabolized in the same pathways as glucose (Whitby *et al.*, 1988).

According to Zilva and Pannall (1988), the liver modifies the potential hyperglycaemic effect of a high carbohydrate meal by extracting the glucose from the portal blood. Some glucose passes through the liver unchanged and the rise in the systemic glucose concentration stimulates the B-cells of the pancreas to

secrete insulin. A large insulin response causes peripheral glucose utilization to increase by stimulating hepatic and muscle glycogenesis. This results in a rapid decrease of plasma glucose, to a level below the baseline. The relatively high insulin activity also inhibits the breakdown of triglycerides (lipolysis) and of protein (proteolysis) and stimulates the synthesis of lipids and protein. If there is a relative or an absolute insulin deficiency, these actions are impaired. During fasting, when exogenous glucose is unavailable and the plasma insulin concentration is low, endogenous triglyceride is reconverted to FFA and glycerol by lipolysis. Much of the FFA is oxidised in tissue and used as a source of energy. Glycerol enters the gluconeogenic pathway and the synthesized glucose is released into the circulation. The uptake and utilization of glucose in tissues is known to be inhibited by raised circulating FFA levels and ketones. This inhibition may eventually lead to an insulin resistance state. The disposal and synthesis of glucose during the absorptive and post-absorptive states, leading to stable blood glucose levels, are summarized in figures 2.4. and 2.5.

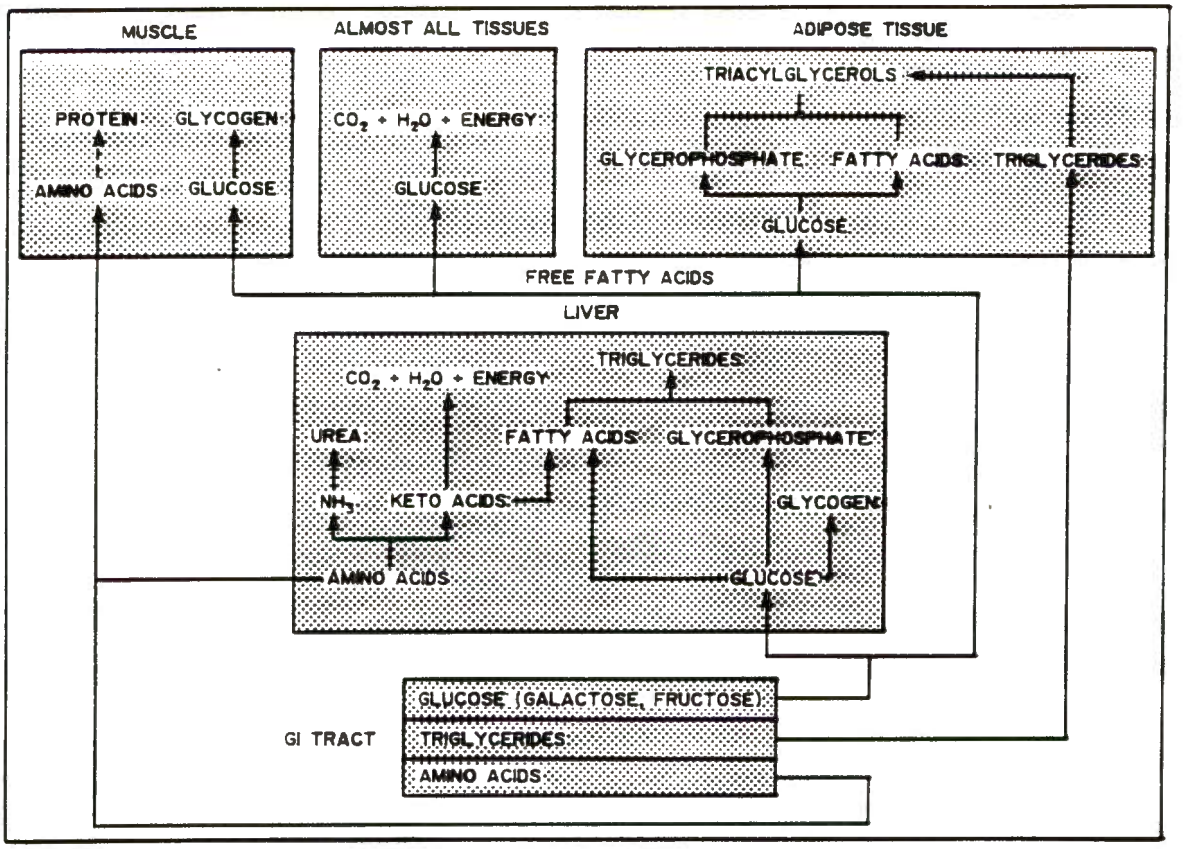

Figure 2.4 Major metabolic pathways of the absorptive phase. Glycerophosphate is the form of glycerol used to synthesize triacylglycerol. (Adapted from Vander et al. 1981)

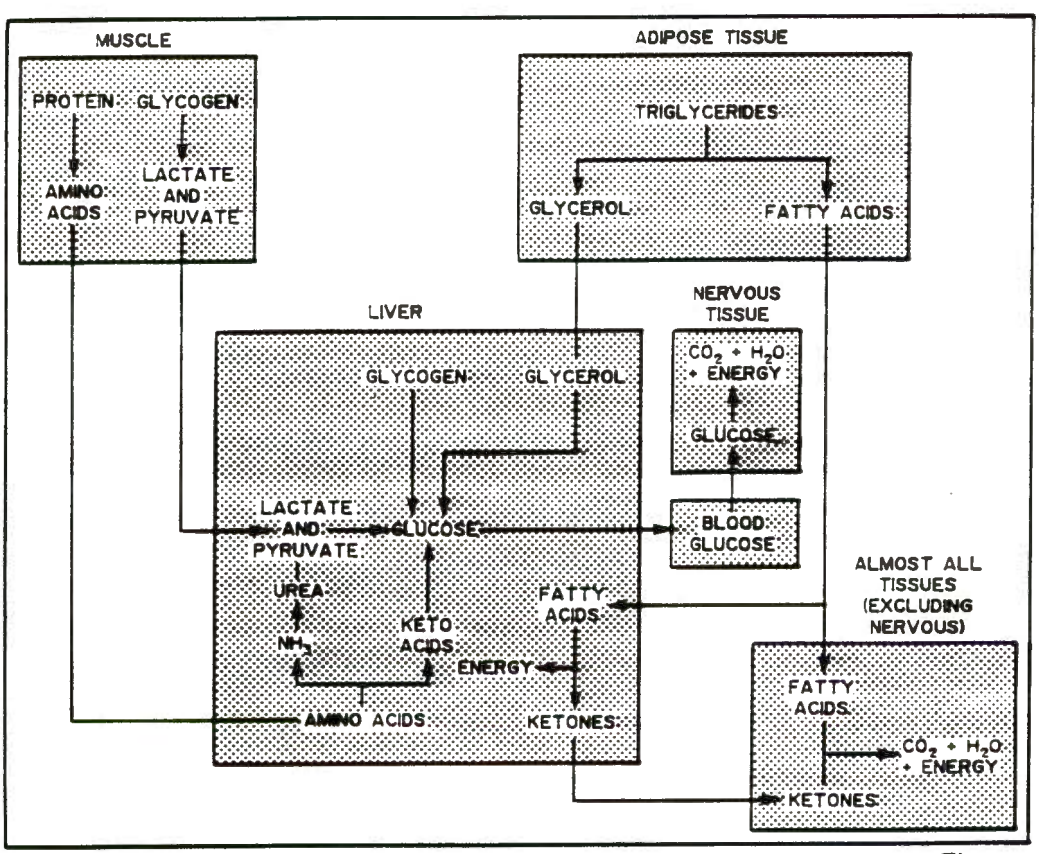

Figure 2.5 Major metabolic pathways of postabsorptive phase or fasting. The central focus is regulation of the blood glucose concentration. (Adapted from Vander et al. 1981)

Other hormones are also involved in glucose homeostasis. According to Whitby *et al.*,(1988a) these are briefly;

Glucose Dependent Insulinotrophic Peptide (GIP):

This is one of the gastro-intestinal peptides. The plasma GIP levels increases after meals containing carbohydrate or fats. The principal action of GIP is to stimulate the release of insulin from the pancreas after a carbohydrate load. It therefore serves as an anticipating mechanism from the gut to "prepare" or "warn" the pancreas of an incoming glucose load (Whitby *et al.*, 1988a).

Glucagon:

Glucagon is synthesized by the α-cells of pancreatic islets. It is secreted in response to hypoglycaemia. It stimulates the breakdown of liver glycogen, thereby increasing plasma glucose. It also promotes gluconeogenesis and increases proteolysis and lipolysis. It therefore has an opposite effect on blood glucose levels than insulin.

Adrenaline:

This catecholamine inhibits insulin release and promotes glycogenolysis and lipolysis.

Cortisol:

Cortisol stimulates gluconeogenesis and FFA production and inhibits glucose metabolism in peripheral tissues. It therefore increases blood glucose levels.

Growth Hormone (GH):

GH inhibits glucose uptake by tissues. GH normally varies inversely with blood glucose and it causes the release of FFA from adipose tissue.

The functions of these hormones as well as the factors which control their secretions, are summarized in Table 2.5.

Table 2.5 ACTIONS OF HORMONE ON INTERMEDIARY METABOLISM

		Insulin	Glucagon	Growth Hormone	Glucocorticoids	Adrenaline
Carbohydrate metabolism						
(a) in liver						
	*glycolysis	+				
	*glycogenesis	+				
	*glycogenolysis		+			+
	*gluconeogenesis	–	+		+	
(b) in muscle						
	*glucose uptake	+		–	–	
	*glycogenesis	+				
	*glycogenolysis				+	
Protein	*synthesis	+		+		
	*breakdown	–			+	
Fat	*synthesis	+				
	*lipolysis	–		+	+	+
Secretion	*stimulated by	Hyperglycaemia	Hypoglycaemia	Hypoglycaemia	Hypoglycaemia	Stress
		Amino acids	Amino acids	Stress	Stress	
		Glucagon	Fasting	Sleep		
		Gut hormones				
	*inhibited by	Adrenaline	Insulin			
		Fasting				
Results		Uses and stores available glucose	Provides glucose	Spares glucose Provide FFA as alternative fuel	Provides glucose	
Plasma FFA levels		Fall			Rise	
Plasma glucose levels		Fall			Rise	

+ stimulates – inhibits

Adapted from Zilva and Pannall 1988

2.3.3 Carbohydrates In The Diet : Effects On Glucose Homeostasis

The type of carbohydrate food which will comprise good diabetic control, has recently been addressed by several researchers. Factors that influence the rate of digestion, thus causing variable metabolic responses, are not adequately listed in food tables. It therefore is not possible to predict the glycaemic response to a food based on its chemical composition alone (Jenkins *et al.*, 1988). Studies by Crapo in normal subjects (Crapo *et al.*, 1976, 1977) and in subjects with IGT (Crapo, 1980) focussed on the difference in glycaemic responses between different starchy foods of similar macronutrient composition. Differences in both glucose and insulin responses were observed and it was postulated that possible difference in rates of digestion of foods were responsible. From the begining of the 1980's many tests of single foods and mixed meals have been undertaken in both normal and diabetic subjects. In 1981 the concept of the glycaemic index was proposed as a method of assessing and classifying the glycaemic response to carbohydrate foods (Jenkins *et al.*, 1981). This concept will now be discussed in more detail.

2.4 THE GLYCAEMIC INDEX

2.4.1 Definition

The term GI is defined as the blood glucose response to a food expressed as a percentage of the blood glucose response to an identical carbohydrate portion of a reference food. The reference or a standard load is usually 50 grams of glucose; but this proved to be less acceptable for routine use in diabetics and therefore an equicarbohydrate portion of white bread is used as a standard (Jenkins *et al.*, 1988). The GI is therefore defined as:

Incremental blood glucose area after a food, divided by the corresponding area after an equicarbohydrate portion of white bread, multiplied by 100. Bread contains some resistant starch which escapes alpha-amylase digestion in the small intestine and ferments in the colon to short chain fatty acids (Englyst, 1985). Vorster, Venter and Silvis (1990) suggest that a standardised pure starch product should be developed for use as a reference food in diabetic subjects.

2.4.2 Factors That Influence Glycaemic Index

Several factors may influence the glucose and insulin responses to a food. Variability in glycaemic response to a particular food can be expected within individuals and also between individuals. The effects of exercise, physical fitness and variations in the background diet of the subjects may be some

of the causes of these variations. The carbohydrate, fat and protein content of the diet up to three days before a glucose tolerance test may also have an influence on the glycaemic response. Factors which will influence insulin receptor number, as well as affinity or function of insulin receptors such as dietary fibre will effect the glucose response (Vorster, Venter & Silvis, 1990). The GI obtained in healthy subjects may differ from that obtained in diabetic patients.

It is noteworthy that in a study by (Gresse, 1991) diabetic men displayed a larger glycaemic response than women. Factors such as type and duration of diabetes, severity and medication, may also influence the variability of the glycaemic response to foods. Despite these differences (Wolever, 1990) suggests that the GI is an effective way of standardising the glycaemic response to different foods. In order to overcome the problem of variability between subjects, the glycaemic response of each subject is expresssed as a percent of his own response to a standard food (Wolever, 1990).

Many food factors, apart from macro-nutrients and fibre, affect glycaemic responses. These include anti-nutrients, lectins, phytates, saponins, tannins and enzyme inhibitors, starch-protein and starch-lipid interactions, the nature of starch, food form, particle size and cooking.

Protein and Fat

Protein and fat decrease the blood glucose response and enhance insulin secretion when added to a carbohydrate test meal (Estrich *et al.*, 1967). Fat delays gastric emptying and therefore will have the effect of decreasing the glycaemic response to a meal. When fat was taken in a breakfast meal, the insulin response to a standard lunch meal taken four hours later was similar to that when no fat was taken at breakfast, despite a significantly increased blood glucose level (Collier *et al.*, 1987). Thus it appears that fat initially enhances insulin secretion. However, four hours later fat may reduce insulin secretion possibly due to the presence of increased FFA levels in the blood (Nestle *et al.*, 1964).

Carbohydrate and Fibre

When carbohydrate and plant fibres are ingested together less hyperglycaemia ensues than when the same amount and type of carbohydrate is ingested without plant fibre (Haber *et al.*, 1977). However, not all fibres are able to reduce the blood glucose and insulin, and insoluble fibres such as wheat bran have little or no effect (Jeffery, 1974; Monnier *et al.*, 1978). Foods containing viscous fibres such as barley and legumes, have low GI (Jenkins 1979; Jenkins and Jenkins 1987). It is not known whether it is the presence of viscous fibre or the effects of food form, particle size or type of starch which is the reason for the low glycaemic response of these foods (Wolever, 1990).

Antinutrients

As reviewed by Wolever (1990), antinutrients are food components which reduce the availability of nutrients in foods. They are found in substantial amounts in legumes, which have a low GI. The GI of a food is closely related to its content of lectins, phytates and polyphenols. These compounds may decrease the amylytic digestion of starch by inhibiting the access of starch to the active site of amylase due to binding of the lectin either to the enzyme or to the starch. Lectins also impair the uptake of sugars across the gut wall by binding to the surface of the cell and blocking the transport site (Wolever, 1990). Amylase inhibitors are present in many raw foods including legumes, wheat, banana and peanuts. They are very unstable to heat and virtually disappear in cooked foods. Purified amylase inhibitors can reduce post-prandial glycaemic and insulinaemic response by inducing carbohydrate malabsorption (Jenkins *et al.*, 1981b).

Nature Of Starch

Amylase and amylopectin are the only two polysaccharides that are hydrolysed in the human small intestine. Amylopectin is more readily gelatinized and readily hydrolysed by amylase than amylose. The low glycaemic response of legumes could possibly also be attributed to its starch granule containing 30-40% amylose. Wheat contains 70% amylose (Halliday, 1990).

Food Processing And Cooking

Increases in the GI have been reported after consumption of cooked starch as opposed to raw starch (Snow and O'Dea, 1981). The starch granule swells and gelatinize in the presence of heat and water, which increases its susceptibility to enzymatic digestion. Commercially processed foods with methods such as extrusion, flaking, popping and explosion have been associated with increased GI (Thorn *et al.*, 1983). In contrast, conventional cooking method such as boiling, cause less physical disruption and only moderate heat, and are therefore less likely to cause starch damage or complete gelatinization (Brand *et al.*, 1985).

Food Form

Physical form and particle size of the food molecule have a great influence on the digestion rate and consequently on the metabolic effect. Larger food particles have a lower surface to volume ratio which reduces the access of enzymes to the interior of the particle (Heaton *et al.*, 1988). In an *in vitro* hydrolysis study, stoneground wholemeal flour was hydrolysed more slowly than white flour (Snow and O'Dea, 1981). Ground rice showed higher glucose increase than whole rice (O'Dea *et al.*, 1981). In an *in vivo* study, insulin responses were also greater with fine maize meal than with whole or cracked maize grains (Heaton *et al.*, 1988).

Mixed Meals

Coulston and colleagues have argued that the differences between the GI values of different foods do not persist in mixed meals owing to the effect of fat and protein in reducing glycaemic response (Coulston *et al.*, 1980, 1984; Calle-Pascual *et al.*, 1986). Others do not agree with this interpretation and state that accurate predictions of the glycaemic response of a mixed meal is closely related to the GI of the individual foods (Wolever *et al.*, 1989; Chew *et al.*, 1988). The properties of foods which influence their digestibility and hence their physiological effects *in vivo* are preserved when the foods are consumed within the context of a meal (Parillo *et al.*, 1985). However, it has been stated that a better approach when planning the use of foods high in carbohydrate in the diabetic diet would be to take into account the glycaemic response of the meal rather than the GI of the food alone (Alfonso *et al.*, 1986).

Ripeness And Storage Of Food

The composition of fruit changes as the fruit ripens. For example, the starch content of banana depends on the ripeness, being 37% of dry weight in the least ripe and 3% in the most ripe (Englyst and Cummings, 1986). Wolever (1988a) demonstrated that the GI of over-ripe, ripe and under-ripe bananas are 90.4 $\pm$ 2.6, 75.3 $\pm$ 5.5 and 59.2 $\pm$ 5.4% respectively. The starch may vary in digestibility during different degrees of ripeness. Potato and other moist-heated starchy foods when cooled and stored, are incompletely digested because of retrogradation of the starch.

The digestibility of the starch in cooked potato falls from 97% to 88% after cooling (Englyst and Cummings, 1987b). Factors affecting digestion of starch will be discussed in more detail in section 2.6.2

2.4.3 Physiologic And Therapeutic Implications Of The Glycaemic Index

2.4.3.1 Blood Glucose And Insulin Levels

The long-term complications of diabetes are related to uncontrolled raised blood glucose levels (Zilva and Pannall, 1988). The rate of cardiovascular disease is also related to serum insulin levels (Krolewski, *et al.*, 1992). To reduce the risk factors of complications in diabetes, reduction of blood glucose and insulin levels are therefore considered important. To prevent large fluctuations in blood glucose, besides drug therapy, carbohydrate foods should be selected that will minimise post prandial blood glucose excursions (Jenkins *et al.*, 1984; American Diabetes Association, 1984). This concept was demonstrated by Jenkins *et al.*, (1987) in a study where six healthy normal subjects were kept on low and high glycaemic index diets in random order. The low GI diet resulted in significant reductions of serum fructosamine, the 12 hour blood gluose profile and serum cholesterol levels. As a measure of insulin secretion, 24 hour urinary C-peptide excretions were 32% lower after the low GI diet than after the high GI diet. In another similar study undertaken in patients with NIDDM, there were significant reductions in fasting blood glucose, HbA_{1c} and

urinary C-peptide levels. In both the high and the low GI diet-period there were significant reductions in body weight, serum fructosamine and cholesterol levels (Jenkins *et al.*, 1988a).

2.4.3.2 Blood Lipids

Disordered carbohydrate and lipid metabolism are both risk factors for cardiovascular disease and are often found in the same individuals, especially in diabetics (Pearson 1991). Insulin stimulates the hepatic production of very low density lipoprotein (VLDL), and therefore, hypothetically, low GI diets which reduce insulin secretion may be of use in the treatment of hyperlipidaemia. This hypothesis was examined in 30 patients with raised triglyceride levels who were studied for three months on a low GI diet (Jenkins *et al.*, 1987b). During the second month starchy carbohydrate foods with a low GI were substituted for those with a higher GI with minimal change in dietary macronutrient and fibre content. In the 24 patients with raised triglyceride levels (types 11b, III and IV) there were significant reductions of serum total cholesterol (9%),low density lipoprotein (LDL) cholesterol (9%) and serum triglyceride (19%) with no change in high density lipoprotein (HDL) cholesterol. Therefore, low GI diets may have therapeutic use in the dietary management of the lipid abnormalities associated with hypertriglyceridaemia (Wolever, 1990).

2.4.3.3 Colonic Metabolism

The colon is the site of fermentation, the anaerobic breakdown of carbohydrate by bacteria into short chain fatty acids (SCFA) (Cummings, 1981; Cummings and Englyst, 1987). This is discussed in detail in Section 2.5.5.

Rapidly digested foods with a high GI provide very little starch to the colon. The exact metabolic effects of SCFA produced in the colon are not known but according to Pomare *et al.*(1985) and Dankert *et al.*(1981) it appears that the butyrate is taken up by colonic epithelial cells and is known to inhibit tumour growth. Acetate passes to peripheral tissues where it is metabolized by the muscle. Propionate is taken up by the liver where it stimulates gluconeogenesis, stimulates insulin secretion and inhibits hepatic cholesterol synthesis. The low GI diet provides increased amounts of substrate for fermentation (Jenkins, *et al.*, 1987d). Therefore, slow absorption of carbohydrate may have additional metabolic effects.

2.4.4 Objections To The Concept Of The GI

Coulsten (1984) raised objections to the GI concept. These objections revolve around four major issues, namely:

* large individual variations for a particular food;
* lack of agreement of GI between different centres for a particular food;
* lack of difference of GI's between mixed meals;
* lack of demonstration of long-term benefits of low GI foods or

diets (Wolever, 1990).

Despite these objections, there are many (Jenkins *et al.* 1981; Gericke, 1987; Vorster, Venter & Silvis 1990) of the opinion that the GI is a concept that can be used in the planning of especially diabetic diets.

According to Wolever (1990), variability of glycaemic responses can be due to: a variability of methods, variation due to test meal, a day to day variability, variability of the same food within the same subject is also possible as well as a variation between different subjects.

Very little information is available regarding the reproducibility of the GI. Because of the wide variety and complexities of factors that influence the GI, variation can be expected for the same food on different occasions in the same individual (Vorster, Venter and Silvis, 1990). In order to overcome the problem of variability between subjects the glycaemic response of each subject is expressed as percent of his own response to a standard food (Jenkins *et al.*, 1981).

Walker and Walker (1984) obtained almost identical results for the GI of 10 foods tested in rural Africans to the results obtained for the same foods in Western Europeans by Jenkins *et al.*(1981).

In diabetes, factors such as type and duration of the disease, severity and the use of medication may influence the glycaemic response to foods (Vorster, Venter and Silvis, 1990). Coulsten (1984),and Peterson (1983) suggest that the mean GI cannot be used in diabetic patients, and that it is not advisable to use the GI as a clinical tool to reduce post-prandial and day long glycaemia. Jenkins and coworkers have however reported long term benefits of low GI diets in healthy and in hyperlipidaemic subjects (Jenkins *et al.*, 1987a, 1987b).

Variability in the GI, inherent in variability of methods, could be reduced if prior to determining the GI of a food, methodology is standardised. These measures will include a correct selection of experimental subjects taking into account factors such as age, sex, healthy or diabetic, number, and exclusion criteria to ensure a homogenous group. The reference food should be selected carefully (glucose or white bread), and the length of time of the test should be standardized (ideally tests should be carried out until the blood glucose reaches baseline (Gannon and Nuttall, 1987). The carbohydrate load should be standardized (usually 50g) as well as methods used in calculating the area under the glucose curves (Vorster, Venter and Silvis, 1990). Furthermore, carbohydrate intakes three days prior to the test should be at least 300 grams. The test should be done in the morning after an overnight fast of ten to twelve hours (Vorster, Venter and Silvis, 1990).

2.5 DIETARY FIBRE (DF)

2.5.1 Introduction

Plant fibres are portions of plant foods that are not digested in the human small intestine. DF has important preventative and therapeutic implications for certain conditions such as diabetes (Anderson *et al.*, 1987), hyperlipidaemia, coronary heart disease (Miettinen 1987), hypertension and several intestinal disorders (Vinik & Jenkins 1988). Considerable attention has been focused recently on the various plant fibres because of their influences on gastro-intestinal physiology. Plant fibres have profound influences on human nutrition because they alter the absorption and metabolism of many nutrients (Anderson *et al.*, 1979).

2.5.2 Definition Of Dietary Fibre Or NSP

The term DF lacks a precise definition. In 1972 Trowell defined DF the skeletal remains of plant cells that are resistant to digestion by the enzymes of man (Trowell, 1972). This physiological definition disregards the chemical characteristics of DF. Englyst suggested that DF should be measured as the non-starch polysaccharides (NSP) in plant foods (Englyst, 1981). NSP includes all the carbohydrate fractions and types of dietary fibre: soluble and insoluble, pectins, gums, hemicelluloses, storage polysaccharides such as guar,also cellulose, glucans and non-cellulosic polysaccharides, as shown in figure 2.6.

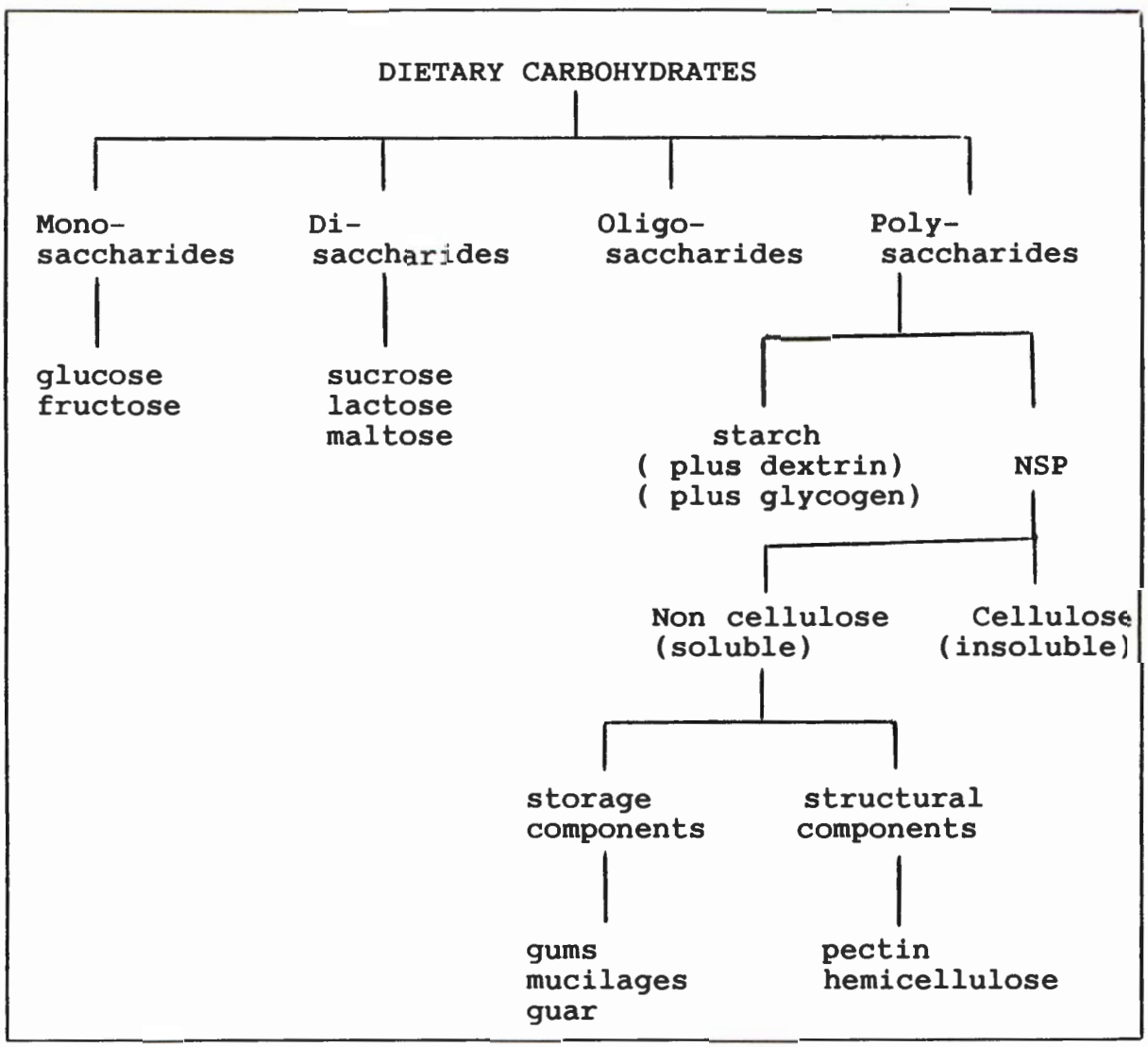

Figure 2.6 Classification of dietary carbohydrates
Adapted from Zilva & Pannall (1988).
NSP classification adapted from Englyst and Hudson (1987).

2.5.3 Difference In Fibre Types

Fibres can be grouped into two broad categories: water soluble and water insoluble. Soluble fibres include pectin, gums, some hemicellulose, mucilages, and guar gum.

Insoluble fibres include cellulose, lignin and many hemicelluloses (Anderson and Chen, 1979). Sources of insoluble

fibres are vegetables, wheat bran and grain fibres, while oats, barley, some fruits and vegetables and legumes contain soluble fibres. None of these are digested by enzymes in the human small intestine.

2.5.4 General Physiological Effects Of DF (NSP) In The Gut

The physiological effects of fibre in the gut is influenced by the environmental factors in the gut, such as osmolality, pH, the presence of other fibres or nutrients, the digestive process, water retention and the presence of bacteria (Anderson and Chen, 1979). Adequate quantities of cellulose rich foods (eg. wheat bran) decrease intestinal transit time and increase faecal bulk. Soluble fibres may delay gastric emptying, increase small intestinal transit time because of their gel formation, and have only a small effect on faecal bulk (Jenkins *et al.*, 1977; Leeds *et al.*, 1975). The water adsorption properties of most fibres contribute to alterations in intestinal transit time (Eastwood *et al.*, 1985). The specific effects of NSP will now be discussed in more detail.

2.5.5 Specific Physiological Effects Of DF (NSP) In The Large Intestine

Many functions of the large intestine are affected by the DF (NSP) namely, bacterial metabolism, transit time, intercolonic pressure, flatus production, faecal composition and water content, faecal bulk, consistency, and defaecation (Eastwood and Brydon, 1985).

Fibre, together with resistant starch, is fermented anaerobically by bacterial enzymes in the colon. This process begins as soon as fibre enters the caecum. Cummings and Englyst (1987) have reviewed the literature extensively and discussed the evidence for fermentation of fibre in the large intestine. The three major events which occur are shown in Fig. 2.7, and are bacterial growth, generation of gases such as carbon dioxide (CO_2) methane (CH_4), hydrogen (H_2), and the production of SCFA mainly acetic, propionic and butyric acid.

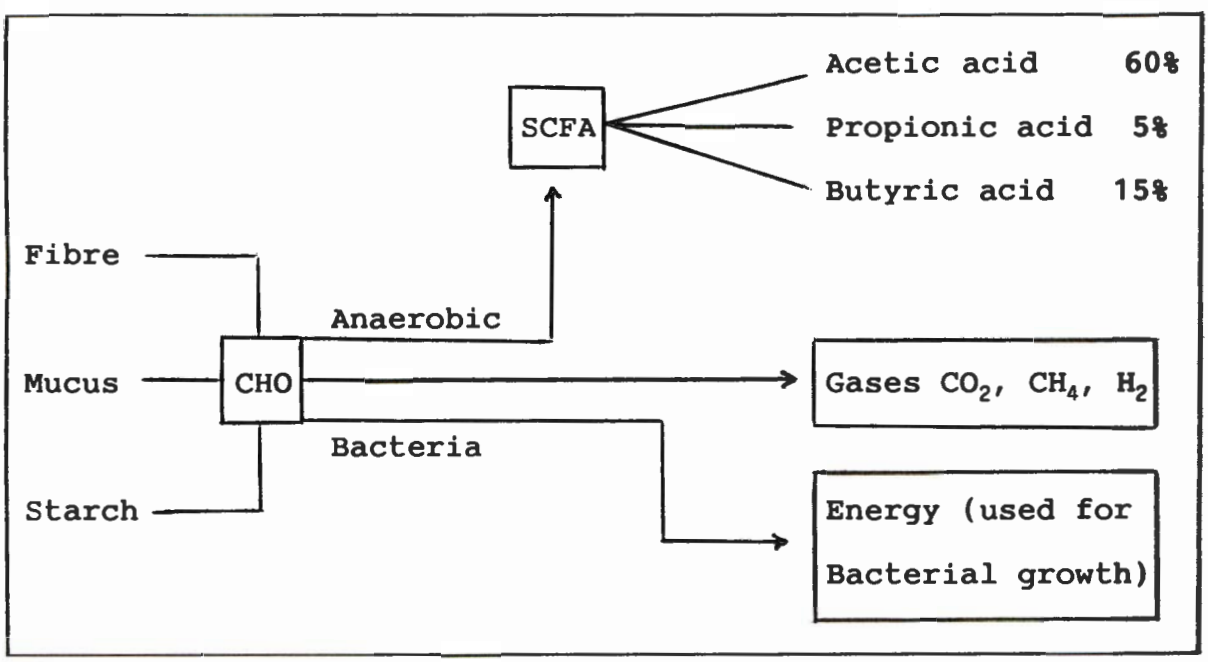

Fig 2.7 Metabolic fate of dietary carbohydrate in the large intestine.
CO_2 = Carbon Dioxide, H_2 = Hydrogen, CH_4 = Methane
CHO = Carbohydrate
Adapted from Cummings, Englyst and Wiggins (1987).

Resistant starch which escape digestion in the small intestine may be more important as a substrate for fermentation than NSP (Cummings and Englyst, 1987).

The endogenous colonic flora are capable of metabolizing soluble and some insoluble dietary fibre. The colon is capable of absorbing products of this metabolism (Fleming and Floch, 1986).

The gases methane and hydrogen are not formed by human tissues and are excreted in breath and per rectum (Gibson *et al.*, 1990).

Fermentation is also the way in which the microflora of the large intestine obtain energy for maintenance of cellular function and growth (Cummings and Englyst, 1987).

SCFA, the principal metabolites, are rapidly absorbed from the colonic lumen (McNeil *et al.*, 1978), but their fate beyond this has not been studied extensively. SCFA absorption stimulates sodium and water absorption (Ruppin *et al.*, 1980) and thus it is an important contributor to salt and water homeostasis in the colon. Colonic epithelial cells metabolize SCFA, especially butyrate. Butyrate has an important regulatory effect on nucleic acid metabolism in colonic cells and therefore may be of importance in maintaining the health of the large bowel epithelium.(Cummings, 1987).

Once absorbed, SCFA pass into the portal vein and then to the liver where propionate and some acetate is taken up. Acetate escapes to peripheral tissues where it is metabolised by muscle

(Cummings *et al.*, 1986). It has been reported that SCFA produced by bacterial fermentation of fibre are absorbed and may decrease liver gluconeogenesis (Cummings *et al.*, 1986). Carbohydrate and lipid metabolisms are related; therefore, if fibre decreases carbohydrate absorption and insulin secretion, it may have an indirect effect on decreasing lipid synthesis (Vinik & Jenkins 1988). Fibre may also affect the enterohepatic circulation of bile acids (Miettinen 1987).

Following fermentation of fibre, a change in caecal pH and a possible increase in adsorption of bile acids to fibre, may influence the loss of bile acids from the enterohepatic circulation (Eastwood, 1985). The specific mechanism of action of SCFA on hepatic metabolism is still not well understood.

2.5.6 Effects Of DF (NSP) On Carbohydrate Metabolism

Blood glucose is a useful index representing the effect of diet on carbohydrate metabolism in the body. The concentration of blood glucose at any point reflects the balance between entry of glucose into blood from the gut or from gluconeogenesis and removal by peripheral tissue for energy metabolism, storage as glycogen or conversion to fat, or in case of hyperglycaemia removal via glycosuria (Zilva and Pannall, 1988). Any of these events may be affected directly or indirectly by DF as shown in Figure 2.8 (Wahlqvist, 1987).

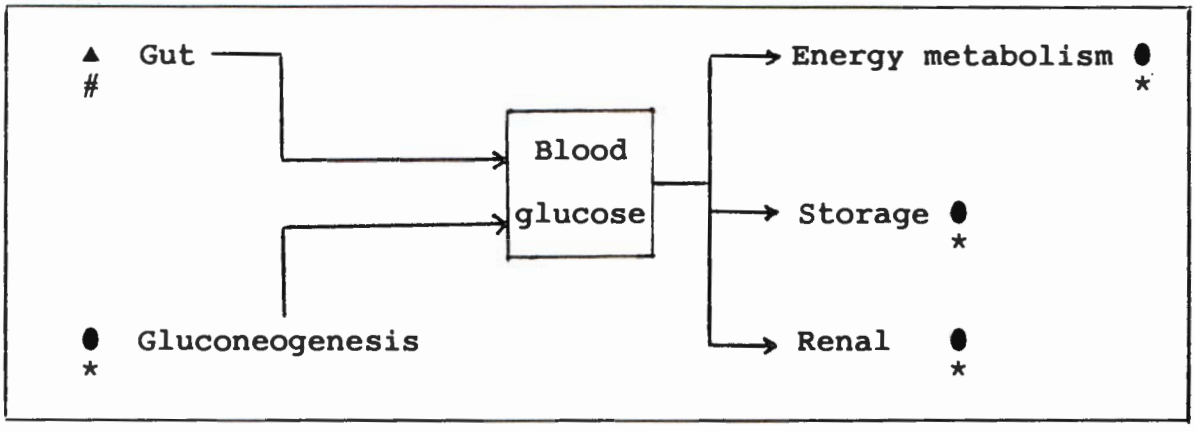

Fig. 2.8 Site of dietary fibre action on glucose flux. Adapted from Wahlqvist (1987).

▲ definite # direct
● possible * indirect

Hormones involved in carbohydrate metabolism may also be affected by gut hormones, and gut hormones are influenced by NSP (Collier *et al.*, 1982).

Haber *et al.* (1977) demonstrated that when carbohydrates and NSP are ingested together, lower blood glucose responses are obtained than when the same carbohydrates are ingested without NSP. The most viscous fibres seem to be the most effective in improving glucose tolerance (Jenkins *et al.*, 1978; Vorster *et al.*, 1988). Studies using urinary xylose excretion measurements have shown that the lower glycaemic response to NSP enriched meals is due to delayed carbohydrate absorption, rather than to malabsorption (Jenkins *et al.*, 1978). When whole and fibre-depleted cereal products were fed to healthy subjects (Jenkins *et al.*, 1981a) or to a group of diabetic subjects (Jenkins *et al.*, 1983), no difference in blood glucose response was obtained between the two meals. This confirmed earlier observations that the effect of bran in reducing the blood glucose rise after taking glucose

syrup, although present, was small (Jeffrey, 1974). The lack of effect of cereal fibre on post-prandial blood glucose levels suggests that the food form rather than fibre may be important in determining the glycaemic response (Jenkins *et al.*, 1983). With hydration, soluble fibre form gels in the small intestine and colon, which may delay the absorption of carbohydrate and other nutrients (Anderson & Chen, 1979). This mechanism is thought to be responsible for the effects of soluble fibre on glucose tolerance.

Dietary fibre also dampens the insulin response to a carbohydrate meal (Potter *et al.*, 1981). The mechanisms by which high fibre diets lower blood insulin responses are not known. The viscosity of DF causing delayed gastric emptying and slowing of carbohydrate absorption has been suggested as a possible mechanism (Jenkins, 1979), as well as enhanced insulin sensitivity following the high fibre meal, through unknown mechanisms (Potter *et al.*, 1981).

Soluble fibres have also been shown to lower LDL cholesterol levels (Vinik and Jenkins 1988). Legumes, rich in soluble fibre, also reduce triglyceride concentrations in hypertriglyceridaemia (Jenkins *et al.*, 1980), even though diets high in carbohydrates are reported to cause hypertriglyceridaemia (Glueck *et al.*, 1969). The possible mechanism by which substitution of soluble fibre, mainly legumes, into the diet may reduce serum triglycerides is not clear. It has been suggested that reduced glucose (Jenkins *et al.*, 1980) and insulin (Albrink *et al.*, 1979) levels, seen with a number of high fibre foods, may

prove less of a stimulus to hepatic triglyceride synthesis. High fibre diets may have an insulin sparing effect. Addition of fibre in up to 70% carbohydrate diets may prevent the increase in triglyceride levels (Albrink *et al.*, 1979).

Amongst high fibre foods with slow rates of digestion and flat glycaemic responses are leguminous foods. These foods, when incorporated into the diets of diabetics have been found to reduce postprandial blood glucose profiles, C-peptide excretion and HbA_1c levels (Simpson *et al.*, 1981). In long-term studies in both NIDDM and IDDM patients, incorporation of soluble fibre such as guar gum has improved many aspects of diabetes control, including HbA_1c levels, urinary glucose losses, insulin requirement and insulin sensitivity (Jenkins *et al.*, 1987c). Increased peripheral insulin sensitivity induced by high fibre diets may be due to an alteration in insulin receptor number (shown on circulating monocytes),or a result of an upregulation of receptors associated with lower circulating insulin levels (Anderson, 1986). Another mechanism by which DF may have an effect on carbohydrate metabolism is through the generation of SCFA by colonic microflora and absorption of SCFA into the splanchnic circulation where it may influence hepatic metabolism (Pomare *et al.*, 1985). In a recent study six healthy female volunteers were given a propionate supplement daily for seven weeks. The propionate decreased the fasting blood glucose levels and maximum increments during a glucose tolerance curve, and increased the HDL cholesterol compared to a control group on a the placebo (Venter, 1989)

2.6 DIETARY STARCH

2.6.1 Introduction

For many years it was assumed that starch was completely digested and absorbed in the small intestine. When Englyst developed a method for measurement of NSP, he identified a fraction of starch resistant to pancreatic amylase *in vitro* (Englyst *et al.*, 1982). Consequently, studies in man indicated that some dietary starch may escape digestion in the small intestine and is probably the major substrate for fermentaton in the colon (Anderson *et al.*, 1981; Englyst and Cummings 1985; 1986; 1987a). It was clear from these studies that a substantial amount of starch escapes digestion in the small intestine. A small proportion of this starch is in the form of retrograded amylose, originally defined as a type of resistant starch (Englyst *et al.*, 1982). For example, banana does not contain any retrograded amylose, but when bananas were fed to illeostomates, up to 89% of the starch escaped digestion (Englyst and Cummings, 1986). The starch in freshly cooked potato is well digested but in cooked and cooled potato, 12% of the starch resist digestion (Englyst and Cummings, 1987b). These observations led to a reclassification of starch according to its digestibility *in vitro* (Englyst and Kingman, 1990). The classification is based on the speed with which glucose is released when the food is incubated with various pancreatic enzymes.

Table 2.6 'IN VITRO' NUTRITIONAL CLASSIFICATION OF STARCH

Type of starch	Example of occurrence	Probable digestion in the small intestine
RAPIDLY DIGESTIBLE STARCH	Freshly cooked starchy food e.g. bread	Rapid
SLOWLY DIGESTIBLE STARCH	Most raw cereals	Slow, but complete
RESISTANT STARCH		
1 Physically inaccessible starch	Partly milled grain and seeds	Resistant
2 Resistant starch granules	Raw potato and banana	Resistant
3 Retrograded starch	Cooled, cooked potato; bread and cornflakes	Resistant

Adapted from Englyst and Cummings (1987a).

The total starch (TS) is measured when starch is milled or homogenized, gelatinized at 100°C, dispersed with potassium hydroxide and then incubated with pancreatin and amyloglucosidase. Three subfractions of starch can then be identified by controlled periods of enzymatic digestion of homogenised food samples.

Rapidly Digestible Starch (RDS)

This is measured as the glucose released from a sample after 20 minutes. It consists mainly of amorphous and dispersed starch.

Slowly Digestible Starch (SDS)

Slowly digestible starch is measured as the glucose released from a sample after a further 100 minutes after RDS of enzyme digestion.

Resistant Starch (RS)

Resistant starch is the starch which potentially resist digestion in the small intestine and passes into large intestine. RS can be subdivided into three fractions.

1) RS1 type of starch is resistant because it is in a physically inaccessible form such as partly milled grain and very dense type of processed starchy foods such as pasta.
2) RS2 are the starch granules with B or C crystalline structure (See 2.6.2).
3) RS3 represents the most resistant starch fraction and is mainly retrograded amylose formed during the cooling of gelatinized starch (Englyst and Macfarlane, 1986).

2.6.2 Factors Affecting Starch Digestion

Structure Of The Granule

The starches are α-glucans i.e. they are composed of chains of glucose molecules joined by α-linkages (Englyst and Kingman, 1990). Amylose and amylopectin varies according to the sources of the granule. Waxy maize contains 99.2% amylopectin and 0.8% amylose (Englyst and Kingman, 1990). Wheat contain 7% amylopectin and 25% amylose and high amylose maize contains 30% amylopectin 70% amylose. The crystalline structure of the starch granule has been studied by X-ray diffraction (Katz, 1934) as quoted by Englyst and Kingman (1990). Three types of structures have been identified: type A found in cereals, type B found in potato and banana and type C found in peas and beans. Type B and C tend to be more resistant to enzyme degradation.

Physical Inaccessibility

Coarsely milled grains provoke smaller plasma insulin responses after ingestion than does finely ground flour (Heaton *et al.*, 1988). When starch is contained within undisrupted grains it will resist digestion. It has been shown that after meals of sweetcorn, peas and beans, up to 20% of faecal solids were undigested starch (Englyst, 1985).

Effect of Heating

When heated to about 50°C in the presence of water the amylose in the starch granule swells, the crystalline structure of the amylopectin disintegrates and the granule ruptures. The starch gelatinises and the extent of gelatinization depends on the amount of water present and the duration of heat treatment. On cooling gelatinized starch retrogrades leading to resistance to hydrolysis by pancreatic enzymes (Sievert and Pomeranz, 1989). Digestibility of leguminous starch is also determined by the nature of the starch and its entrapment in fibrous thick walled cells, which prevents its complete swelling during cooking (Wursch *et al.*,1986).

GUT-FACTORS

Digestion of starch also depends on the availability of amylase in the gut. Factors such as chewing, transit time and concentration of amylase also affect the digestibility *in vitro* (Englyst and Cummings, 1987a). This classification is based on rapidity with which glucose is released when the food is incubated with various enzymes under specified laboratory conditions.

2.6.3 Selected Biological Effects Of Starch

2.6.3.1 Fermentation Of Starch In The Large Intestine

Once RS reaches the large intestine it can be fermented by the colonic bacteria along with NSP to produce lactate, SCFA, carbon dioxide and hydrogen. The fermentation of starch results in a larger proportion of butyrate to acetate than fermentation of NSP. Butyrate is used by colonic epithelial cells. Up to 69% malabsorbed starch from banana has been measured in illeostomates (Englyst and Cummings, 1986). In the rural African diet maize is the main part of the diet. When maize is eaten cold, large amounts of retrograded starch could therefore escape digestion and absorption in the small intestine. Depending on the type of starch and its processing, up to eight times more starch than NSP may be available for fermentation (Cummings *et al.*, 1986). Therefore starch, not fibre, may probably be the major substrate for fermentation in the human colon. Approximately 10% of all starch in Western diets is thought to be resistant starch (Cummings *et al.*, 1986).

2.6.3.2 Effects On The Glycaemic Index

As mentioned in 2.4.2, different starchy foods produce different glycaemic responses when fed individually. Recently, it has been indicated that the glycaemic response may be predicted from the rate at which a food is digested *in vitro*. Slowly digested foods produce flatter glycaemic responses. These foods have been termed '*lente* carbohydrate foods' (Jenkins *et al.*, 1982a).

Cooked legumes are digested slower and have a very low GI. Cold maize porridge resulted in a smaller blood glucose and insulin response than hot porridge, reflecting the extent to which starch resisted hydrolysis after cooking (Venter *et al.*, 1990). The maize starch used in this study contained 26% amylose and 74% amylopectin. The difference in blood glucose and insulin responses observed with cooled and reheated maize porridge was ascribed to the presence of RS. The amylose fraction of maize starch has a gel-forming ability with cooling and these gels cannot be reliquified with reheating (Venter *et al.*, 1990).

2.7 THE SECOND MEAL EFFECT (SME)

A low GI meal improves the carbohydrate tolerance of the subsequent or second meal (Jenkins *et al.*, 1982), a phenomenon known as the second meal effect (SME). The hypothesis upon which the SME is based, is that when carbohydrate absorption is slow there is less rapid rise of blood glucose and no undershoot of the baseline glucose levels. This results in a smaller counterregulatory response, namely a smaller secretion of glucagon, growth hormone catecholamines, and a lower rise in FFA levels. This is supposed to lead to an improved glucose disposal after the next meal (Wolever,1990).

Improved glycaemic responses to lunch were seen four hours after viscous fibre (Jenkins *et al.*, 1980a) or slowly absorbed carbohydrate foods (Jenkins *et al.*, 1982) were eaten in a breakfast test meal. SME has been demonstrated between breakfast and lunch (Jenkins *et al.*, 1982) and between dinner and breakfast

(Wolever *et al.*, 1988). This effect may be dependent on the GI of the first meal. Suppression of FFA release after slow absorption of carbohydrate may be responsible for the SME (Wolever 1990). Evidence for a reduced counterregulatory response was lower FFA and 3-hydroxy-butyrate levels four hours after the guar-containing breakfast (Jenkins *et al.*, 1980a).

Shaheen and Fleming (1987) could not demonstrate a SME after a standard lunch four hours after consuming either red kidney beans or bran cereal or white bread as breakfast meal. Jenkins *et al.* (1982) also suggest that not only the amount but also the rate of delivery of carbohydrate in the first meal may influence the glycaemic response to a subsequent meal. Carbohydrate in the upper gastro-intestinal tract stimulates GIP release. A reduced GIP response to a carbohydrate load indicates a reduced concentration of substrate for absorption in the gut. Lentils release their carbohydrate products slowly and show a flattened GIP response; this in turn reduces the stimulation to insulin release. A large rise in insulin, followed by falls in blood glucose may in turn stimulate FFA release. The increase in FFA levels may be associated with impaired carbohydrate tolerance (Jenkins *et al.*, 1982). Significant increases in FFA and 3-hydroxybutyrate synthesis was noted at four hours, when a glucose load was taken at breakfast compared to a glucose load taken with guar gum (Jenkins *et al.*, 1978). The question now arises how FFA could possibly influence carbohydrate metabolism and be responsible for the SME.

Several abnormalities of carbohydrate metabolism is associated with high circulating concentrations of FFA. These abnormalities include insulin resistance and impaired glucose tolerance. Studies have shown that the release of FFA is inhibited by glucose and insulin, and enhanced in diabetes, starvation and carbohydrate deprivation. Growth hormone corticosteroids and adrenaline also stimulate FFA release (Randle *et al.*, 1963). Nestel *et al.*, (1964) demonstrated that raised FFA levels induced by noradrenaline interfered with the removal of intravenously administered glucose; when the noradrenaline induced FFA was prevented, glucose tolerance improved. Ferrannini (1983) also demonstrated that when insulin is deficient, elevated level of FFA leads to hyperglycaemia not by competition of fuel utilization, but through an enhancement of endogenous glucose output.

Randle proposed in 1963 that there are interactions between glucose and fatty acid metabolism in muscle and adipose tissue, which take the form of a cycle, the so-called glucose fatty acid cycle (Randle *et al.*, 1963). This cycle provides a mechanism, independently of hormonal control, to maintain a constant plasma-glucose concentration. Control of the cycle is regulated by insulin. Insulin enhances glucose uptake in muscle and adipose tissue, inhibits release of fatty acids in adipose tissue and increase esterification of fatty acids in adipose tissue and muscle. Growth hormone, corticosteroids and adrenaline also can modify the glucose fatty acid cycle by accelerating release of fatty acids from adipose tissue and muscle. This action may

induce insulin insensitivity due to inhibition of uptake of glucose, and may therefore be responsible for an effect of FFA on glucose disposal during meals (SME).

2.8 THE STAUB-TRAUGOTT EFFECT

When glucose loads are given in succession, significant and progressive improvement in glucose tolerance will occur. This facilitated disposal of a glucose load is known as the Staub-Traugott phenomenon, first described by Hamman and Hirschman (as quoted by Metz and Friedman, 1970). The mechanism responsible for this effect is not fully understood but it seems as if the effect is not dependent on plasma FFA nor insulin levels (Metz and Friedenbrg, 1970). Abraira and Lawrence (1978) also demonstrated that the Staub-Traugott effect was independent from FFA suppression.

A Straub-Traugott effect was seen in black NIDDM patients when a second carbohydrate load was given within three hours after the first carbohydrate load (Gresse, 1991). A SME was also observed in the same study when the GI of the first meal was low.

2.9 SUMMARY AND MOTIVATION EXAMINED

In recent years interest has increased in allowing diets to be designed on the physiological basis of the glycaemic response of foods, rather than on the chemical composition of foods. From the literature survey it is clear that a low GI of a first meal may have a SME, defined as an improved disposal of glucose during

the second meal. Wolever (1990) presented the hypothesis that supression of FFA release after slower but prolonged absorption of carbohydrate is responsible for the SME.

Hyperglycaemia in NIDDM patients should be first treated with a diabetic diet. The modern diabetic diet prescribed by several authorative organisations, has a nutrient composition very similar to the typical traditional diet of the rural blacks in South Africa (Silvis, 1989). The incidence of Western diseases are low in blacks in South Africa (Walker and Walker, 1985; Segal and Walker, 1986). The consumption of starch is reported to be very high while intakes of dietary fibre in the South African black population are decreasing (Vorster, 1987, as quoted by Venter, 1989).

The staple in the diet of South African blacks is maize meal and it is known that maize porridge is often eaten cooled. It has been shown that when starchy foods are cooked and then cooled, they are incompletely digested due to retrogradation of starch (Englyst and Cummings, 1987a). The consumption of large quantities of resistant and partially resistant starch in the form of cooled maize porridge may result in smaller blood glucose and insulin responses and an improved SME due to suppression of FFA release. This may be the result of the slow digestion and absorption of starch.

The maize mainly used in South Africa contains 26% amylose and 74% amylopectin (Cronje, 1985). It is reported that the amylose fraction of maize starch has a gel forming ability on cooling,

which cannot be reliquified with reheating (Englyst and Cummings 1987a). It has been shown that the GI of maize porridge is low and that it can further be lowered by cooling the porridge (Venter, 1989).

No information is available on the GI of maize porridge with added barley fibre, a relatively new fibre in South Africa,and which contains 6.3% (analyzed in our laboratory by the Englyst method) soluble or gel fibre. The effect of maize porridge on the SME is also not known. The mechanism responsible for the SME is still not well understood. Therefore, the objectives of this study were to determine the GI of cooked, frozen and reheated maize porridge with and without barley fibre, and to examine the SME with both the above porridges as first meal. The underlying mechanism responsible for the SME obtained with maize porridge as first meal and glucose as second meal, was also examined.

Based on the available literature, the hypotheses examined in this study were that barley fibre should lower the GI of maize porridge because of its gel fibre content and that the suppression of FFA, glucagon and GH release by a slowly absorbed first meal, will result in an improved SME.

The results of this study should provide information that could be used in the planning of diets, eg. diabetic diets, where the composition of the first meal is manipulated to ensure an improved glycaemic response to the subsequent meal.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

In this chapter general methodology, composition and preparation of the diets and details on experimental methods used will be discussed. Laboratory work was executed by the researcher at the Gastro-enterology laboratory of Baragwanath Hospital and at the Carbohydrate Research Laboratory at the University of the Witwatersrand.

The coefficient of variation (CV) of repeated measurements (intra and inter assay) of standards or control sera were calculated as the standard deviation/ mean x 100 and will be reported as percent of coeffiecient of variation(% CV)

3.2 SUBJECTS

Six healthy black women volunteers of ages between 20 and 36 years and body mass index (BMI) between 22 and 28 kg/m^2 who tested HIV negative, participated in the study. For randomization of the meals, the six subjects were divided into three groups. All subjects gave informed consent to the study which was approved by the University of the Witwatersrand Human Research Committee. Characteristics of subjects are given in Table 4.1.

Published GI results of specific foods are usually obtained in groups of 5 to 10 subjects, (Vorster *et al.*, 1990; Wolever *et al.*, 1990).

Pretest dietary standardization and limited ranges of age and BMI, as well as using subjects of the same race and sex, and indexing the glucose response to a food to a control glucose tolerance in each subject, will obviate individual difference in GI. Therefore, a small number of subjects could result in significant differences in glycaemic indices of various foods.

3.3 STUDY DESIGN AND PROCEDURES

3.3.1 Design

A Latin square design was used to randomly test the effect of 50g of carbohydrate as glucose (Meal A), maize meal porridge (Meal B) maize meal porridge plus barley fibre (Meal C) on post-prandial blood glucose fluctuations for 180 minutes (first meal effect). After a wash-out period of 60 min, a standard 50g glucose meal was given and subjects were monitored for another 180 min. The wash - out period avoids a Staub-Traugott effect (Metz and Friedenberg, 1970). The design is illustrated in Figure 3.1.

STUDY DESIGN : HEALTHY SUBJECTS

LATIN SQUARE : 3 VISITS

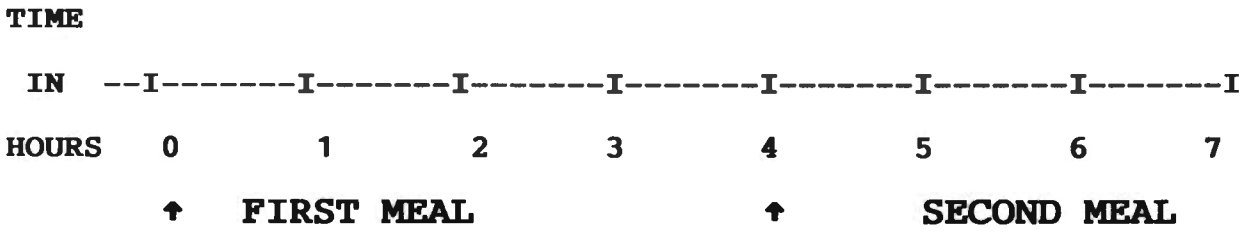

Figure 3.1 Study Design
50g carbohydrate as maize
CHO = carbohydrate

3.3.2 Background Diet

Subjects were put on a minimum of 300g of carbohydrate diet for 3 days prior to the test. A 24 hour dietary recall, taken on each subject during the test period, showed that the subjects consumed at least 300g carbohydrate per day, which allowed optimal substrate induced enzyme synthesis and thus minimum variation in glucose tolerance (Vorster, Venter and Silvis 1990).

3.3.3 Experimental Meals

The meals given were as follows:

Meal A

A control glucose tolerance test was performed with a 50g glucose load (100ml of SABAX 50% glucose solution with 200ml distilled water).

Meal B

Individual portions of 62.7g of refined maize meal (Iwisa No.1) was soaked for 10 minutes in 150ml boiling water with 1.0g table salt. The porridge was then cooked in a microwave oven at maximum power for three periods for 2 min each and stirred for 1.0 min inbetween. It was then allowed to cool to room temperature and frozen at -20°C. The porridge was removed from the freezer the night before the test and thawed at 4°C in the refridgerator overnight.

One and a half hours prior to consumption of the porridge it was reheated for 2 min at full power in the microwave. The porridge was then consumed at approximately 40°C without any water.

Meal C

62.7g Iwisa No. 1 maize meal was mixed with 15g of barley fibre and was prepared, stored and consumed as Meal B.

3.3.4 Test Procedure

Subjects fasted for 12hours prior to the test and reported at our laboratory at 07:30. To reduce stress and anxiety they were familiarized with all aspects of the study. They were not allowed to smoke and move around unnecessarily during the test.

Capillary blood samples (finger prick) were taken to measure fasting blood glucose with the Glucometer Reflectance photometer model 5529 (Ames division, Miles laboratories).

If the fasting glucose was < 5.5 mmol/l an indwelling catheter (IV Jelco TM. 18 Swg. I.D. 0.59mm) was inserted into a vein in the arm to allow multiple blood sampling without having to re-enter the vein. The vein was kept open by infusing 0.9% saline (SABAX) with SABAX Plexitron solution administration set with a Y injection site (Approx 15 drops/ml). Approximately 100ml of saline per hour was allowed to infuse (Approx 25 drops/min). When collecting blood the first saline mixed blood (Approx 2.0ml) was discarded. The blood was collected in sterile syringes and dispensed in appropriate tubes for various tests.

3.3.5 Glucose Tolerance Test

Meal A

Fasting blood samples were collected after inserting the catheter. The subject then drank 50g of glucose dissolved in distilled water (total volume 300ml). Blood samples were then collected at 15, 30, 45, 60, 90, 120, 180 and 240 min after drinking the last sip of glucose solution, which was taken within 5 min. After 240 min, 50g glucose dissolved in distilled water (total volume 300ml) was given. Blood samples were taken again at 4hrs, (4)15 min, (4)30, (4)45, (5)00, (5)30, (6)00 and 7.00hrs.

Meal B and C

After collection of fasting blood samples the subjects ate either maize meal porridge (meal B) or porridge with barley fibre (meal C) within 5 min. Blood samples were drawn at 15, 30, 45, 60, 90, 120, 180 and 240 min. After 240 min, 50g glucose dissolved in distilled water (total volume 300ml) was given. Blood samples were taken again at 4hrs, (4)15min, (4)30, (4)45, (5)00, (5)30, (6)00 and 7.00hrs.

3.4 COLLECTION AND HANDLING OF BLOOD SAMPLES

3.4.1 Collection Of Blood

The following tubes were used for sample collections:

* 1.0ml blood was taken in sodium flouride tubes for glucose estimation.
* 3.0ml blood for glucagon estimation in sodium EDTA tubes with (3000 U) of Trasylol^{R} [Bayer 200,000 KIE].
  EDTA tubes with Trasylol was stored at 4°C.
* 6.0ml blood for insulin, growth hormone and triglycerides determinations in plain glass tubes without any anti-coagulant.
* 4.5ml of blood in 0.5M sodium citrate (0.1mol/L) for free fatty acid determination.

3.4.2 Times Of Collections For Various Tests.

The times of collection of a blood sample for a particular variable is indicated with an asterix in table 3.1.

Table 3.1 Time Of Collection Of Blood Samples.

TIME (min)	0	15	30	45	60	90	120	180	240	255	270	285	300	330	360	420
GLUCOSE	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
INSULIN	*		*		*	*	*	*	*		*		*	*	*	*
TRIGLY-CERIDES	*		*		*	*	*	*	*		*		*	*		
GROWTH HORMONE	*		*		*	*	*	*	*		*		*	*	*	*
GLUCAGON	*							*	*							
FREE FATTY ACIDS	*								*							

All the blood samples were stored and then centrifuged within an hour of collection at 4°C at 2000rpm.
Aliquots of serum and plasma were frozen at -20°C.

3.5 ASSAY PROCEDURES

3.5.1 Capillary Blood Glucose

Capillary blood glucose concentrations were monitored using a Glucometer II Reflectance photometer Model 5529 (Ames Division Miles Laboratories, Elkhart, Indiana, U.S.A.) and Glucostix^{R} reagent strips. A drop of blood from a finger prick was placed on the pad of the reagent strip. The glucose oxidase in the strip catalyses the oxidation of glucose in the blood by oxygen in the atmosphere producing gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of hydrogen peroxidase reacts with the chromogen in the strip and produces a colour. The intensity of the

colour is directly proportional to the glucose concentration in the blood. The Glucometer II measures the colour changes and a digital read-out displays the blood glucose levels. These values were only used to monitor the experiment, for example to be prepared for hypoglycaemic incidents.

3.5.2 Serum Glucose

Glucose in the serum was estimated by using a colorimetric method with a manual glucose oxidase kit available from Laboratory Reagent Service, SAIMR, cat no. C472468. The method is based on the procedure of Trinder where glucose in the blood is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Introduction of peroxidase and a chromogenic oxygen acceptor results in the formation of colour. The intensity of the colour was measured on a Beckmann Spetrophotometer, model DU 42, at 550nm. The controls used for the assay were Beckmann "Decision" Level I, II and III, cat no. M808145. The CV of repeated measurement of control sera varied between 2.89% to 4.16%.

3.5.3 Immuno-Reactive Insulin (IRI)

IRI was determined in duplicate on non-haemolyzed serum with Phadeseph^{R} Insulin RIA radio-immunoassay kit, cat no.6410, available from Pharmacia Diagnostics Uppsala, Sweden. The radio-immunoassay is based on a double antibody solid phase technique. The insulin in an unknown sample is allowed to compete

with a fixed amount of a 125 I-labelled insulin for the binding sites on the highly specific antibodies. The concentration of insulin is then determined by comparing its competitive capacity to that of insulin standards of known concentrations. When the test was performed, serum samples, Insulin 125 I solution and anti-insulin solution were incubated at room temperature. Bound and free insulin were separated by adding double antibody suspension, followed by incubation at room temperature, centrifugation and decanting. The radio-activity of the solid phase pellet was then measured in a gamma counter. The amount of bound radio-activity was inversely proportional to the amount of insulin present in the sample. The CV of repeated measurements of control sera varied between 8.7% - 11.3%.

3.5.4 Growth Hormone

Growth hormone was determined in duplicate on non-haemolyzed serum using Pharmacia^{R} GH RIA radio-immunoassay kit available from Pharmcia Diagnostics Uppsala, Sweden. The GH RIA is a two site immunoradiometric assay (2 site IRMA) serum using two different antibodies in excess. During incubation growth hormone in the serum reacts with anti-hGH 125 I antibodies (raised in rabbit) and anti-hGH antibodies (raised in sheep). Bound and free anti hGH 125 I is seperated by centrifugation and decanting. The radio-activity in the pellet is then measured. The radioactivity is directly proportional to the concentration of growth hormone in the sample. The count of standard or unknown samples is expressed

as a percent of the mean counts of the total activity. The percent values of the standards are plotted on a lin-log paper to construct a standard curve and the concentrations of the unknown samples are read from the standard curve. The CV of repeated measurements of control sera varied between 1.0% to 7.5%.

3.5.5 Glucagon

Glucagon was estimated on non-haemolyzed plasma. The blood was collected in EDTA tubes with Kallikrein inactivator (1000U Trasylol^{R} per ml of blood) to prevent proteolytic degradation of glucagon. A Medgenix^{R} GLU-RIA-50 radio-immunoassay kit available from Medgenix Diagnostics, Belgium, was used. The radioimmunoassay is based upon the principle of a competition between a labelled (Ag*) and an unlabelled antigen (Ag^{0}) for specific antibodies (Ab). In the first step an unlabelled antigen (Ag+) is incubated with an anti-glucagon anti-serum for 3 hours at 4°C. The 125 I glucagon is then added, the incubation thereafter continued for 18-24 hours at 4°C. Anti-rabbit gammaglobulin anti-serum mixed with PEG (DA-PEG Solution) is then added to all tubes. After a brief incubation (20 min) all the 125 I glucagon antibody complex is precipitated. The tubes are then centrifuged and the DA-PEG bound radioactivity is determined. A standard curve is constructed and the glucagon concentration of the samples are determined by dose interpolation from this curve. The CV of replicates was 6.4 %.

3.5.6 Free Fatty Acids (FFA)

FFA in the citrated plasma was determined at T_o and T_{240} min using an enzymatic kit (NEFA QUICK "BMY"(R) Boehringer Mannheim Yamanouchi, Minato-KU, Tokyo, cat no. 450459). Oleic acid (1000umol /l) was used as the standard. The inter-assay CV of the was 2.2 -6.4 % and that of the intra-assay, was 2.86 %.

3.5.7 Triglycerides

Triglycerides concentration was determined with enzymatic-colorimetric kits from Boehringer Mannheim (cat no.701882). In the first step of this test, triglycerides are hydrolysed by lipase to glycerol and 3 molecules of fatty acids. Glycerol is then converted to glycerol 3 phosphate in the presence of ATP and then to dihydroxyacetone phosphate and hydrogen peroxide in the presence of oxygen by glycerokinase and L glycerol 3 phosphate oxidase respectively. The hydrogen peroxide reacts with 4 amino-phenazone and 4 chlorophenol in the presence of peroxidase to form 4-(p- benzoquinone-mono-amino)-phenazone, water and hydrochloric acid. The intensity of the colour developed is proportional to the amount of glycerol and thus triglyceride in the sample, and is measured at 500nm in a spectrophotometer. Beckmann Decision level 1, level 11, level 111 (cat no. M808145) were used as control sera. The CV of the inter-assay varied between 4.0-5.2% and the intra-assay was 2.99%.

3.6 CALCULATIONS AND STATISTICS

The incremental areas under glucose response curves were calculated with a computer programme (based on a simple integrated method) using the lowest blood glucose value as baseline (Vorster, Venter and Silvis 1990). Significant differrences between treatments were calculated with an analysis of variance and the Student- Newman-Keuls test, using the SAS^R package (SAS^R users guide, 1985). Pearson correlation coefficients between various variables at different times were also determined with this package.

CHAPTER 4

RESULTS

4.1 SUBJECTS

The baseline characteristics of the volunteers are shown in table 4.1. The mean age was 30.0 ± 7.7 years, weight 57.5 ± 8.2 kg, and mean height 160,0 ± 10.4 cm. Body mass index (BMI) ranged from 20.1 to 27.9 kg/m^2 and waist-to-hip ratios from 0.64 to 0.73. None of the subjects were on oral contraceptives, or had any gastro-intestinal disorders. All tested HIV negative. The HIV tests were done by the SAIMR laboratory at Baragwanath Hospital. Previous tests showed that the glucose tolerance status were normal in all subjects.

The meals were consumed within the allotted 5 min period although some participants reported difficulty in swallowing the meal with fibre due to its consistency. The participants found the taste of the porridge made from maize meal and added fibre palatable.

TABLE 4.1 MEAN (± SD) CHARACTERISTICS OF EXPERIMENTAL SUBJECTS

VARIABLES	MEAN	±SD	MIN	MAX	%COEFFICIENT OF VARIATION
Age (Years)	30.0	7.7	20.0	39.0	25.7
Weight (Kg)	57.5	8.2	44.5	70.0	14.3
Height (cm)	160.0	10.4	146.0	169.0	6.5
Body Mass Index (kg/m^2)	22.5	3.3	20.1	27.9	14.6
Waist (cm)	70.0	6.5	62.0	78.5	9.3
Hip (cm)	101.3	5.7	94.5	108.0	5.6
Waist/Hip ratio	0.69	0.04	0.64	0.73	5.8

4.2 GLUCOSE RESPONSES

The mean glucose tolerance curves after ingestion of the carbohydrate meals are illustrated in figure 4.1. Meal A evoked the highest response. Meal B and C showed a dampened response compared to meal A although meal C showed a slightly higher response than meal B, but no statistically significant differences were elicited. The highest glucose response during the second meal was observed when glucose was the first meal.

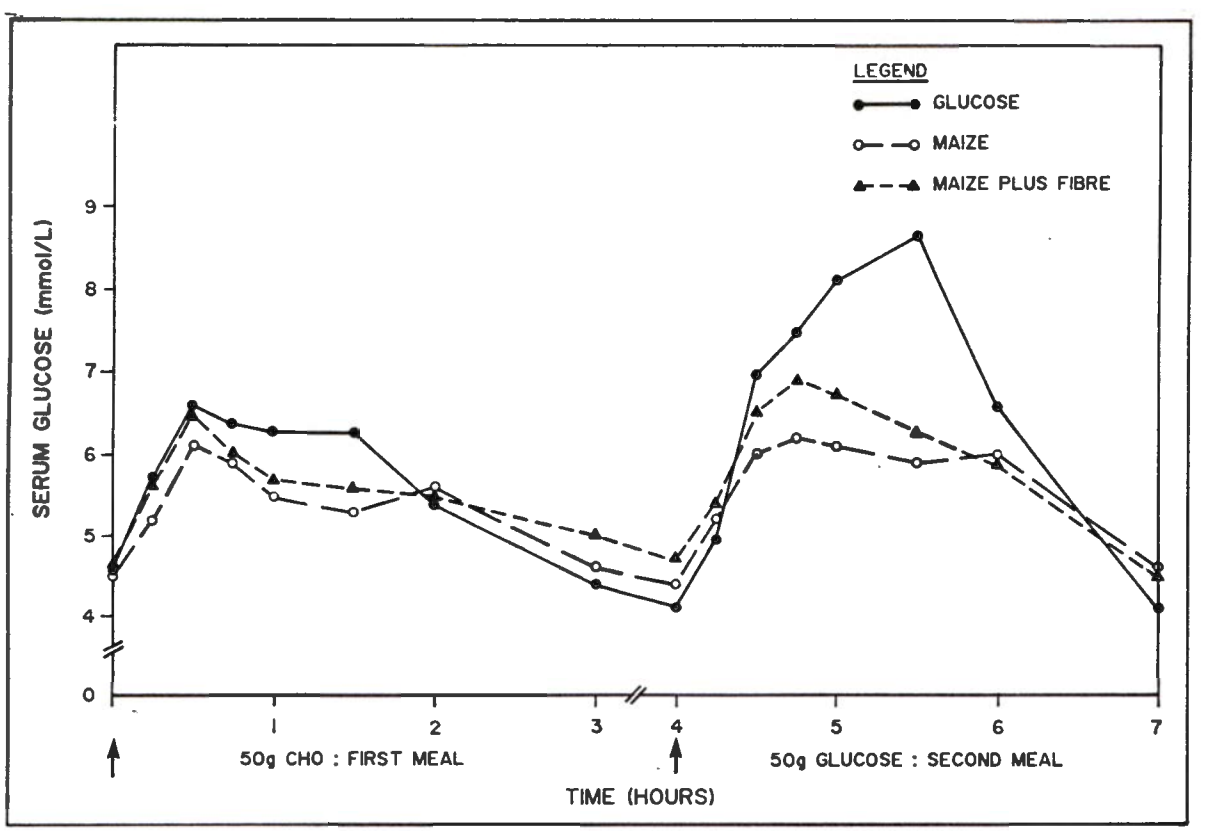

Figure 4.1 : Mean glucose tolerance curves after ingestion of 50g carbohydrate in the form of glucose (•—•) meal A, maize porridge (o--o) meal B, and maize porridge with barley fibre (▲--▲) meal C, during the first and the second meal.

Table 4.2 shows the mean incremental areas under the glucose tolerance curves as illustrated in figure 4.2. There was an increased response during the second meal, compared to the first meal.

TABLE 4.2 : INCREMENTAL AREAS UNDER GLUCOSE TOLERANCE CURVES (MEAN ± SD) (n=6)

INTERVENTION	MEAN (± SD) INCREMENTAL AREA mmol/L/min)	
	FIRST MEAL	SECOND MEAL
GLUCOSE (MEAL A)	336.3 ± 45.7 AB	486.0 ± 113.0 * AB
MAIZE (MEAL B)	190.3 ± 69.4 A	241.0 ± 104.6 A
MAIZE PLUS FIBRE (MEAL C)	195.7 ± 64.1 B	257.3 ± 84.1 B

*** 2 subjects**
AB : Means with the same symbol differs significantly

(ANOVA, P = < 0,005; SAS^R - package)

Figure 4.2 also illustrates a significant difference in incremental areas between meal A (control glucose) and meal B ($p = 0.05$) and between meal A and meal C ($p = 0.05$). There was no significant difference between maize meal porridge (meal B) and barley fibre added porridge (meal C). The figure further indicates that the area under the glucose tolerance curve during the second meal after meals B and C differed significantly from the area when the glucose was taken as first meal (meal A).

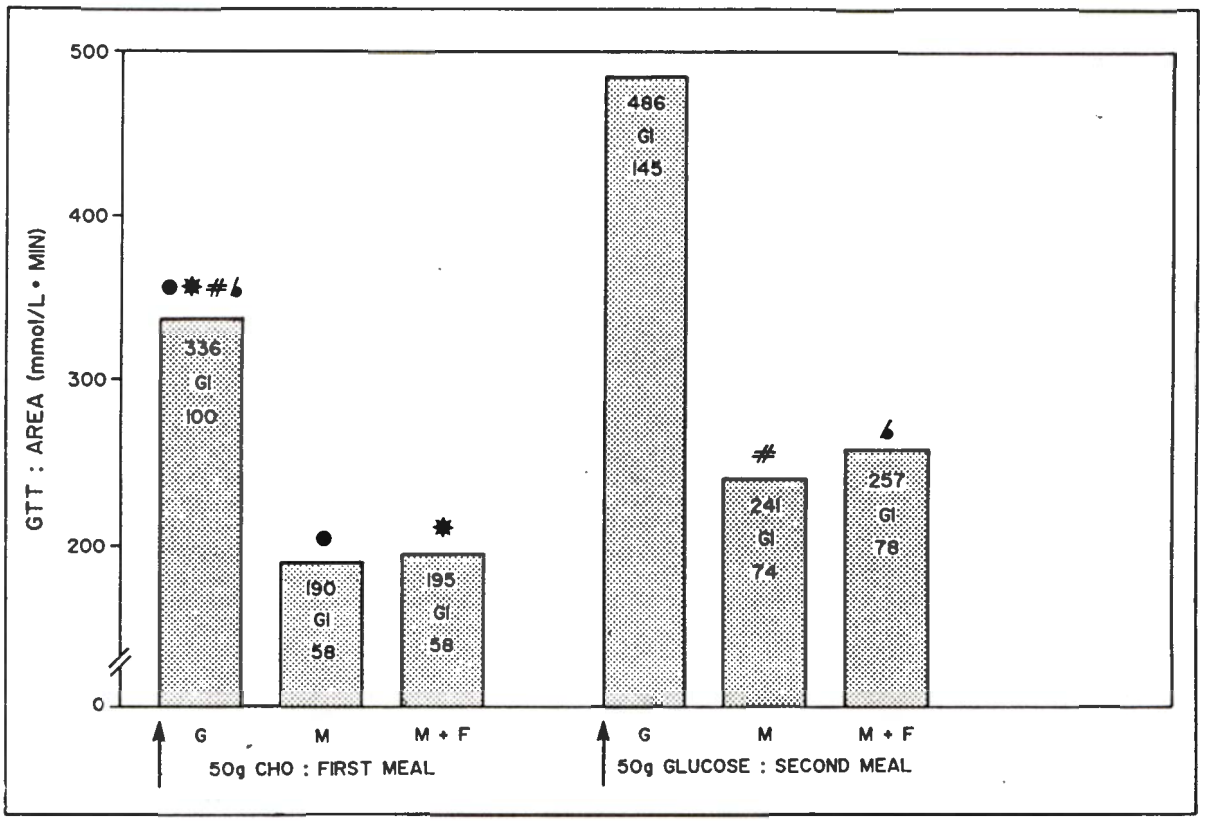

Figure 4.2 : Area under the glucose response curve and the glycaemic index for the first and the second meal.
GTT = Glucose tolerance test M = Maize meal porridge
G = Glucose meal CHO = Carbohydrate GI = Glycaemic index
M + F = Maize meal porridge plus barley fibre
o * # *b* : Means with the same symbol differs significantly

ANOVA, p = < 0,005; (SAS^R package)

Table 4.3 shows the GI of maize porridge with and without barley fibre. The mean GI of maize porridge was 57.7 ± 25.6% and of maize porridge plus fibre 57.5 ± 16.3%. These results compare well with the GI of reheated maize porridge reported by Venter *et al.* (1989) as 55.7 ± 20.5%. The mean values in our study were therefore very similar to those reported in the literature. However, the standard deviation was large. This could be the result of a large inherent individual variation in glycaemic responses.

TABLE 4.3: MEAN (± SD) GLYCAEMIC INDEX (GI) OF MAIZE PORRIDGE WITH AND WITHOUT BARLEY FIBRE AND ITS EFFECT ON THE SECOND MEAL.

INTERVENTION	GI % MEAN	± SD	MIN	MAX	% COEFFICIENT OF VARIATION
Maize : first meal	57.7	25.6	43.2	108.7	44.4
Glucose : second meal*	74.3	38.1	17.6	132.4	51.3
Maize plus Fibre first meal	57.5	16.3	30.4	72.5	28.3
Glucose : second meal*	77.6	26.4	27.4	103.3	34.0

Maize and maize plus fibre as first meal - glucose as second meal

*** Second meal effect was calculated as:**

$$\frac{\text{Mean incremental area of second meal glucose}}{\text{Mean incremental area of first meal glucose}} \times 100$$

The second meal GI was calculated as the mean incremental area of the second meal (glucose) divided by the mean incremental area of the first meal (glucose) multiplied by 100.

When meal B and C were taken as first meal it reduced the GI of the second meal in comparison to glucose (meal A) as first meal. The second meal GI after meal B and C was 74.3 ± 38,1% and 77.6 ± 26,4% respectively, compared to 145 ± 33,6% after meal A.

4.3 INSULIN RESPONSES

Figure 4.3 illustrates the mean insulin response during the three different first meals as well as during the glucose load (second meal). No statistically significant differences were observed. During the first meal glucose gave the largest response. The responses to meal B and C were very similar, which was unexpected because of the reports in the literature (Jenkins *et al.*, 1978; Haber *et al.*, 1977; Albrink *et al.*, 1979) that dietary fibre blunts not only the glucose response, but also the insulin response.

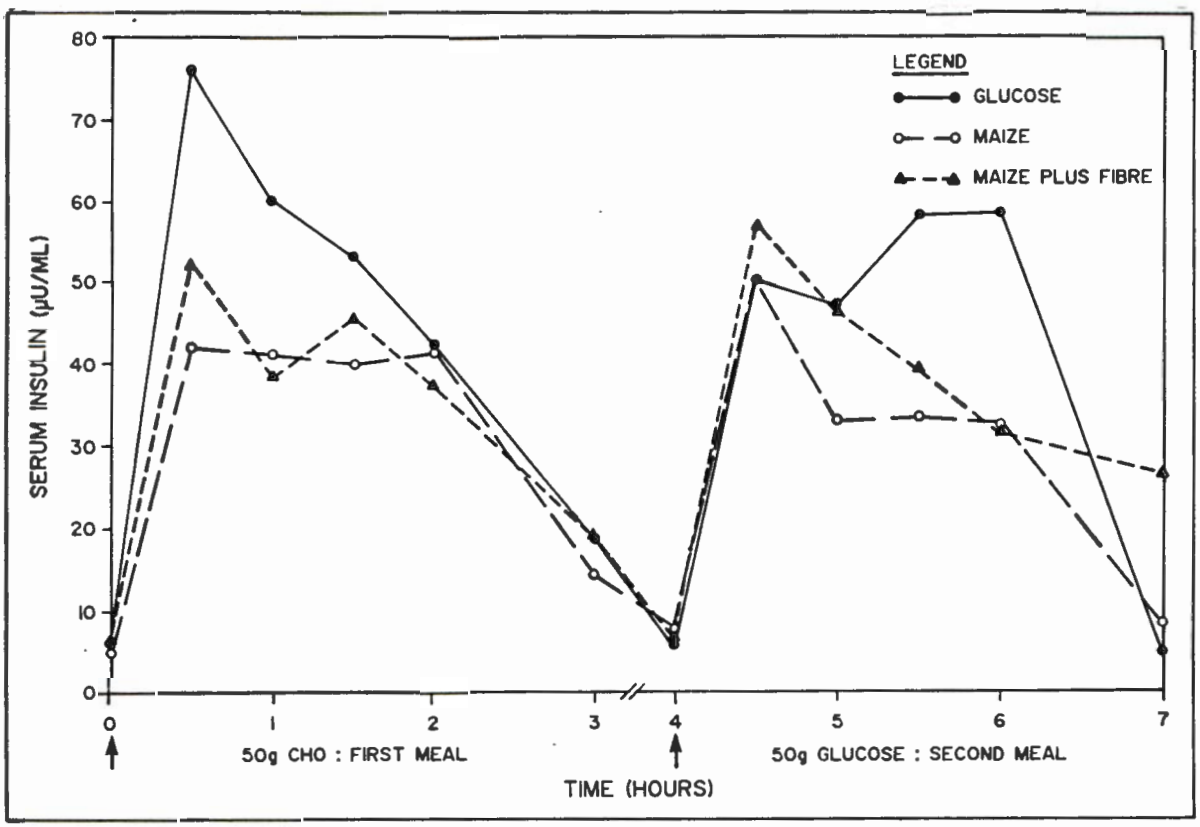

Figure 4.3 : Mean insulin response curves after ingestion of 50g carbohydrate in the form of glucose (•—•) meal A, maize porridge (o--o) meal B, and maize porridge with barley fibre (▲--▲) meal C, during the first and the second meal.

4.4 GLUCOSE-INSULIN PRODUCT

The mean products of serum glucose and insulin levels during the first and second meals are illustrated in figure 4.4. The figure clearly demonstrates that the product was substantially lower during meal B and C than during the glucose load (meal A). It also shows that the first meal influenced the product substantially during the second meal. These differences did not reach significance (ANOVA).

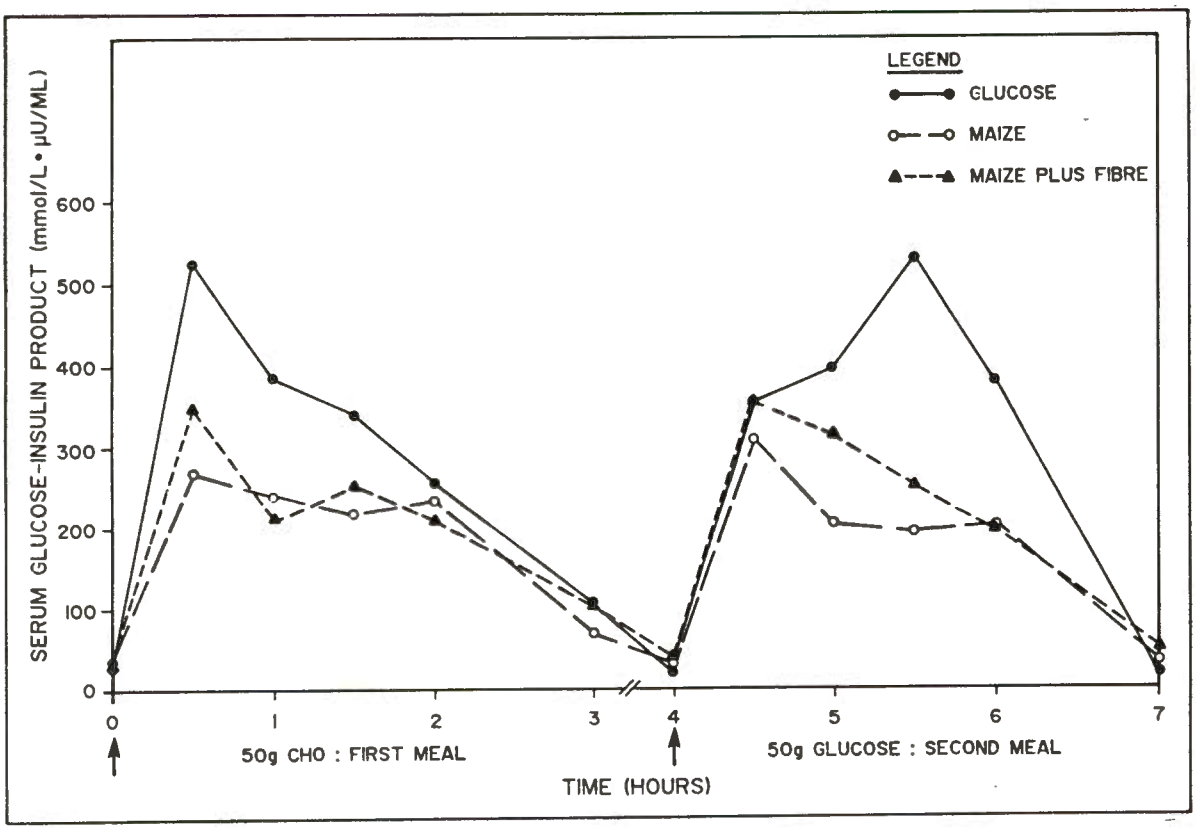

Figure 4.4 : Mean products of serum glucose insulin response curves after ingestion of 50g carbohydrate in the form of glucose (•—•) meal A, maize porridge (o--o) meal B, and maize porridge with barley fibre (▲--▲) meal C, during the first and the second meal.

4.5 GLUCAGON, TRIGLYCERIDES AND FFA LEVELS

Glucagon levels were low following consumption of all the meals and returned to basal levels at four hours as shown in table 4.4. There were no significant differences in the glucagon and growth hormone values between the three meals.

Table 4.4 compares the mean (± SD) of glucose, hormones, FFA and triglyceride profiles during the glucose tolerance test in meal A, B and C at 0, 30, 180 and 240 mins. The glucose incremental area after the different carbohydrate loads are also illustrated in the table.

From Table 4.4 it seems that mean fasting triglycerides levels were in the normal ranges (0.9 mmol/L) and that only small and not significant changes occurred during the glucose (meal) tolerance tests. During meal A and B slight decreases and during meal C slight increases were observed.

Fasting FFA levels were higher when the subjects took meal A and were still higher than levels observed with meals B and C after 4 hours. However, the percentage reduction in FFA levels during meal A was 8.4%, meal B 31.8% and meal C 17.3%, indicating that the maize porridge alone suppressed FFA release during the glucose tolerance test (GTT) more efficiently than glucose (meal A) or maize meal porridge with barley fibre (meal C).

TABLE 4.4 : HORMONAL AND FFA PROFILES DURING GLUCOSE TOLERANCE TEST IN MEAL A, B AND C. MEAN (± SD)

VARIABLE	TIME MINUTES 0		30		180		240		AREA 2	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
MEAL A : GLUCOSE										
Glucose	4.6	0,25	6.6	1,2	4.4	1,3	4.1	0,29		
Area					336	45,7			486	113
Insulin	6.0	2,5	75.9	33	18.6	20,5	5,7	3,2		
Insulin-glucose product	28	13,0	524	288	108	132	24	14		
Glucagon	120	57			99	40	120	54		
Growth Hormone	3.9	3,3	0.65	0,78	8.7	8,9	6.7	9,3		
Free Fatty Acids	609	258					558	277		
Triglycerides	0.9	0,4	0.9	0,37	0.8	0,35	0,8	0.36		
MEAL B : MAIZE										
Glucose	4.5	0,28	6.1	0,99	4.6	0,62	0,68			
Area					190	69			241	105
Insulin	6.0	1,6	42.0	26.5	14.6	12,7	7.6	6,0		
Insulin-glucose product	27	8	271	187	71	67	36	34		
Glucagon	123	48			101	38	107	39		
Growth Hormone	8.8	13.8	1.7	2,3	3.5	3,8	12.7	10.2		
Free Fatty Acids	443	117					302	113		
Triglycerides	0.9	0,39	1.0	0,3	0.8	0,2	0.8	0,2		
MEAL C : MAIZE + FIBRE										
Glucose	4.7	0,36	6.5	0,60	5.0	1,2	4,7	0,71		
Area					196	64			257	84
Insulin	6.9	3,0	52.2	33,3	19.2	13,4	7.7	4,2		
Insulin-glucose product	33	16	350	249	109	95	38	25		
Glucagon	126	63			98	46	114	70		
Growth Hormone	6.33	5,5	0.85	0,71	7.9	11,9	3.85	3,8		
Free Fatty Acids	444	148					367	144		
Triglycerides	0.9	0,4	1,1	0,6	1.0	0,4	1.0	0,4		

Glucose = mmol/L
Glucagon = pg/ml
FFA = umols/L
Insulin = uU/ml
Growth Hormone = uU/ml
Triglycerides = mmol/L

4.6 OTHER HORMONAL RESPONSES

Growth hormone changes over the seven hour period are illustrated in Figure 4.5. The figure indicates that the growth hormone response was suppressed compared to fasting values from 30 min to two hours following all three meals. The highest growth hormone value was observed at four hours after meal B.

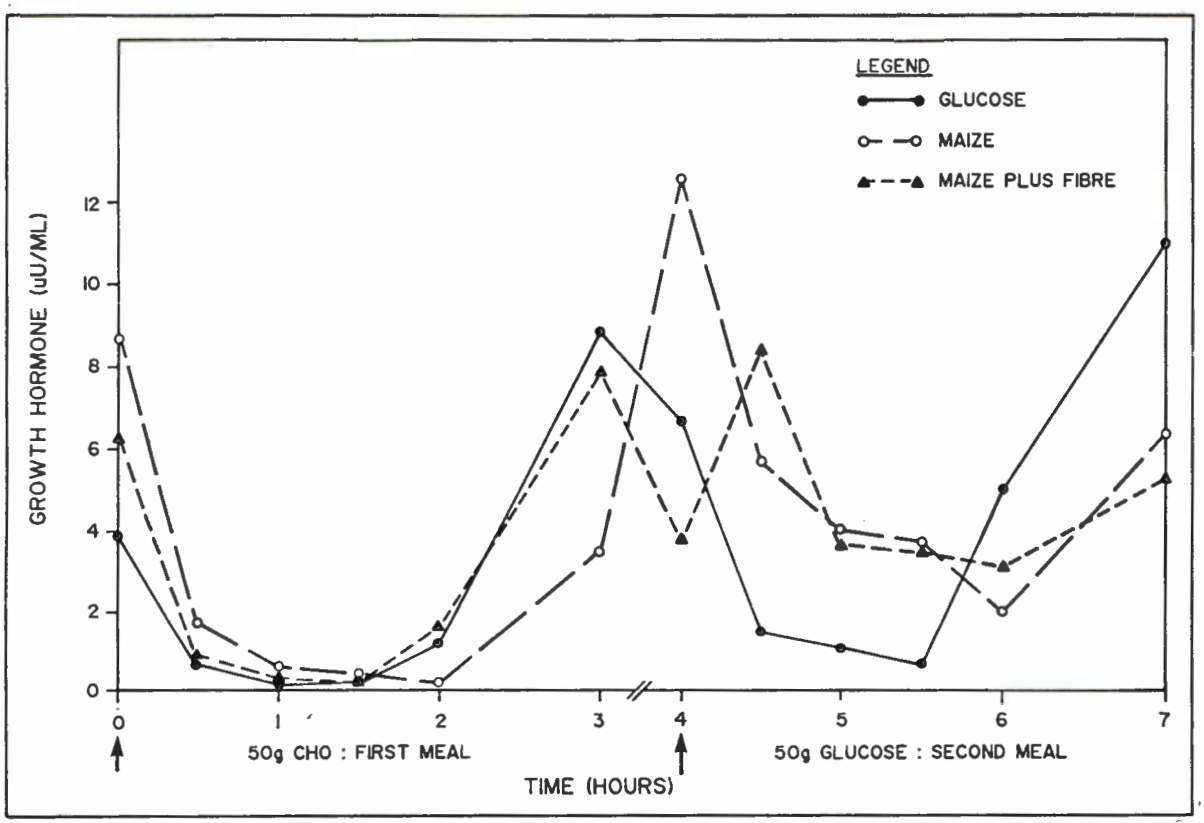

Figure 4.5 : Mean growth hormone response curves after ingestion of 50g carbohydrate in the form of glucose (•—•) meal A,maize porridge (o--o) meal B, and maize porridge with barley fibre (▲--▲) meal C, during the first and the second meal.

Figure 4.6 gives the values of all measured variables immediately before the second meal (glucose in all instances) was given. When glucose was the first meal (meal A), glucose and insulin levels were much lower than with meal B and C at 240 min, while glucagon, growth hormone and FFA levels were lower for meal B and C (with the exception of growth hormone for meal B).

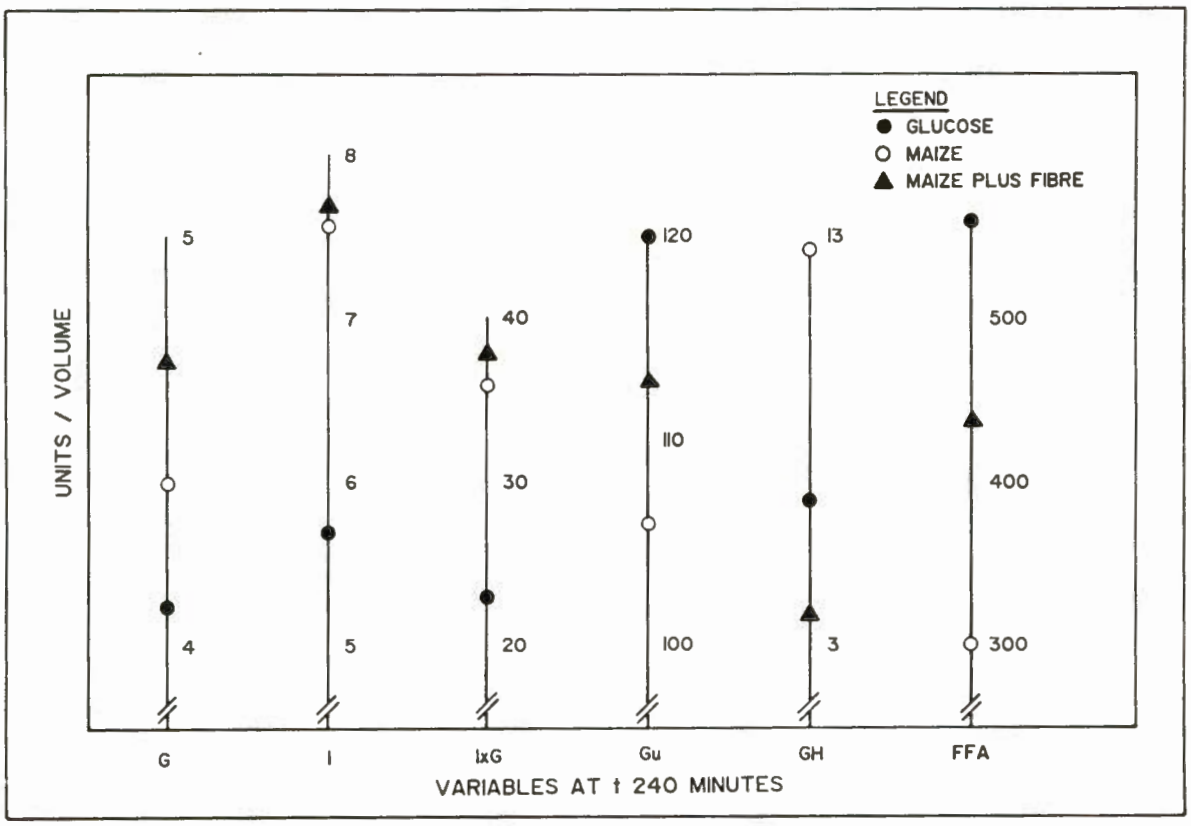

Figure 4.6 : Measured variables at 240 min immediatly before the second meal.
G=Glucose I=Insulin Gu=Glucagon
GH=Growth Hormone IXG=Insulin-Glucose Product
FFA=Free Fatty Acid
•=Glucose o=Maize Porridge ▲=Maize Porridge plus Fibre.

The effect of these variables on the glycaemic response during the second meal was examined by calculating the correlation between the variables and the area under the glucose curve of the second meal and will be discussed below.

TABLE 4.5 : CORRELATIONS BETWEEN VARIABLES MEASURED AT 240 MINUTES AND AREA UNDER THE CURVE DURING THE SECOND TOLERANCE TEST

VARIABLES CORRELATED	CORRELATION		
	r	p	
Glucose X Area 2	- 0.53	0.05	
Glucose X Insulin	0.65	0.004	
Glucose X IXG	0.74	0.0004	
Glucose X Glucagon	- 0.26	0.29	NS
Glucose X Growth Hormone	- 0.33	0.17	NS
Glucose X Free Fatty Acids	- 0.37	0.13	NS
Glucose X Triglycerides	- 0.28	0.26	NS
Area 2 X Insulin	- 0.7	0.005	
Area 2 X IXG	- 0.7	0.005	
Area 2 X Glucagon	0.2	0.50	NS
Area 2 X Growth Hormone	0.15	0.61	NS
Area 2 X Free Fatty Acids	0.80	0.0005	
Area 2 X Triglycerides	0.24	0.40	NS
Insulin X IXG	0.99	0.0001	
Insulin X Glucagon	- 0.11	0.66	NS
Insulin X Growth Hormone	- 0.36	0.14	NS
Insulin X Free Fatty Acids	- 0.60	0.008	
Insulin X Triglycerides	- 0.002	1.00	NS
IXG X Glucagon	- 0.13	0.62	NS
IXG X Growth Hormone	- 0.36	0.14	NS
IXG X Free Fatty Acids	- 0.58	0.01	
IXG X Triglycerides	0.09	0.72	
Glucagon X Growth Hormone	0.20	0.42	NS
Glucagon X Free Fatty Acids	0.001	1.0	NS
Glucagon X Triglycerides	0.16	0.52	NS
Growth Hormone X FFA	0.03	0.92	NS
Growth Hormone X Triglycerides	- 0.30	0.22	NS
FFA X Triglycerides	- 0.14	0.58	NS

NS = Not Significant
Area 2 = Area under the curve during the second meal

IXG= Insulin-glucose product
FFA= Free fatty acid

4.7 CORRELATIONS

Table 4.5 summarizes the correlations between variables measured at four hours (240 min) with the glucose area during the second tolerance test. Correlation between all variables at 240 min are also given. There was a significant correlation between glucose values and insulin values at four hours but no statistical significant correlation between glucose values and glucagon, growth hormone, FFA and triglyceride. No significant correlation was observed between insulin values and the other hormones but a significant negative correlation was seen between insulin and FFA values ($r=-0.6$, $p = 0.008$). Glucagon, growth hormone and triglyceride had no significant correlation with any of the variables.

Insulin showed a negative correlation with area 2 (second meal tolerance) of $r = 0.7$, $p = 0.005$ and with FFA $r = 0.6$, $p = 0.008$. The most significant correlation was seen with FFA and the second meal area ($r = 0.8$, $p = 0.0005$). as shown in Table 4.5.

In summary, the important observations regarding the determinants of the second meal response as seen with correlations between the area under the glucose curve of the second meal and the variables immediately before the meal was given, were : the high and highly significant negative correlations with glucose and insulin and the high significant positive correlation with FFA.

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

The results of this study showed that when the second meal was given to the subjects four hours after the first meal, no Staub-Traugott effect (as defined in the literature) was seen. The SMR was lower when the first meal was maize meal porridge or maize meal porridge with barley fibre, compared to the glucose as a first meal.

The results further indicated that the consumption of cooked, cooled and reheated maize meal porridge, with or without barley fibre, reduced the post-prandial blood glucose and insulin concentrations in healthy subjects, when compared to the same amount of carbohydrate as glucose. Both the porridges elicited a similar GI and SMR. Barley fibre did not reduce the GI or SMR of maize porridge further.

A negative correlation was observed between glucose and insulin and the area under the glucose tolerance curve during the second meal. A positive and statistically significant correlation was found between FFA levels, four hours after the first meal and the area under the glucose tolerance curve during the second meal. The reasons and underlying mechanisms for these observed effects will now be discussed in more detail.

5.2 STAUB-TRAUGOTT EFFECT

The Staub-Traugott effect is defined as an improved or facilitated glucose disposal or utilization of a second glucose (carbohydrate) load taken within one half to three hours after a first glucose load. The effect was first described by Hamman and Hirschman in 1919 (as quoted by Metz and Friedenberg, 1970). The underlying mechanisms has not yet been elucidated, but it seems as if the effect is not dependent on insulin release (Metz and Friedenberg, 1970) nor on plasma FFA and GH levels (Abraira and Lawrence, 1978). In this study no Staub-Traugott effect was present, because the incremental area under the glucose tolerance curves for the second meal was higher than during the first glucose meal, as shown in Table 4.3 and Fig 4.2. The reason for the absence of the Staub-Traugott effect is possibly because the second meal was given at four hours after the initial meal. A Staub-Traugott effect was clearly noted when the second meal was given at three hours following the traditional African meal in black NIDDM patients (Gresse, 1991).

5.3 GLYCAEMIC INDEX

An important observation in this study was the low GI of the maize meal porridges. The consumption of cooked, frozen and reheated porridge resulted in a GI of 58% compared to the 100% of a glucose solution containing the same amount of carbohydrate.

This is in agreement with the results reported by Venter (1990). She showed that cooled maize porridge resulted in significantly lower increments in blood glucose than hot or reheated porridges. The GI of reheated and cooled porridge was 55.7 ± 20.5% compared to the findings of the present study of 57.7 ± 25.6%. In both the studies the calculation of incremental glucose area was with the lowest blood glucose value as baseline.

The low GI of cooked, frozen and reheated maize meal porridge in this study could possibly be ascribed to the presence of retrograded or resistant starch, which could have escaped amylytic digestion in the small intestine (Venter, 1990). Englyst and Cummings (1987b) observed in illeostomy patients that 12% starch was recovered in the illeal effluent of illeostomates when potato was fed cold compared to a 3% recovery of starch when potato was fed hot.

The finding of this study that the addition of barley fibre to the porridge did not change the glycaemic index or the SMR to the porridge, is of interest. These results are in agreement with the data from studies which examined the effect of insoluble fibre on the GI. No significant differences in the GI of white bread and whole meal bread could be found (Wolever *et al.*, 1988). Spagetti produced a smaller rise in blood glucose than either whole meal or white flour bread (Jenkins *et al.*, 1983). Early work of Jefferys (1974), who gave wheat bran with glucose syrup, also showed a very small reduction in glycaemic response in healthy volunteers.

Soluble dietary fibre components are known to reduce the glycaemic response to glucose (Wolever, 1990). Soluble dietary fibres such as pectin and guar gum have a greater effect on carbohydrate metabolism and the glycaemic response than do water insoluble fibres such as wheat bran (Vinik and Jenkins, 1988).

A possible explanation why the addition of barley fibre which contained soluble components, had no effect in lowering the GI of maize porridge any further, could be due to the high amount of retrograded starch in the cooled maize porridge. These porridges were cooked, cooled, frozen, and reheated before eating. The maize starch used in this study is reported to contain 26% amylose and 74% amylopectin (Cronje, 1985). The starches are characteristically insoluble in cold water but produce colloidal sols on heating. On cooling the linear amylose molecules in concentrated solutions form a gel. The latter process is known as retrogradation. During retrogradation solubility of the starch molecule decreases and so does its susceptibility to hydrolysis by acid and enzymes (Sievert and Pomeranz, 1989). Englyst and Cummings (1987b) showed that 12.0% of starch from cold potato resisted digestion compared to 3.0% from hot potato. Cold potato starch is therefore more resistant to digestion than hot potato (Englyst and Cummings, 1987b). Banana starch is also poorly digested, with up to 75% escaping digestion (Englyst and Cummings, 1986), probably because of the crystalline structure of the raw starch granules. Measurement of available carbohydrate from different starchy foods in illeal effluent demonstrated a wide

range of recoveries, from 2.7% to 18%. The available carbohydrate losses correlated well with the in vitro digestibility of the food and its GI (Jenkins *et al.*, 1987d). The maize starch in our study could have also retrograded and escaped digestion. It therefore seems reasonable to suggest that the effect of the resistant starch in the maize porridge on rates of digestion and absorption, was so great that it over-rided the effect of the added barley fibre.

When Syrian Golden hamsters were fed a gallstone provoking diet for six weeks with or without barley fibre, a significantly lower frequency of gallstones were found in animals fed with the barley fibre supplement. Serum and bile concentrations of cholestrol was also lower in the supplemented group (Zhang *et al.*, 1990). Barley fibre may therefore be of importance in a long-term glycaemic control, rather than in an acute glucose response to meals.

5.4 THE SECOND MEAL RESPONSE

Compared to glucose, maize porridge with and without barley fibre elicited a clear, easily detectable SMR. As illustrated in Figure 4.2 when the maize porridges were taken as first meal they reduced the GI of glucose as second meal to 74.3 $\pm$ 38.1% and 77.6 $\pm$ 26.4% respectively, compared to 145 $\pm$ 33.6% when glucose was taken as first meal.

The current hypothesis which tries to explain the second meal effect (Wolever, 1990) is that when carbohydrate absorption is prolonged, there is a smaller tendency for blood glucose levels to undershoot basal levels. This may result in a smaller counter-regulatory response, with a smaller rise in glucagon, growth hormone, catecholamines and FFA secretions and relatively low insulin resistance. This is supposed to lead to an improved glucose disposal after the next meal. The evidence for this hypothesis was obtained from studies where guar gum was used to slow absorbtion of glucose (Jenkins *et al.*, 1980a). When 50g of a glucose solution was taken as a bolus or sipped at an even rate for 3.5 hours (Ocana *et al.*, 1988), or when a breakfast of lentils (a low GI food) or wholemeal bread were followed by a standard bread lunch four hours later (Jenkins *et al.*, 1982), a SMR was observed.

Figure 4.6 gives the values of variables measured immediately before the second meal of glucose was given. When glucose was the first meal serum glucose and insulin levels were much lower than with the porridges at four hours. Glucagon and FFA levels were lower when the porridges were the first meal. Insulin and glucose levels at four hours showed a highly significant negative correlation with the area under the second meal glucose response curve ($r = -0.7$, $p = 0.005$ and $r = -0.53$, $p = 0.05$ respectively). A highly significant positive correlation was obtained between FFA levels at 4 hours and this area ($r = +0.8$, $p = 0.0005$). The results of this study therefore supports the hypothesis which states that if the first meal is slowly digested and absorbed,

which results in plasma insulin and glucose levels not to undershoot the baseline, and lower FFA levels at four hours prior to the second meal, then a SMR will be observed (Wolever 1990; Wolver *et al.*, 1988; Jenkins *et al.*, 1982).

No significant differences were seen in the glucagon and growth hormone levels at four hours, although the growth hormone levels were the highest and glucagon levels were the lowest after consuming maize porridge without the fibre. Glucagon, growth hormone, corticosteroids and adrenaline modify glucose control by stimulating glucose synthesis and accelerating the release and inhibiting the uptake of fatty acids from adipose tissue. Through this action, these hormones also inhibit uptake of glucose by muscle at a particular insulin concentration (Randle *et al.*, 1963). In this study the glucagon and growth hormone at four hours showed no correlation with FFA levels or the second meal area. The data therefore indicates that glucagon and growth hormone were probably not responsible for the SMR. The interpretation of our data is compatible with the concept that an increased plasma FFA level leads to the impairment in glucose utilization (Randle *et al.*, 1963).

5.5 OTHER OBSERVATIONS

A negative correlation between FFA and insulin levels were observed at four hours. Therefore, while insulin levels remained high, FFA release was suppressed. The glucose fatty acid cycle provides a

mechanism independently of hormonal control to maintain a constant plasma glucose concentration (Randle *et al.*, 1963). Insulin enhances glucose uptake in muscle and adipose tissue and inhibits the release of FFA. Several abnormalities of carbohydrate metabolism are associated with high plasma concentrations of FFA. Nestle *et al.* (1964) demonstrated that when FFA in plasma was raised by means of noradrenaline, it impaired the removal of intravenously administered glucose; but when the nor-adrenaline induced rise in FFA was prevented, glucose tolerance improved. In this study, the low FFA levels which correlated negatively with insulin levels prior to the second meal, may be responsible for the improved glucose disposal during the second meal.

5.6 APPLICATION OF RESULTS

Diets that decrease post-prandial hyperglycaemia and hyperinsulinaemia, and which improve the glucose tolerance of the subsequent meal are important for diabetics. These diets may possibly also help non-diabetics to reduce their risk of developing long-term degenerative diseases. Low GI meals which are slowly digested and absorbed from the gastrointestinal tract, facilitate the disposal of glucose absorbed from a subsequent meal (SMR). This study showed that the SMR was present even when the interval between the first and the second meal is more than four hours. Therefore, the concept of the SMR can be utilized in the planning of diabetic diets, especially regarding the frequency and composition of meals.

5.7 RECOMMENDATIONS

This study was done on a homogeneous group of six healthy female volunteers testing only three different meals. If the number of subjects are increased and a larger variety of carbohydrate foods or meals are tested for the SMR, it may give additional results which could be used in the planning of therapeutic diets. It could also provide more information regarding the possible effects of growth hormone and glucagon on the SMR. Our group was possibly too small to obtain significant results on these two hormones.

From the literature review it is clear that much more is to be learned in terms of reducing the GI of carbohydrate containig foods or meals and their effects on physiological functions. Various methods could be applied to lower the GI of a meal, such as addition of soluble fibres, (Jenkins *et al.*, 1980) protein, (Collier *et al.*, 1987), antinutrients such as phytates, lectins or amylase inhibitors to the carbohydrates (Thorne *et al.*, 1983), reducing the cooking time, (Brand *et al.*, 1983), selecting starches containing high amylose contents such as legumes (Thorne *et al.*, 1983), and avoiding refining and least disrupting the organized starch granules (Heaton *et al.*, 1988). Legumes exemplify a class of food, high in fibre, protein and antinutrients with a starch which is slowly digested. They produce relatively small blood glucose rises after consumption by both healthy and diabetic subjects (Thorne *et al.*, 1983; Jenkins *et al.*, 1983). Legumes also improve long-term metabolic control in diabetics (Anderson *et al.*,

1979). Consumption of resistant and partially resistant starch in the form of cooked and cooled starch (Englyst and Cummings, 1978b; Venter, 1990) and unripe bananas (Englyst and Cummings, 1986; Wolever *et al.*, 1988a) could also lower the GI and improve the SMR.

Identification of more foods which could lower the GI of meals and understanding of the factors determining starch digestibility will possibly allow a greater therapeutic use of diet in the management of disorders of carbohydrate metabolism.

CHAPTER - 6

CONCLUSION

This study was designed to:

* determine the glycaemic index of maize porridge with and without added barley fibre.
* examine the effect of the composition of these maize porridges as a first meal on the glycaemic response to a second meal (glucose load) given four hours after the first meal.
* to study the underlying mechanisms responsible for the second meal response to maize porridge.

The results of this study clearly indicated that the consumption of the cooked-cooled-frozen and then reheated maize porridge resulted in low blood glucose and insulin responses. The FFA level at four hours was lowest for maize meal porridge and highest for a glucose solution. There was also a positive correlation ($r = +0.8$, $p = 0,0005$) between FFA levels at four hours and the area under the glucose response curve for the second meal.

The results obtained support the hypothesis of Wolever (1990) and Jenkins *et al.* (1982) that a low GI meal will lead to raised glucose and insulin and lowered FFA levels at four hours after the meal. This will lead to an improved glucose disposal of the subsequent meal, named the second meal response (SMR). From the literature review and our observations we can conclude that the

blood levels of glucose, insulin and FFA prior to the intake of the second meal had an effect on the second meal glucose tolerance.

Another main observation was that barley fibre had no effect on the GI of maize porridge, possibly because the amount of soluble fibre components in the barley fibre was too small. There is still a possibility that the addition of barley fibre to maize porridge may have long-term beneficial effects.

The results of this study has led to a better understanding of the short-term effect of cooked-cooled-frozen and reheated maize porridge on post-prandial carbohydrate tolerance and its effect on subsequent meals.

Maize meal porridge is the staple in the diet of black Africans. It is acceptable, inexpensive and also easily available. Provided that maize meal porridge is supplemented with the correct types and amount of foods to ensure an adequate balance in the diet (such as legumes, fruits, vegetables, milk and some animal protein foods) it forms an important part of the diet of many South Africans. Black NIDDM patients are clearly in a process of Westernization (Gresse, 1991). These patients can be motivated to revert back to their traditional eating habits. In rural areas maize porridge is often cooked once a day and then consumed cold throughout the day. As seen above, this practice may contribute to the beneficial effects of maize porridge.

The concept of the glycaemic index was developed using data mainly obtained as the first meal of the day. Very little research is done on the potential effect of the first meal on subsequent meal responses. A better understanding of the effects of food preparation and meal composition on post-prandial glucose responses and handling of subsequent meals is needed.

This study indicated that blood levels of glucose, insulin and especially FFA prior to the ingestion of the second meal, determined to a large extent glycaemic response to this meal. More research is needed to examine if this is also the case in diabetic patients. Because the concept that the composition of a first meal will influence the body's response to a subsequent meal will find its greatest application in the dietary treatment of diabetic patients, it is concluded that this study should be repeated in these patients.

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