

**Assessment of β -cell function and insulin
sensitivity in established Non-insulin
dependent diabetes mellitus - the influence
of diet and sulphonylurea therapy**

By

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University of Leicester

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Assessment of β -cell function and insulin sensitivity in established NIDDM - the influence of diet and sulphonylurea therapy.

MD Submission - September 1995

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Abstract

This thesis reports on studies validating a modification of 'minimal' model analysis of the FSIVGTT to measure insulin resistance specifically in NIDDM subjects and its use along with a mixed meal test incorporating measurement of specific insulin and proinsulin concentrations to quantify changes in β -cell function and insulin resistance following two years of diet or sulphonylurea treatment in established NIDDM.

All NIDDM subjects displayed severe β -cell dysfunction (post-prandial insulinopenia and hyperproinsulinaemia) and insulin resistance when compared to age and sex matched normals. Therapeutic interventions failed to normalise either of these abnormalities. Dietary therapy resulted in improved glycaemic control, weight loss and improved insulin sensitivity but most especially improved β -cell function (increased post-prandial insulin secretion, reduced proinsulin concentrations) at the one year assessment. Two years post-diagnosis post-prandial proinsulin concentrations continued to fall whilst insulin concentrations mirrored those at the time of presentation. Sulphonylurea therapy also resulted in improved glycaemic control but with significant weight gain. Insulin sensitivity tripled over the two year period and β -cell function also improved after initial increases in both post-prandial insulin secretion and proinsulin concentrations at the one year assessment

Reduced 'glucose toxicity' appeared to be a major factor affecting the changes in the measured parameters in both groups of subjects. For diet treated individuals, it is suggested that this reduction rapidly maximises β -cell function and insulin sensitivity to a predetermined level. Maintenance of glycaemic control subsequently is dependent of factors more difficult (diet, exercise, weight) or impossible to control (time). For sulphonylurea treated subjects, reduction in 'glucose toxicity' was important in improving β -cell function and insulin sensitivity but the drugs themselves exerted an independent effect, especially on sustained increases in insulin secretion. It is suggested that the continuous use of sulphonylureas may play a causal role in the ultimate deterioration in glycaemic control frequently seen in patients who initially appear to benefit from their effects.

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Glossary of abbreviations

ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
CIGMA	Continuous infusion of glucose with modelling analysis
CV	Coefficient of variation
DAG	Diacylglycerol
DHAP	Dihydroxyacetone phosphate
ELISA	Enzyme linked immunoassay
FAD	Flavin adenine dinucleotide
FFA	Free fatty acids
FI	Fasting insulin (ELISA)
FPG	Fasting plasma glucose
FPI	Fasting proinsulin
FSIVGTT	Frequently sampled intravenous glucose tolerance test
GLP-1	Glucagon-like peptide - 1
GLUT(n)	Glucose transporter (n = 1-5)
GTP	Guanosine triphosphate
HbA _{1c}	Glycosylated haemoglobin
HDL	High-density lipoprotein
HGO	Hepatic glucose output
HOMA	Homeostasis model assessment
IDDM	Insulin dependent diabetes mellitus
IGF-1	Insulin-like growth factor-1
IGF-2	Insulin-like growth factor-2
IGT	Impaired glucose tolerance
IP3	Inositol triphosphate

IRAS	Insulin resistance and atherosclerosis study
IRI	Immunoreactive insulin
IRS-1	Insulin receptor substrate-1
IVGTT	Intravenous glucose tolerance test
LDL	Low-density lipoprotein
MAP	Mitogen-associated protein
MODY	Maturity onset diabetes of the young
MTT	Meal tolerance test
NAD	Nicotinamide adenosine dinucleotide
NEFA	Non-esterified fatty acids
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerance test
PGE2	Prostaglandin E2
PI 3-K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol biphosphate
PKA	Protein kinase A
PKC	Protein kinase C
RE	Residual error
SD	Standard deviation
SE	Standard error
SFU	Sulphonylurea
S _G	Glucose effectiveness
S _I	Insulin sensitivity index
SITT	Short insulin tolerance test
TG	Triglyceride

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Chapter 1 - Introduction

1.1 Epidemiological aspects of Non-insulin dependent diabetes mellitus

Diabetes mellitus is a major world-wide public health problem. In the United Kingdom it is a common disease, affecting up to 2% of the population and accounting for at least 4% of the National Health Service budget [Higginson,1994], much of which (approximately 80%) relates to the cost of complications of the disease [Williams,1985]. Diabetes related problems account for 3% of all hospital admissions and acute bed occupancy rates are 6 times those of non-diabetic individuals [Williams,1985]. Of the affected individuals, at least 75% suffer from 'non-insulin dependent diabetes mellitus' (NIDDM), a disease characteristically of middle aged and elderly people [Gatling et al,1985], with a particularly high incidence amongst certain ethnic groups (e.g. Afro-Caribbeans, Asians) [West,1978; Mather et al,1985; Forrest et al,1986]. As a result of the changing socio-economic structure of Westernised populations, the prevalence of NIDDM is in the region of 10% in those over the age of 60 years and it is likely that this estimate will increase with time.

Since the recognition of NIDDM as a clinical entity [Himsworth,1936], it has traditionally been thought to be a 'mild' form of diabetes [Gill,1986] however morbidity and mortality from the common complications of NIDDM - macrovascular disease, retinopathy and nephropathy - is high and rising with the increased life expectancy of Western populations [Pirart,1978; Panzram,1987] (see Table1). In population terms this leads to significant and increasing demands on health care resources.

Mortality from macrovascular disease in NIDDM is 2-3 times higher than that in non-diabetic individuals with an average reduction in life-expectancy of 5-10 years [Goodkin,1973; Panzram,1987]. In addition, morbidity from microvascular complications particularly retinopathy and nephropathy is increasingly common with currently 30% of end stage renal disease secondary to diabetes of which 50% is due to NIDDM [Wood,1985].

Table 1.1. Causes of death in NIDDM subjects

Cause of death	%	
Vascular disease	59.4	
Ischaemic heart disease		34.7
Cerebrovascular disease		22.0
Peripheral vascular disease		2.7
Renal disease	2.9	
Metabolic emergencies	3.1	
Infections	7.6	
Malignancies	10.0	
Accident/Suicide	2.1	
Others	11.4	
Unknown	3.4	
Total	100	

Adapted from Panzram, G (1987). Diabetologia 30:123-131

1.2 Determinants and Risk Factors for NIDDM

1.2.1 Age

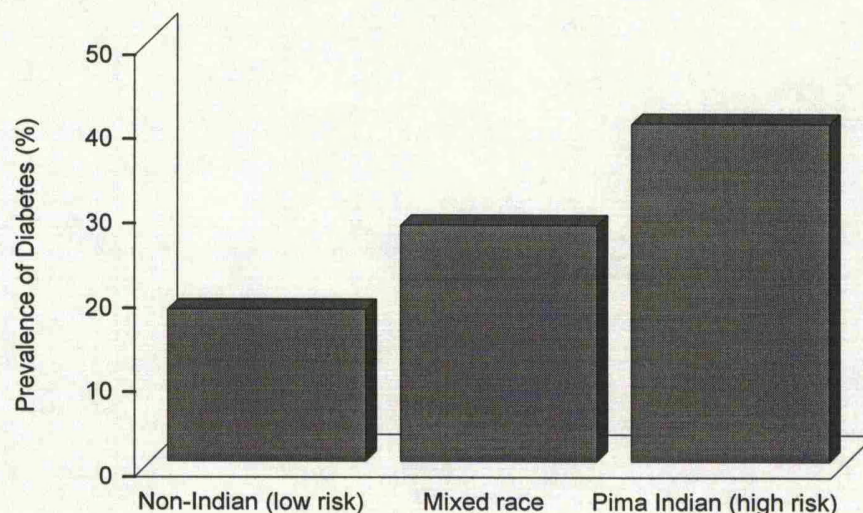
Non-insulin dependent diabetes mellitus typically affects middle-aged to elderly individuals although an identical disease occurring in young adults

(maturity onset diabetes of the young - MODY) secondary to a mutation in the glucokinase gene is recognised [Tattersall,1974].

1.2.2 Genetic determinants

The disease has a strong genetic element with approximately 40% of individuals with NIDDM having a family history of the disease. The risk is also apparently increased if the affected parent is the mother [Alcolado et al,1991]. The genetic basis to the disease is supported by twin studies in which concordance rates between 40 and 100% have been reported in monozygotic (identical) twins [Barnett et al,1981; Newman et al,1987]. In addition the prevalence of NIDDM in 'hybrid' populations i.e. the interbreeding of populations with extremes of susceptibility to NIDDM can be shown to be intermediate (see Figure 1.1).

Figure 1.1. Prevalence of NIDDM in 'hybrid populations



Adapted from: Alberti KGMM, DeFronzo RA, Keen H, Zimmet P. (Eds). The International Textbook of Diabetes. Chichester: John Wiley and Sons, 1992

Different ethnic groups sharing the same environment also show variations in the prevalence and incidence of the disease [Poon-King et al,1968; Mather et al,1985].

1.2.3. Environmental determinants

Metabolic programming

In recent years the concept of 'metabolic programming' has gained in popularity. This concept stems from the results of a number of studies on individuals with hypertension, ischaemic heart disease and NIDDM [Barker et al,1986; Barker et al,1990; Hales et al,1991; Barker et al,1992] in which birth weight was found to be inversely related to the development of the disease in adult life. As birthweight is intimately linked with the intra-uterine environment, the potential for adverse intra-uterine events resulting in 'programmed' development of a range of metabolic diseases has been suggested. In the case of NIDDM metabolic programming forms the basis of the 'thrifty phenotype' hypothesis of the causation of the disease [Hales et al,1992] (vide infra).

Obesity

At least 60% of individuals with NIDDM are obese at the time of diagnosis. The association between obesity and diabetes has been recognised for centuries [West,1978] however a causal relationship between the two has not been demonstrated in man. Many patients with NIDDM are obese but only a small proportion of the obese develop NIDDM whilst a significant number of the non-obese also develop the condition. Importantly, a number of studies in different ethnic groups e.g. Israelis [Modan et al,1986], Caucasian North

Americans [Wilson et al,1981], and Pima Indians [Knowler et al,1981] stress the relationship of *previous* obesity to the development of the disease. In addition, the distribution of the obesity has been shown to relate to the incidence of NIDDM with central (android) obesity significantly associated with the development of the disease [Ohlson et al,1985; Lemieux et al,1994].

Exercise

The level of physical exercise taken by an individual may also be linked to the susceptibility for the development of NIDDM. In at least one study the incidence of the development of NIDDM was inversely related to the degree of physical exercise undertaken historically [Helmrich et al,1991]. The protective effect of exercise was seen predominantly in those individuals deemed at 'high risk' of developing NIDDM i.e. those with a positive family history of the disease with concomitant hypertension and/or obesity.

Diet

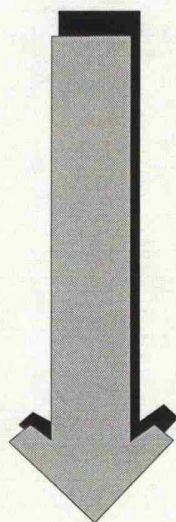
Diet may also be an important environmental risk factor for NIDDM, although its effects are difficult to define in a case-control approach as diet is a major part of the therapy of NIDDM. One study [Bennett et al,1984] demonstrated that excessive food intake, especially of carbohydrates and fat was positively related to the incidence of NIDDM. This study took place in Pima Indians, a racial group with an extraordinarily high incidence of NIDDM and therefore its conclusions may not be relevant to other NIDDM population groups. Other studies lend circumstantial evidence e.g. the prevalence of NIDDM in the general Japanese population is relatively low, however the prevalence amongst retired Sumo wrestlers is up to 40%. During their years of training they consume up to 6500 kilocalories per day compared to 2500 kilocalories

per day for the average citizen [Irie et al,1983]. In this group of individuals therefore, the development of NIDDM may be related to the combination of continuing high energy intake in the face of decreased exercise levels

The influence of diet and exercise may also help to explain the effect of migration on the prevalence of NIDDM in a variety of different ethnic groups. For example for certain Asian groups in the UK the prevalence of NIDDM is up to 5 times greater than that in India [Mather et al,1985], whilst Japanese Americans living in Hawaii have twice the prevalence of NIDDM found in Japan [Kawate et al,1979]. A model summarising the theoretical role of genes and the environment on the development of NIDDM is shown in Figure 1.2.

Figure 1.2. The possible role of genes and the environment in the pathogenesis of NIDDM

Normal - Predisposed



Initiation
Progression

Genes	Environment
Primary "diabetogenes" Diabetes-related genes - Primary (e.g. obesity)	Toxins
Diabetes-related genes - Secondary e.g. Diabetes-induced	Diet Activity

Diabetes mellitus

1.3 The pathophysiological basis of NIDDM

1.3.1 Normal physiology

Postabsorptive state

The postabsorptive (fasting or 'basal') state refers to the period of time after a meal when plasma glucose concentrations are at a near steady-state i.e. the rate of glucose utilisation is matched by the rate of appearance into the circulation. During this time virtually all the glucose entering the circulation is derived from glycogenolysis in the liver [Dietze et al,1976] with gluconeogenesis contributing during prolonged fasting [Rothman et al,1991] supported by a relatively small contribution from renal gluconeogenesis [Owen et al,1969]. A variety of three-carbon precursors including lactate, alanine and glycerol provide the main precursors for gluconeogenesis. Lactate and alanine are produced as a result of glucose metabolism, mainly in skeletal muscle [Felig,1973] whereas glycerol is derived from low grade lipolysis occurring during fasting.

Glucose uptake and utilisation in the fasting state is mainly insulin-independent and occurs in erythrocytes, neural and splanchnic tissues, accounting for up to 80% of the glucose utilisation [Ferrannini et al,1989]. The major insulin-dependent tissue responsible for glucose metabolism is skeletal muscle however little glucose is metabolised by muscle in the postabsorptive state because of the low circulating insulin concentrations [Andres et al,1956]. The main fuels utilised by skeletal muscle during fasting are free fatty acids (FFAs) [Dagenais et al,1976].

Fuel availability in the postabsorptive state is controlled by the relationship between insulin and glucagon secretion. Low circulating concentrations of insulin allow sufficient FFAs to be generated to maintain muscle metabolism and have a permissive effect on hepatic gluconeogenesis and glycogenolysis. The relatively high concentrations of glucagon stimulate these hepatic processes, providing fuel for the insulin-independent tissues.

Postprandial state

Glucose from ingested food typically appears in the systemic circulation within 5-10 minutes and up to 40% is extracted by the liver and splanchnic tissues [Firth et al,1986a]. The elevation of the glucose concentration in the portal circulation results in the secretion of insulin and suppression of glucagon release. The increasing portal concentrations of insulin suppress hepatic glycogenolysis and thus reduce hepatic glucose output (HGO), leading to maximal suppression of HGO usually 2 hours after a meal [Ferrannini et al,1985]. Increasing concentrations of insulin also inhibit lipolysis and stimulate glucose uptake by skeletal muscle. Of the glucose taken up by muscle, $\approx 50\%$ is completely oxidised; $\approx 35\%$ is converted into glycogen and $\approx 15\%$ is released as lactate and alanine which subsequently become available for hepatic gluconeogenesis [Kelley et al,1988]. Glucose plays a vital role in its own disposal by both increasing its own uptake in peripheral tissues especially the central nervous system [Edelman et al,1990], and inhibiting further glycogenolysis and gluconeogenesis in the liver [Sacca et al,1978].

Systemic insulin concentrations following a meal are not the result simply of β -cell stimulation by increasing plasma glucose concentrations. A number of gut-derived hormones e.g. cholecystokinin, gastrointestinal inhibitory polypeptide, glucagon-like peptide-1-(7-37) [Porte Jr,1991; Nathan et

al,1992], and amino acids e.g. arginine [Floyd Jr et al,1968], contained within foodstuffs potentiate insulin secretion. Thus insulin secretion in the post-prandial state occurs as a result of both glucose and non-glucose secretagogues.

1.3.2 Carbohydrate metabolism in NIDDM

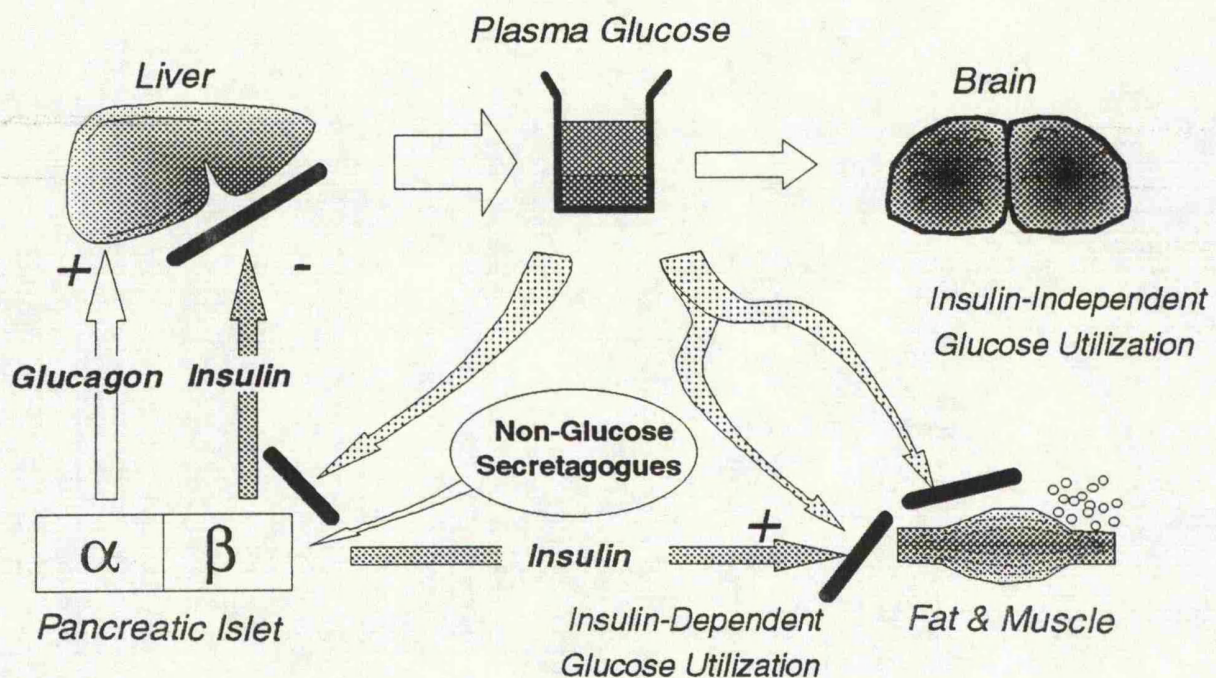
Postabsorptive state

The postabsorptive state in NIDDM is characterised by hyperglycaemia with both the production and utilisation of glucose increased in proportion to the fasting plasma glucose [Bogardus et al,1984a; Firth et al,1986a]. Hepatic production of glucose is said to be moderately increased in severely hyperglycaemic NIDDM subjects [DeFronzo et al,1987; Golay et al,1988b] and tissue disposal of glucose also increases to a level approximating production to allow a new steady-state to emerge. The mechanism by which increased HGO occurs in the postabsorptive state is not clear but at least three factors could contribute. Firstly, NIDDM is known to be associated with hyperglucagonaemia and increased hepatic sensitivity to the effects of glucagon would result in increased HGO [Unger et al,1970]. Secondly, the increased circulating FFA concentrations in the fasting state in NIDDM are known to fuel increased hepatic gluconeogenesis [Nurjan et al,1992]. Thirdly, increased disposal of glucose by skeletal muscle in the postabsorptive state has been shown to result in increased delivery of alanine and lactate to the liver with resultant increased gluconeogenesis - the so-called 'glucose paradox' [Consoli et al,1990].

Postprandial state

The ingestion of carbohydrates in subjects with NIDDM leads to a characteristic prolonged elevation of plasma glucose concentrations - glucose intolerance. This is almost entirely a result of the inadequate insulin secretion and action characteristic of NIDDM. There is impaired suppression of HGO in the postprandial state in NIDDM which in combination with the already mildly elevated basal HGO results in a markedly increased release of glucose from the liver following ingestion of a meal or glucose [Firth et al,1986a] (see Figure 1.3).

Figure 1.3 Postprandial glucose metabolism in NIDDM



Adapted from Kahn, CR. Diabetes:43, 1066-1084, 1994

There are several potential reasons for the impairment in suppression of HGO in the postprandial state. It is clear that the early phase ('first phase') of insulin release is essential for subsequent normal carbohydrate tolerance and that its function may be the priming of insulin sensitive tissues including the liver [Bruce et al,1988]. In subjects with NIDDM the first phase of insulin release is characteristically deficient [Perley et al,1967] and consequently this priming effect is lost. Additionally, studies in subjects with NIDDM have repeatedly shown that the liver is relatively resistant to the effects of insulin in suppressing HGO.[Firth et al,1986c; Ferrannini et al,1988].

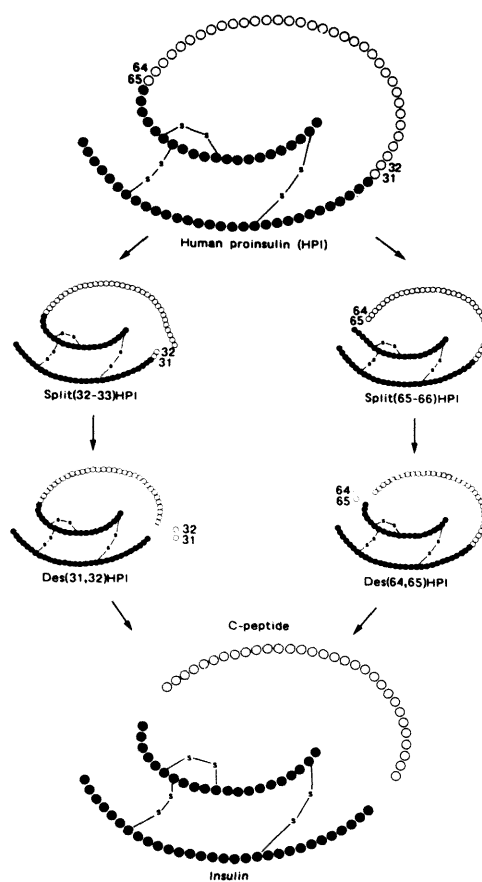
Hyperglycaemia itself is also known to inhibit HGO [DeFronzo et al,1982a] and as such NIDDM may represent a state of partial glucose resistance. Finally, glucagon secretion continues in the postprandial hyperglycaemic state supporting continuing gluconeogenesis [Butler et al,1991].

Failure to suppress HGO is estimated to account for approximately one-third of the defect in postprandial glucose homeostasis found in NIDDM [DeFronzo,1992]. The remaining two-thirds is accounted for by a defect in skeletal muscle glucose uptake and metabolism felt to represent one of the major metabolic defects in NIDDM and this will be addressed in greater detail later.

1.4 Insulin

Insulin is a polypeptide hormone secreted by the β -cells of the pancreatic Islets of Langerhans. Transcription of the insulin gene leads to synthesis of preproinsulin (an 11.5 kiloDalton (kDa) polypeptide) on the membrane of the rough endoplasmic reticulum. Cleavage to proinsulin (a 9kDa polypeptide)

Figure 1.4. Insulin biosynthesis



and discharge into the cistern of the rough endoplasmic reticulum follows. Proinsulin consists of the complete insulin molecule (A and B chains) along with C-peptide which facilitates folding of the chains and their linkage by disulphide bonds [Steiner et al,1972]. Proinsulin is transported to the Golgi apparatus and packaged into vesicles. During vesicle maturation, protease cleavage of proinsulin by proinsulin endopeptidases [Davidson et al,1988] results in equimolar quantities of C-peptide and insulin with insulin precipitating in the presence of zinc to form dimers and hexamers [Howell et al,1969]. Figure 1.4 shows the conversion of proinsulin to insulin in a schematic fashion.

1.4.1 Insulin secretion

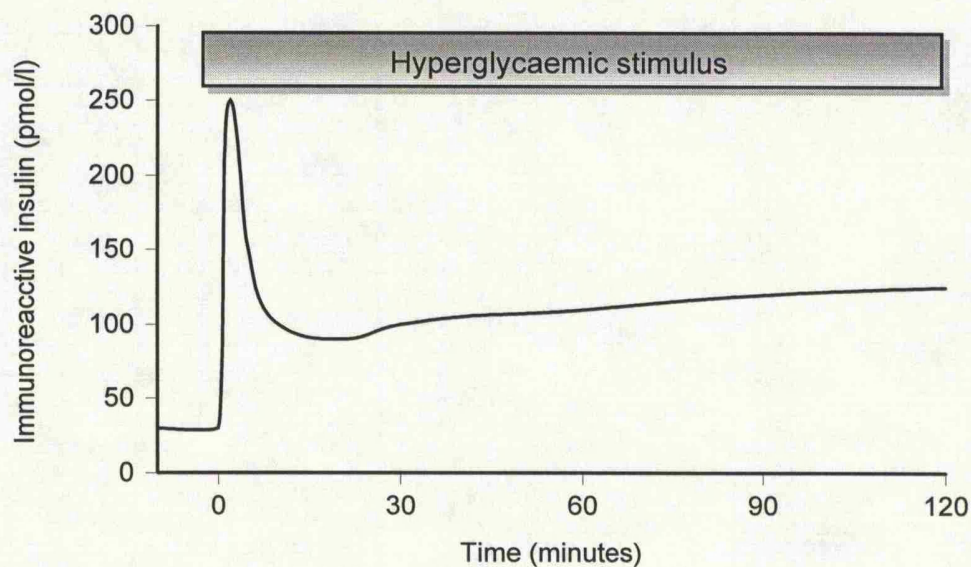
Insulin is secreted in response to specific secretagogues but in common with many other hormones has a background independent pulsatility to its secretion. Basal pulsatile insulin secretion has a periodicity of approximately 13 minutes with 10-15 superadded larger pulses throughout the day, not necessarily related to the presence of secretagogues [Lang et al,1979].

Although the main regulator of insulin secretion is the plasma glucose concentration [Hedsekov,1980] a number of other moieties are able to either 'initiate' insulin secretion, or 'potentiate' the release of insulin stimulated by glucose or an 'initiator'. The release of a number of gut-derived hormones ('incretins'), is stimulated by ingested glucose. These hormones have a direct stimulatory effect on the β -cell leading to acute increases in plasma insulin concentrations when administered in-vivo and in-vitro and a *potentiating* effect on insulin secretion brought about by previous glucose stimulation [Porte Jr,1991]. Additionally, a number of amino-acids, of which arginine appears the most potent, act in the same way [Floyd Jr et al,1968].

The study of insulin release to glucose via an oral glucose challenge is therefore complicated by the secretion of other gut hormones and may be influenced by other factors such as transit time and absorption rate. In an attempt to avoid these confounding factors, insulin secretion is frequently investigated by the use of an intravenous glucose challenge.

Insulin secretion in response to intravenous glucose occurs in two phases. The first phase (see Figure 1.5) begins within 50-100 seconds of the plasma glucose rising above 5.5 mmol/l and persists for 5 to 10 minutes, followed by a second-phase of insulin secretion of lower magnitude which is maintained as long as the hyperglycaemic stimulus persists [Curry et al,1968].

Figure 1.5. Insulin responses to continuous intravenous glucose in normal subjects



The secretion of insulin is usually an exocytic process involving active transport of secretory granules to the β -cell surface (see Figure 1.6), fusion with the cell membrane and extrusion of contents (insulin and C-peptide) on an equimolar basis - the regulated pathway [Rhodes et al,1987]. Secretion of insulin can however occur via a constitutive pathway i.e. without prior granule maturation, however secretion in this manner appears to be limited to a small number of subjects with insulinomas and is not a feature of insulin secretion in NIDDM subjects [Nagamatsu et al,1992].

Figure 1.6. Insulin granule biogenesis, sorting and release

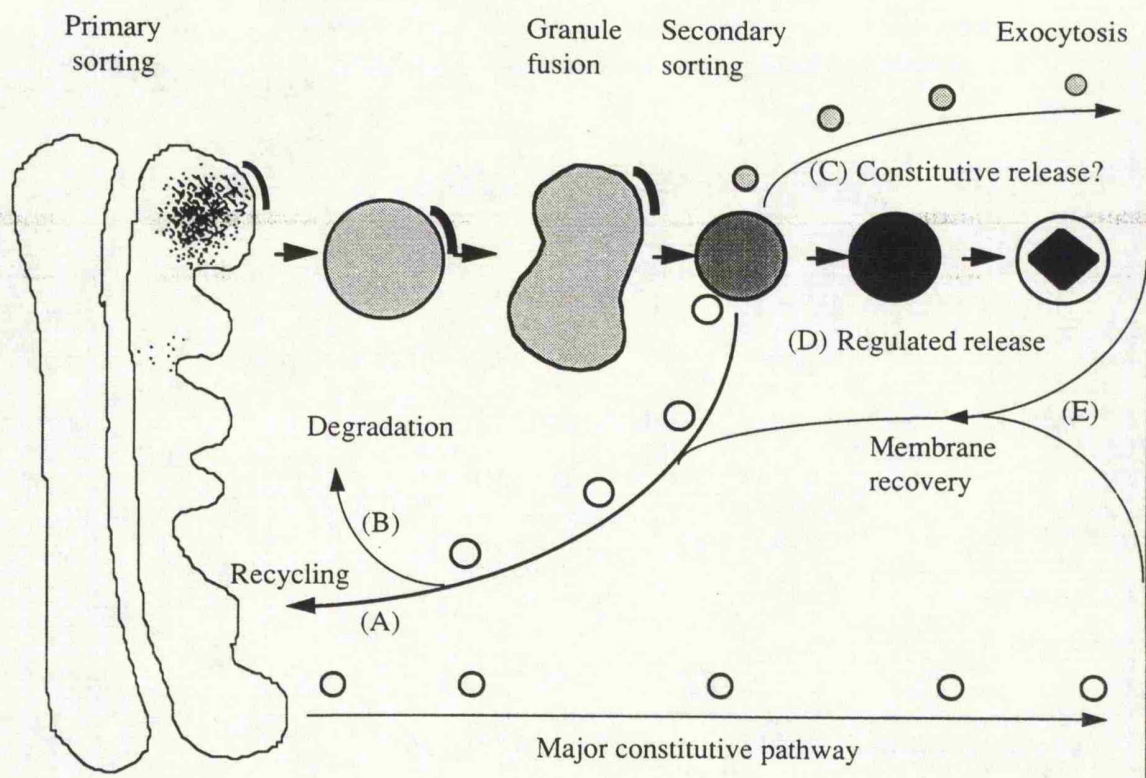
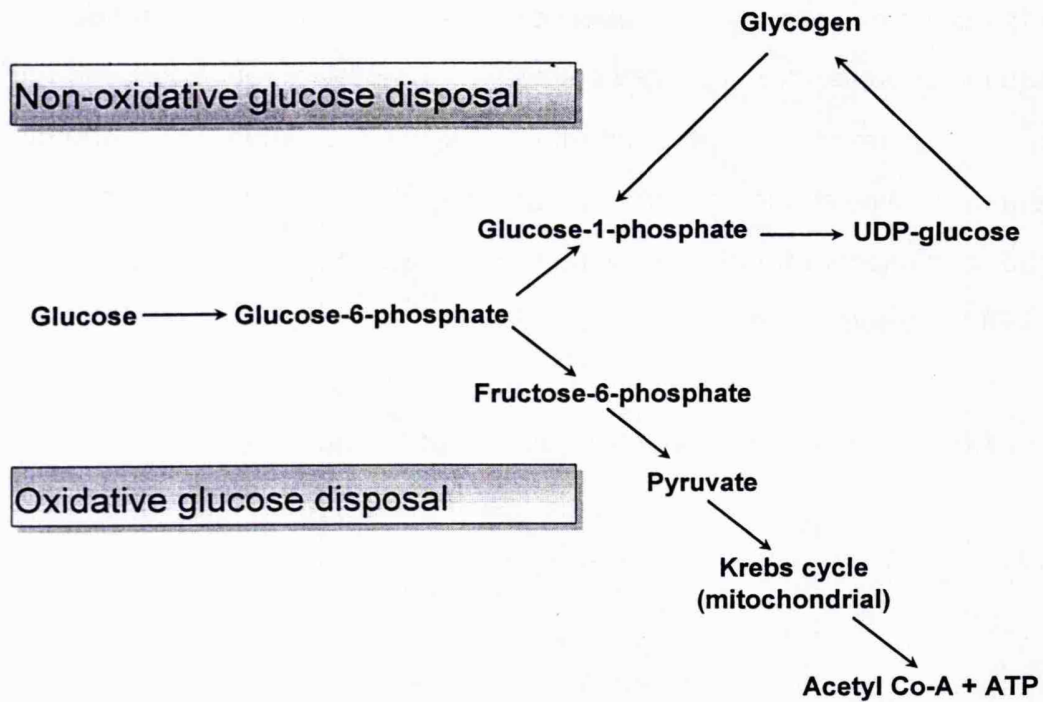


Figure 1.7. Intracellular glucose disposal



1.4.2 Cellular uptake and metabolic fate of glucose

Glucose uptake by virtually all cells is mediated by membrane transporter proteins. Following the first description of a human glucose transporter (GLUT1) in 1986 [Mueckler et al,1986] four other mammalian glucose transporters have since been identified [Bell et al,1990]. Their distribution and other properties are summarised in Table 1.3.

Table 1.3. Mammalian glucose transporter classes

Name	Tissue distribution	Chromosome	K _m (mmol)
GLUT1	Brain, Erythrocytes, Kidney	1p35	2 - 38
GLUT2	Liver, Kidney, Small intestine, β -cell	3q26	17-20 (hepatocytes)/17 (β -cells)
GLUT3	Brain	12p13	11
GLUT4	Skeletal muscle, adipose tissue, heart	17p13	6-7 (muscle)/3-6 (adipocytes)
GLUT5	Small intestine	1p31	N/A

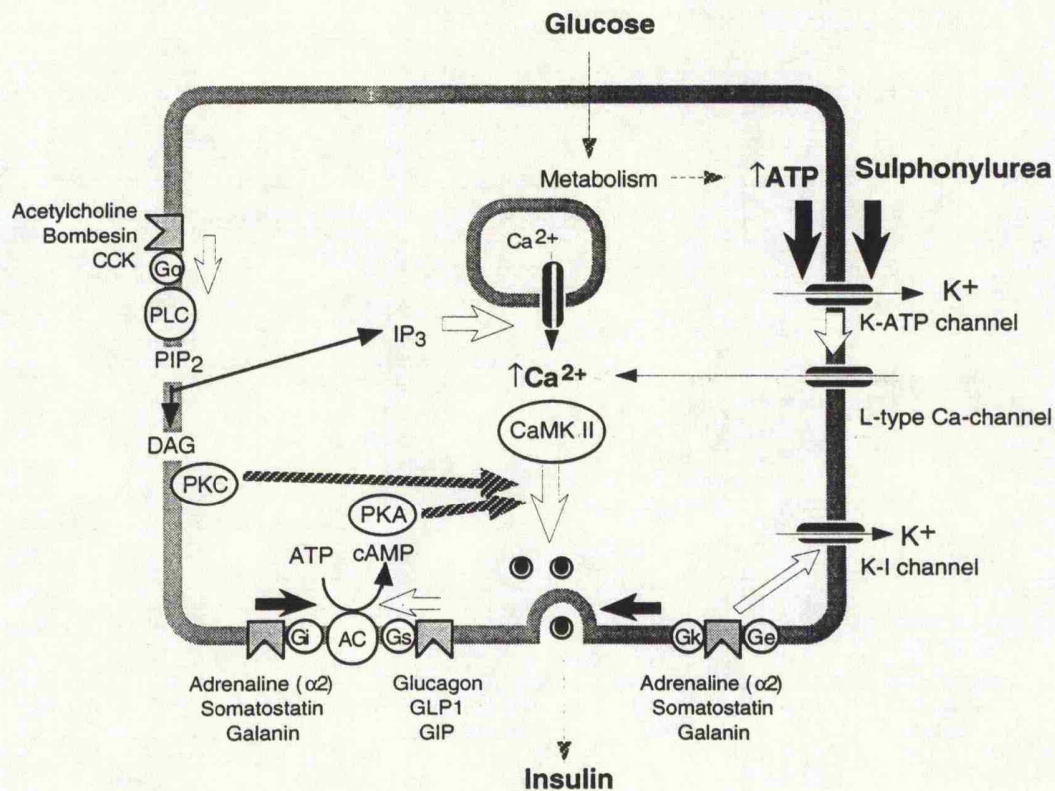
After entry to the cell, glucose is usually phosphorylated by glucokinase and then either oxidised or stored (see Figure 1.7).

1.5 Glucose control of insulin secretion

The high K_m GLUT2 transporter ensures that glucose transport into the β -cell is proportional to plasma glucose over a wide range of concentrations. Transport of glucose into the β -cell is followed by phosphorylation by glucokinase, a step which is rate-limiting in this process and as such glucokinase is widely regarded as the 'glucose sensor' for the β -cell [Matschinsky,1990]. Oxidation of glucose-6-phosphate follows with

subsequent generation of ATP. The β -cell membrane ATP-sensitive potassium channel (the site of action of sulphonylurea drugs [Sturgess et al,1985]) closes as a result of rising intracellular ATP concentrations with subsequent cellular depolarisation following the opening of membrane bound voltage dependent calcium channels and release of intracellular calcium stores [Rajan et al,1990] (see Figure 1.8)

Figure 1.8. Mechanisms of insulin secretion



Adapted from Ashcroft, SH. Diabetologia:37(Suppl 2), S21-S29, 1994

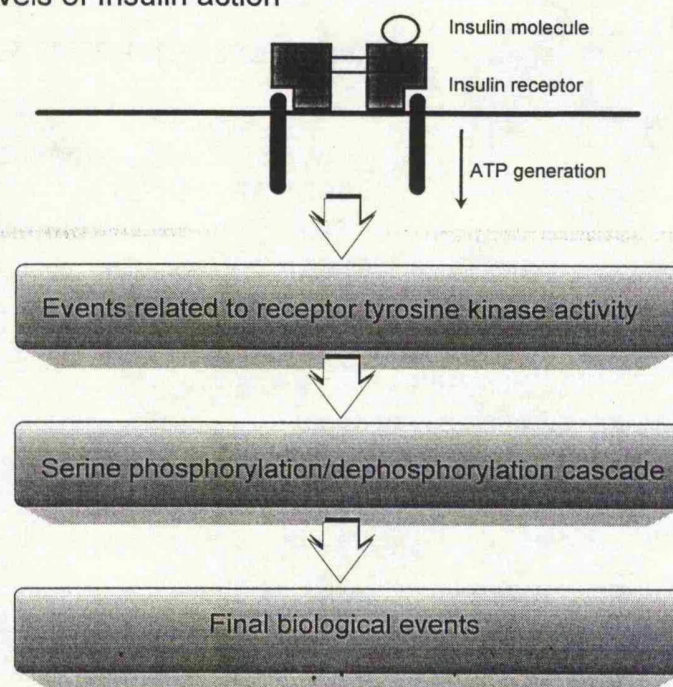
Calmodulin-dependent protein kinase II (CaMK II) is thought to subsequently mediate granule recruitment and insulin secretion. Potentiation of insulin secretion is brought about by activation of other kinases e.g. protein kinases

A and C (PKA, PKC) by cyclic AMP generated by the binding of other hormones e.g. cholecystinin, glucagon, GLP-1 to β -cell surface receptors. Inhibition of insulin release by for example somatostatin or adrenaline is thought to occur via inhibition of cAMP generation.

1.6 Mechanism of insulin action

Insulin has a variety of biological actions including glucose transport, protein, lipid and glycogen synthesis in addition to the stimulation of growth and gene expression [Kahn,1994] (see Figure 1.9)

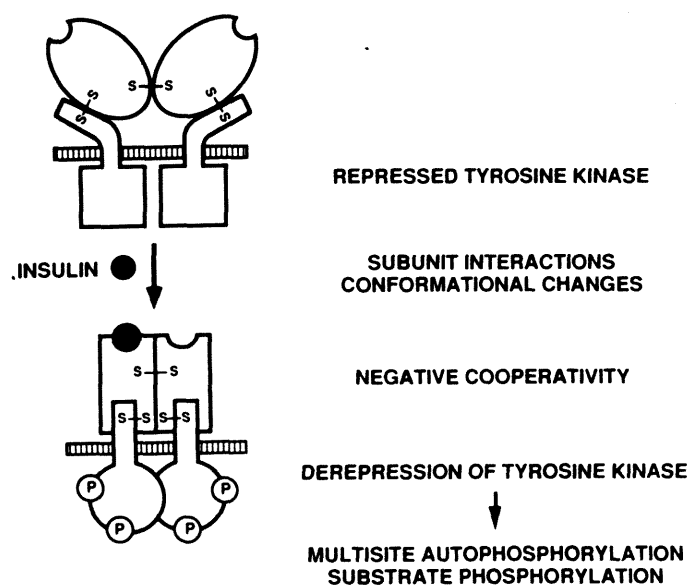
Figure 1.9. Levels of Insulin action



Adapted from Kahn, CR. Diabetes:43, 1066-1084, 1994

1.6.1 Receptor interactions/tyrosine kinase activity

Figure 1.10. Events associated with insulin binding



The insulin receptor

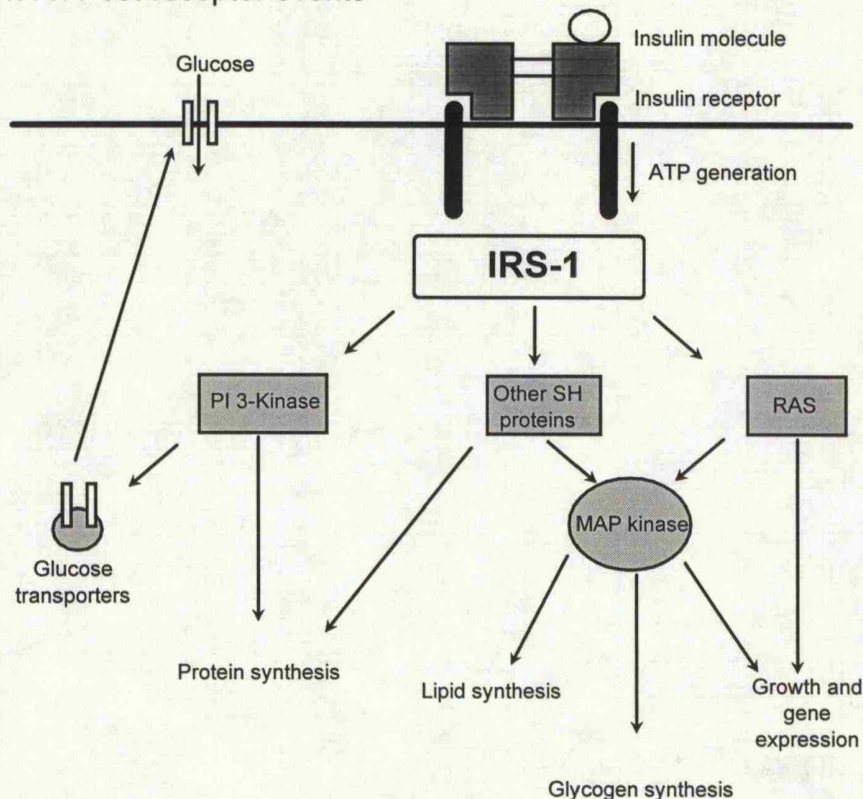
The insulin receptor gene lies on the short arm of chromosome 19 and consists of 22 exons [Seino et al,1989]. The insulin receptor itself is a large transmembrane glycoprotein with a molecular weight between 300 and 400 kilodaltons. Each receptor consists of two α -subunits and two β -subunits linked by disulphide bonds to form a tetrameric structure [Kasuga et al,1982]. The α -subunits contain the insulin binding site and are entirely extracellular whereas the β -subunits are both transmembrane and intracellular and contain the insulin-regulated tyrosine kinase activity [Kahn et al,1988]. Despite the presence of two binding sites for insulin on each insulin receptor, the binding of an insulin molecule to one of these sites results in reduced affinity of the other site for a further insulin molecule - so-called 'negative co-operativity' - resulting in each receptor having the capacity to bind between one and two insulin molecules [DeMeyts,1980]. Binding of an insulin molecule to an α -subunit of the receptor results in conformational change within the receptor structure producing activation of the β -subunit tyrosine kinase activity [Roth et al,1983]. This tyrosine kinase activity appears essential as mutations in this region result in severe insulin resistance [O'Rahilly et al,1992]. Activity of this enzyme leads to autophosphorylation of the β -subunit and a variety of intracellular substrate proteins [Rosen,1987]. Events associated with insulin binding are schematically represented in Figure 1.10.

Post receptor events

Of the insulin receptor cellular substrates the best defined is insulin receptor substrate 1 (IRS-1) [White et al,1985]. It is a 131 kDa cytoplasmic protein containing at least 22 tyrosine phosphorylation sites which are rapidly phosphorylated following insulin stimulation [Sun et al,1991]. These

phosphorylated sites are able to bind to specific domains on a variety of target proteins. Many of the proteins which interact with IRS-1 have now been isolated. Of these, the best understood are the enzyme phosphatidylinositol 3'-kinase (PI 3-K) which is known to be associated with cellular growth [Cantley et al,1991] and increasing activity of this enzyme has been demonstrated in insulin stimulated cells [Backer et al,1993]: the RAS signalling protein which when activated by insulin, binds GTP and activates a further enzyme cascade - the MAP kinases - leading to dephosphorylation and activation of the principle enzyme in glycogen synthesis, glycogen synthase [Dent et al,1990] and altered nuclear protein phosphorylation [Csermely et al,1993] (see Figure 1.11).

Figure 1.11. Post-receptor events



In addition and prior to the activation of enzymes responsible for glycogen synthesis, insulin also promotes the recruitment and translocation of glucose transporters to the cell membrane. The mechanisms by which this occurs are also somewhat obscure however there is evidence to suggest a direct link between the phosphorylation of IRS-1 and its association with PI 3-kinase and the subsequent translocation of GLUT4 receptors in muscle and adipose tissue [Okada et al,1993]. The translocation of the GLUT4 receptor from its intracytoplasmic site to the cell membrane is known to be ATP-dependent [Suzuki et al,1980] and other hormones including insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2) and growth hormone are known to induce such a translocation [Sinha et al,1990; Dimitriadis et al,1992; Scharf et al,1992]. Translocation of glucose transporter vesicles is followed by fusion to the cell membrane and activation of the transporter with subsequent cellular glucose uptake. Glucose transporters are also recycled following insulin stimulation with evidence of a constant flux between the intracellular transporter pool and the cell membrane [Jhun et al,1992].

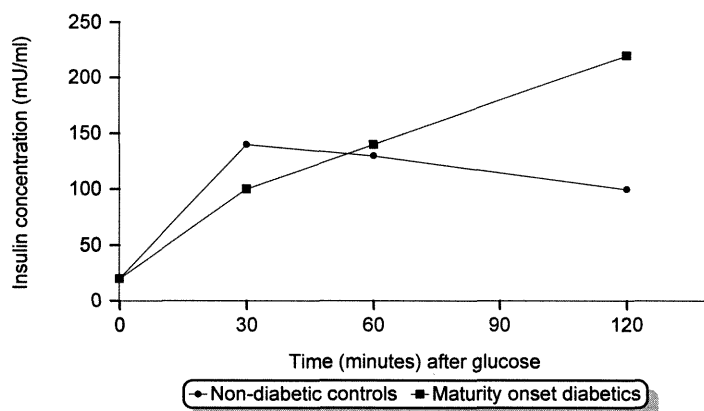
1.7 β -cell dysfunction in non-insulin dependent diabetes mellitus

1.7.1 Patterns of β -cell dysfunction

The investigation of individuals with what is now described as NIDDM by Himsworth in the mid 1930s [Himsworth,1936] suggested the presence of circulating insulin in a disease previously thought to be ".....scientifically definable only as a deficiency of the internal secretion of the pancreas" [Allan et al,1929].

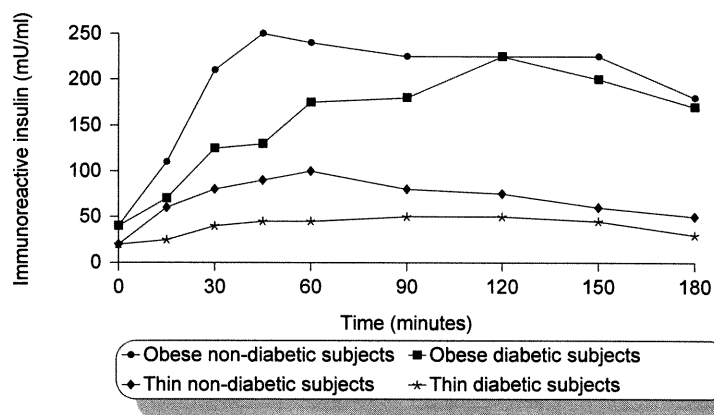
Prior to the description of the radioimmunoassay for insulin [Yalow et al,1960a], several techniques were developed capable of demonstrating insulin-like action in the plasma of subjects with NIDDM [Bornstein et al,1951; Vallance-Owen et al,1954; Martin et al,1958]. These methods were however too complex to be used routinely, gave widely differing results and could not be guaranteed to measure insulin activity alone. Yalow and Berson in their landmark paper presented data on immunoreactive insulin (IRI) concentrations measured during oral glucose tolerance tests in normal healthy individuals and obese individuals with NIDDM (see Figure 1.12). In the fasting state, the NIDDM subjects had mean immunoreactive insulin concentrations identical to those in normal individuals although there was considerable between subject variability.

Figure 1.12. Immunoreactive insulin concentrations following oral glucose



After glucose, the NIDDM subjects had a poor initial IRI response but often displayed late 'hyperinsulinaemia' in the face of ongoing high plasma glucose concentrations. Yalow and Berson suggested that these results indicated the presence of both β -cell dysfunction (poor early response to glucose) and insulin resistance (late hyperinsulinaemia) in these individuals. Subsequent work by Karam showed that obese non-diabetic individuals also displayed fasting and glucose stimulated hyperinsulinaemia and as a result the hyperinsulinaemic response to glucose by the NIDDM subjects was felt to be secondary to their obesity alone [Karam et al,1963]. The issue was resolved by the study of non-diabetic and diabetic subjects carefully matched for weight [Bagdade et al,1967]

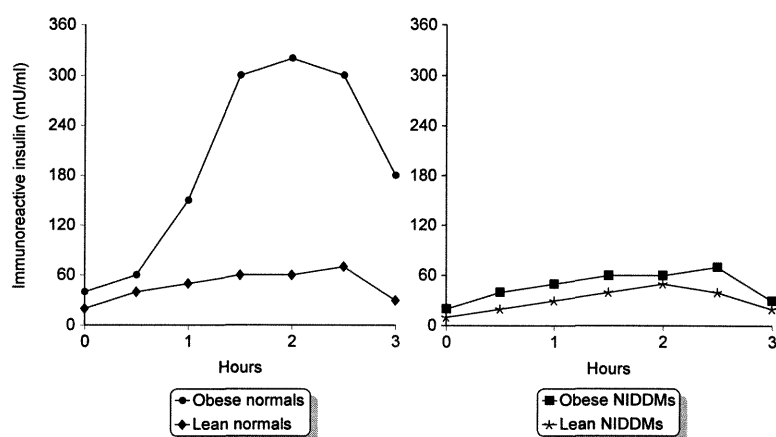
Figure 1.13. Insulin responses to glucose in weight matched healthy and NIDDM subjects.



In this study, fasting insulin concentrations appeared to depend on the degree of obesity. Mean fasting concentrations were identical in the lean subjects regardless of degree of glucose tolerance, whereas in the obese subjects fasting values were elevated but equivalent whether diabetic or not. Following oral glucose, obese normals had higher insulin concentrations than lean normals with the same pattern repeated for the diabetic subjects. However, both the obese and lean diabetic subjects had lower insulin concentrations throughout the test than their carbohydrate tolerant weight-matched counterparts. For the diabetic subjects this difference was both absolute i.e. in terms of measured insulin concentrations, and relative i.e. in relation to hyperglycaemic.

The conclusion from this was that obesity was associated with insulin resistance and 'hyperinsulinaemia' but that non-insulin dependent diabetes mellitus was primarily associated with β -cell dysfunction for two main reasons. Firstly, the initial poor early insulin response to glucose regardless of obesity in the NIDDM subjects, and secondly the later insulin response, although equivalent to that of normal subjects, occurred in the face of significant ongoing hyperglycaemia.

Figure 1.14. Insulin responses in healthy and NIDDM subjects matched for fasting plasma glucose



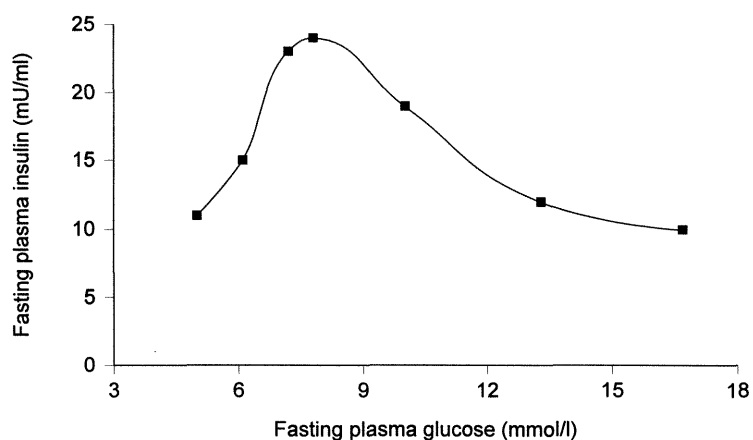
Adapted from Perley MJ, Kipnis DM. J Clin Invest:46, 1954-1962, 1967

As a result of concern that matching for degree of obesity only was insufficient, these experiments (see Figure 1.14) were also repeated at matched fasting glucose concentrations, achieved by infusing the normal subjects with glucose [Perley et al,1967]. In these studies the late hyperinsulinaemia was not seen at all in the NIDDM subjects.

The conclusions were that obesity was associated with insulin resistance and hyperinsulinaemia, but that NIDDM was associated with β -cell dysfunction throughout the test.

Despite these findings, the subsequent development of techniques to measure insulin action, the universal finding of insulin resistance in NIDDM subjects and the concentration on insulin resistance as a potentially major pathophysiological factor in the genesis of non-insulin dependent diabetes mellitus lead to the widespread view that overt insulinopenia in NIDDM was rare [Leahy,1990]. This has been aggravated by reviews of subsequent experimental data by influential investigators who concluded that even in lean NIDDM subjects, insulin concentrations in response to glucose are not subnormal [DeFronzo,1988]. The position relating to fasting insulin in particular has been enshrined in 'Starlings curve' of the pancreas [Reaven et al,1968] (see Figure 1.15).

Figure 1.15. Starlings curve of the pancreas



This describes an inverted 'U' shaped curve of fasting insulin concentration plotted against fasting plasma glucose. Fasting insulin concentrations apparently increasing in individuals with a fasting glucose concentration of up to of 7.8 mmol/l and then falling back into the 'normal' range .

Day profiles of plasma insulin, except for in the most severely hyperglycaemic individuals are also said to be maintained within the normal range [Liu et al,1983; Reaven et al,1988]. Nevertheless, many groups, particularly the Japanese continued to argue that established non-insulin dependent diabetes mellitus was associated with subnormal insulin responses to glucose in all but the most mildly hyperglycaemic individuals [Kosaka et al,1977].

1.7.2 Proinsulin

In the late 1960s, several papers had reported the existence of proinsulin, a circulating precursor of insulin [Rubenstein et al,1968; Steiner et al,1968]. It has become clear that the standard radioimmunoassay for insulin, measures total 'insulin-like' molecules i.e. insulin, proinsulin and other products of proinsulin cleavage, rather than intact insulin. Yoshioka [Yoshioka et al,1988] showed, using a sensitive proinsulin assay that fasting and glucose stimulated proinsulin concentrations were significantly higher in subjects with impaired glucose tolerance and NIDDM compared to normal subjects. Plasma insulin concentrations after glucose (IRI minus proinsulin) were mildly elevated in subjects with IGT but were subnormal in all NIDDM subjects, with progressive decreases as FPG increased.

It would now appear that in the majority of subjects with IGT and NIDDM subjects at the time of diagnosis, fasting and glucose stimulated total proinsulin and proinsulin split-product concentrations are elevated, whilst true

insulin concentrations are either equivalent to or reduced compared to matched non-diabetic controls [Davies et al,1993b; Davies et al,1993c; Krentz et al,1993]. A recent retrospective analysis of 14 studies from 1970 to 1989 in which there was sufficient data to define the participants as either having IGT or NIDDM, and in which age and weight matched controls were studied has shown that early and late insulin (IRI) secretion was reduced in all but the very mildest NIDDM subjects, even without taking insulin assay method into account [Temple et al,1992]. It must be stressed that in these studies, measured insulin concentrations were reduced both in absolute terms and relative to the prevailing plasma glucose concentrations.

In terms of the secretory pathway of insulin within the β -cell, there is little evidence to suggest that the constitutive pathway is active in NIDDM [Halban,1994]. Insulin secretion remains via the regulated pathway which has become dysfunctional, possibly as a result of increased demands placed upon it (although hyperproinsulinaemia does not necessarily occur in other situations of increased β -cell stress e.g. nicotinic acid treatment [Kahn et al,1989]) and possibly as a result of a defect in proinsulin processing [Porte Jr et al,1989] with resultant secretion of intact and split proinsulins in addition to insulin.

In summary, previous findings of hyperinsulinaemia in NIDDM subjects, interpreted as a physiological β -cell response to insulin resistance may well be artefactual and relate to the inadequate matching of subjects and controls, variations in the diagnostic criteria for NIDDM, failure to interpret insulin concentrations in the context of ambient blood glucose concentrations and especially the nonspecific radioimmunoassay for insulin employed.

1.7.3 Other β -cell secretory abnormalities in NIDDM

The biphasic secretion of insulin in response to intravenous glucose has been shown to be abnormal in NIDDM subjects with the characteristic 'first phase' of insulin secretion either missing or significantly defective in subjects with only very mildly impaired glucose tolerance [Perley et al,1967; Seltzer et al,1967]. A clear threshold - usually around 6.4 mmol/l - appears to exist between the fasting plasma glucose concentration and the presence or absence of a first phase insulin response to intravenous glucose [Brunzell et al,1976]. This early insulin deficiency has subsequently been shown to be of major significance in subsequent carbohydrate intolerance [Bruce et al,1988; Luzio et al,1991]. The insulin response to other secretagogues e.g. arginine, was initially reported as being normal [Floyd Jr et al,1968], however as basal hyperglycaemia represents a significant pre-stimulus to the β -cell the documented normal responses to arginine probably occurred solely as a result of potentiation. If plasma glucose concentrations were normalised in NIDDM subjects, then the acute response to arginine and other secretagogues was clearly subnormal [Porte Jr,1991].

The oscillatory secretion of insulin is also abnormal in NIDDM subjects [Lang et al,1981; Matthews et al,1987; Polonsky et al,1988] and their first degree relatives [O'Rahilly et al,1988] being of both reduced amplitude and regularity. It is well recognised that pulsatile delivery of insulin results in greater biological effect [Matthews et al,1983; Paolisso et al,1987], and as a result, the findings in first degree relatives were felt to indicate potential pathophysiological significance to the disrupted oscillatory secretion of insulin.

1.8 Causes of abnormal β -cell function in NIDDM

A variety of mechanisms have been postulated to explain the range of β -cell secretory abnormalities in NIDDM.

1.8.1 Reduced β -cell mass

β -cell mass appears to be reduced by the order of 50%, in both obese and non-obese NIDDM subjects when compared to weight matched controls [Kloppel et al,1985]. This is in sharp contrast to individuals with insulin resistance but normoglycaemia e.g. the obese, or individuals with impaired glucose tolerance secondary to other endocrine dysfunction e.g. Cushing's disease or acromegaly, where β -cell mass is increased. The reduction in β -cell mass in NIDDM is consistent and provides a connection to the 'thrifty phenotype' hypothesis of the pathophysiology of the disease [Hales et al,1992].

The 'thrifty phenotype' hypothesis

This hypothesis arose from work suggesting that cardiovascular disease in adult life resulted from a 'programmed' effect of interference with foetal development [Barker et al,1986]. In this study, death rates from cardiovascular disease in the adult populations studied were highly correlated with neonatal mortality rates at the time of the adults birth. As neonatal mortality is closely related to low birthweight the connection between low birthweight and cardiovascular disease was proposed. Subsequent studies of elderly populations in Hertfordshire and Preston have confirmed associations between low birth-weight and ischaemic heart disease [Barker et al,1989], hypertension [Barker et al,1990] and elevated plasma fibrinogen

concentrations [Barker et al,1992]. These associations suggested interference with the development of specific organ systems in the foetus (blood vessels, liver) and the most likely environmental influence was felt to be poor maternal nutrition.

As has been seen above, NIDDM is characterised by a reduction in the total β -cell volume which could be explained by adverse intrauterine conditions affecting foetal β -cell growth and development. Studies in the elderly male population of Hertfordshire for whom birth and one year data were available revealed that the percentage of men with either IGT or NIDDM fell progressively with increasing birthweight and weight at one year. Obesity in later life appeared to add to the risks from poor early growth [Hales et al,1991].

This provides an interesting counter-point to the popular concept that insulin resistance is the prime abnormality resulting in a number of other common metabolic abnormalities (impaired glucose tolerance, hyperlipidaemia, elevated plasma fibrinogen concentrations) and diseases (coronary artery disease, hypertension, obesity) - [Reaven,1988]. The studies in Hertfordshire showed inverse relationships between birth weight and hypertension, impaired glucose tolerance and ischaemic heart disease and a subsequent study has documented that low birth weight is associated with insulin resistance in later life, irrespective of adult obesity [Phillips et al,1994a].

Of greater interest however is the study of insulin secretion in adults known to have growth retardation in-utero. A large number of glucose tolerant and intolerant subjects in Preston for whom similar data to that from Hertfordshire were available were recently studied using the intravenous glucose tolerance test [Phillips et al,1994b]. No correlation could be established between

defects in insulin secretion and measures of prenatal growth in either the glucose tolerant or intolerant subjects.

Alternative explanations for the associations between low birth weight and adult diseases do however exist. The Hertfordshire cohort was born at a time when mortality for low birth weight infants was high. Low birthweight is associated with an increased frequency of hypoglycaemia and insulin resistance may have conferred protection against this resulting in selective survival of small infants predisposed to diabetes - the so-called "surviving baby phenotype" [McCance et al,1994]. Additionally other studies have shown that low birthweight is associated with socioeconomic and lifestyle disadvantage at the time of birth and subsequently throughout life. The association between low birthweight and disease in later life may therefore be equally due to the persistence of this disadvantage [Elford et al,1991]. Finally, studies in low birth weight children have so far failed to demonstrate a subsequent association with elevations in blood pressure during early adulthood [Williams et al,1992; Matthes et al,1994].

The 'thrifty phenotype' hypothesis is clearly attractive in explaining many features of the metabolic syndrome, including insulin resistance. At the present time however, there is no evidence of impaired glucose-stimulated insulin release in adults of low birthweight and it is not clear whether the associations between adverse intrauterine conditions and subsequent adult disease are causal or otherwise.

1.8.2 Islet amyloid polypeptide (Amylin)

Amyloid deposits have been observed in the islets of Langerhans for over 90 years and amylin was sequenced from deposits extracted from a resected

human insulinoma [Westermarck et al,1987]. It is estimated to be present in the pancreatic islets of 90% of NIDDM subjects [Cooper et al,1987] and is a 37 amino acid polypeptide, normally produced by the β -cell, packaged and subsequently co-secreted along with insulin in the secretory granule [Lukinius et al,1989].

Amylin is amidated at its C-terminus, a feature common to biologically active hormones, and has some 45% homology with calcitonin gene-related peptide, a potent vasodilator [O'Halloran et al,1991]. Recent examination of families with a strong history of NIDDM found no evidence of linkage between the amylin gene and NIDDM on the basis of restriction fragment length polymorphisms [Cook et al,1991] and it is unlikely that the risk of development of NIDDM is carried by the amylin gene.

Plasma concentrations of amylin correlate well with plasma insulin concentrations being typically absent in IDDM subjects, 30% reduced in NIDDM subjects on insulin and 50% increased in NIDDM subjects on sulphonylureas [Hartter et al,1991]. On this basis there is little evidence to suggest that NIDDM results from alteration in amylin release.

Amylin has been shown to reduce glucose stimulated insulin secretion both in rats and in humans [Silvestre et al,1990; Bretherton-Watt et al,1992], however this only occurs at supraphysiological plasma concentrations of amylin, making it unlikely that the peptide has any significant autocrine effects. Additionally, at similarly supraphysiological concentrations, amylin has not been shown to alter systemic glucose metabolism either during intravenous glucose tolerance tests or euglycaemic hyperinsulinaemic clamps [Bretherton-Watt et al,1992; Wilding et al,1994]. These results again suggest that amylin does not play a significant role in the development of NIDDM.

1.8.3 Mutations of the glucokinase gene

Glucokinase, which phosphorylates glucose after entry into the β -cell, is widely regarded as the 'glucose sensor' because of its rate limiting role in glucose-induced insulin secretion [Matschinsky, 1990]. As a result of this it is a potentially important regulator of insulin secretion. Several glucokinase alleles have been identified and suggested as resulting in an increased risk of NIDDM in certain ethnic groups [Chiu et al, 1992]. Mutations in the glucokinase gene have now been repeatedly described in French families with a high incidence of maturity-onset diabetes of the young (MODY) [Vionnet et al, 1992; Frougel et al, 1993]. Similar studies on pedigrees from other ethnic groups e.g. Mauritian Indians and Welsh whites have not demonstrated linkage with a defective glucokinase gene [Yki-Jarvinen, 1994]. Clearly then, these mutations account for the β -cell defect found in only a minority of the general NIDDM population.

1.8.5 Glucose toxicity

Glucose was shown to induce diabetes when repeatedly injected into partially pancreatectomised cats as long ago as 1948 [Dohan et al, 1948]. More recent data in both experimental animals and human subjects suggests that rather than being a simple marker of NIDDM, chronic hyperglycaemia may contribute to the natural history of the condition.

Some support for this hypothesis is available from animal models of NIDDM. Firstly, rats rendered diabetic by either 90% pancreatectomy, or by streptozotocin given in the neonatal period, fail to produce a first phase insulin response to glucose and have markedly impaired second phase insulin responses [Weir et al, 1981; Bonner-Weir et al, 1983]. The insulin

response to other secretagogues is however normal or even exaggerated [Weir et al,1986; Leahy et al,1992b]. These rat models combine hyperglycaemia with relative hypoinsulinaemia, thus the contribution of insulin deficiency to this phenomenon can not be discounted. However, using normal rats rendered hyperglycaemic and hyperinsulinaemic by continuous glucose infusions, the same β -cell unresponsiveness to glucose can be demonstrated [Leahy et al,1986]. Clearly, this effect is not related to prevailing insulin concentrations but to the hyperglycaemia per se.

Further evidence for the toxic effect of glucose on insulin secretion in experimental animals comes, for example, from Zucker *fa/fa* rats. Males of the species are hyperglycaemic with abnormal insulin responses to glucose whereas genetically identical females are normoglycaemic and retain normal insulin responses to glucose [Leahy et al,1992a]. Additionally, a 60% pancreatectomy in normal rats does not lead to hyperglycaemia but the addition of 10% sucrose to their drinking water leads to the development of both hyperglycaemia and the loss of glucose stimulated insulin secretion [Leahy et al,1988].

A further strand of evidence from diabetic animal models comes from the effect of a reduction in the prevailing levels of glycaemia on glucose-induced insulin secretion. Phlorizin lowers plasma glucose levels whenever above basal, by reducing renal tubular glucose reabsorption without having any influence on plasma insulin, free fatty-acid, amino acid or other hormone or substrate levels [Rossetti et al,1990]. The administration of phlorizin to 90% pancreatectomised rats results in restoration of glucose-induced insulin secretion with respect to both first and second phase insulin secretion [Rossetti et al,1987], with a similar effect on normal rats rendered hyperglycaemic by glucose infusions [Leahy et al,1987]. This positive effect of

reducing hyperglycaemia on glucose-induced insulin secretion can be observed following the introduction of insulin by infusion in the intact 90% pancreatectomised animal [Leahy et al,1991] or in vitro using perfused pancreata when glucose is removed from the perfusate [Grill et al,1987]. The restoration of glucose-induced insulin secretion also occurs very rapidly, with normal responses recorded after as little as 40 minutes of normoglycaemia [Grill et al,1987].

Evidence for a toxic effect of elevated plasma glucose concentrations on β -cell function in human subjects is equally compelling. A large number of clinical studies have demonstrated that treatment of subjects with NIDDM by any means which results in a reduction in fasting and/or glucose stimulated glucose levels, results in improved insulin secretion which is often maintained for prolonged periods after the intervention has ceased. In this context both acute and chronic caloric restriction [Kosaka et al,1980; Henry et al,1986], intensive insulin therapy [Turner et al,1976; Hidaka et al,1982] and sulphonylureas [Kosaka et al,1980; Firth et al,1986c] have been shown to exert this effect. In addition, in some studies improved glycaemic control has been associated with a partial return of first phase insulin secretion [Turner et al,1976; Vague et al,1982], although this has not been universally the case.

In contrast to the experimental data on the desensitisation of the β -cell to glucose and the restorative effects of reducing ambient glucose concentrations, the data on the mechanism of this defect are less clear cut. Transport of glucose into β -cells by GLUT 2 is known to exceed glucose phosphorylation by glucokinase by more than 10 fold [Megalsson et al,1986], thus making phosphorylation rather than a glucose transporter defect the more likely rate limiting step. Additionally, GLUT 1 receptors in the brain are down regulated under the influence of sustained hyperglycaemia [Pardridge

et al,1990], presumably in an attempt to 'normalise' intracellular glucose concentrations and prevent accumulation of intracellular glucose. This may therefore equally apply to GLUT 2 and the β -cell. β -cell glycogen stores are also characteristically elevated in chronic hyperglycaemia [Hellman et al,1969] which would be unlikely if active transport of glucose into the cell was significantly reduced.

The metabolism of glucose to three carbon compounds within the β -cell also appears to be intact. Islets from streptozotocin-induced diabetic rats or control rats made hyperglycaemic with glucose infusions demonstrate loss of glucose-induced insulin secretion. However, when islets are exposed to either 0.5mmol/l or 10mmol/l glucose, glucose metabolism is either normal (0.5mmol/l) or increased (10mmol/l) [Collela et al,1987].

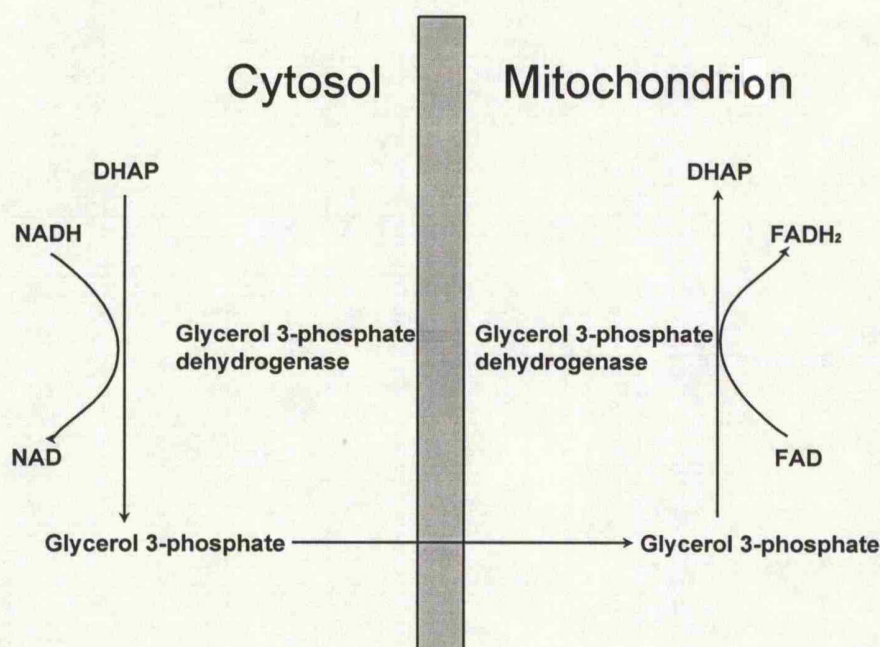
Depletion of the main intracellular messengers/energy transporters associated with insulin secretion (ATP, cAMP and NAD) has not been shown to occur in response to sustained hyperglycaemia in diabetic rat models [Hoenig et al,1986; Collela et al,1987] and the synthesis of insulin mRNA and the conversion of proinsulin to insulin have also been shown to be normal or increased in diabetic rat islets in response to chronic hyperglycaemia [Bolaffi et al,1986; Nagamatsu et al,1987].

Intracellular glucose disposal is by glycolysis in the cytosol and oxidation within the mitochondria. Pyruvate as the final product of glycolysis, was felt to provide the link between the cytosol and the oxidative processes within the mitochondria leading to insulin secretion and therefore abnormalities of pyruvate metabolism may be linked to impaired glucose stimulated insulin secretion. Pyruvate has, however, been shown to be well oxidised by β -cells

from diabetic rat models and although it augments glucose-induced insulin secretion, it has no ability to cause insulin release by itself [Sener et al,1978].

In addition to pyruvate, an additional link between the cytosol and the mitochondria appears to exist in the form of the 'glycerol phosphate shuttle' (see Figure 1.16) in which dihydroxyacetone phosphate (DHAP) is converted in the cytosol to glycerol-3-phosphate with the generation of NAD from NADH.

Figure 1.16. The Glycerol phosphate shuttle



Within the mitochondria, glycerol-3-phosphate is reoxidised to DHAP by a flavin adenine dinucleotide (FAD) dependent glycerol-3-phosphate dehydrogenase, with the generation of FADH₂ an increase in intramitochondrial calcium and hydrogen ion concentration and ATP synthesis resulting in insulin secretion. This FAD-dependent glycerol-3-phosphate dehydrogenase is 40-70 fold more active in islets than in other tissues [MacDonald,1981]. Krebs cycle appears to be intact in the mitochondria of

streptozotocin induced diabetic rats [Grill et al,1991] whereas the activity of the glycerol phosphate shuttle is reduced to 30% in islet mitochondria compared to normoglycaemic controls, despite its normal function in liver mitochondria from the same diabetic animals [Giroix et al,1991].

The significance of these changes is as yet unclear as the system in which they were studied is by no means typical of human diabetes. Nevertheless, these abnormalities present an insight into one of the possible mechanisms of impaired glucose-induced insulin release.

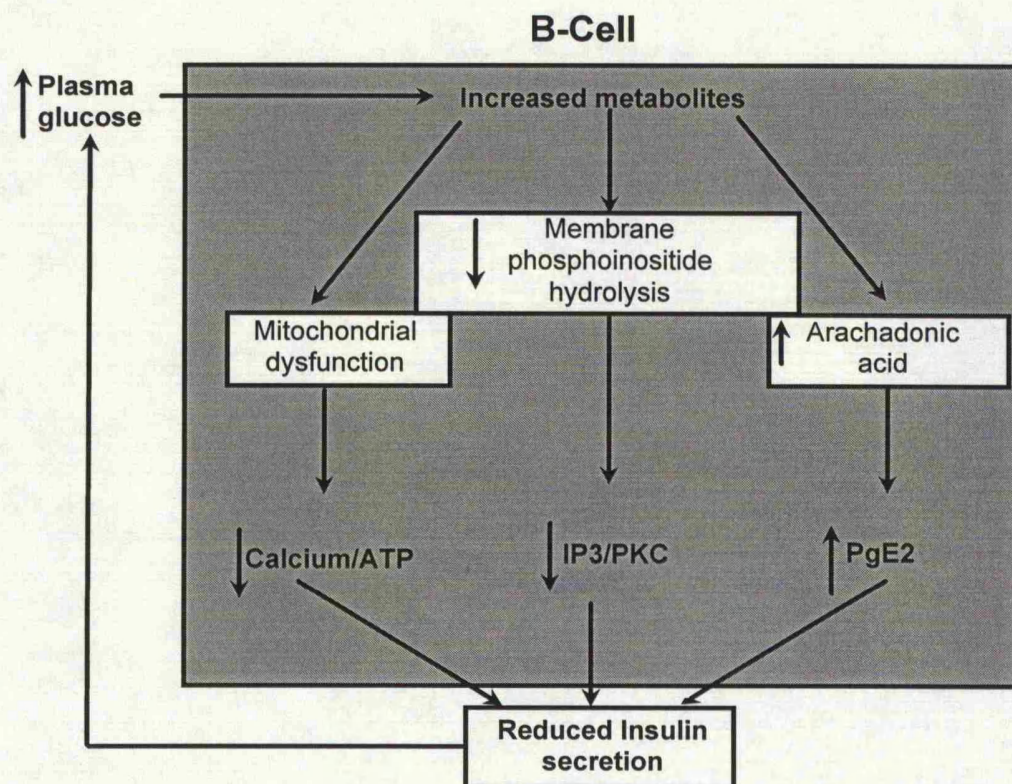
Other possible contributory mechanisms involve the abnormal hydrolysis of membrane phospholipids and the local generation of prostaglandins within the islet. Several of the 'incretins' such as cholecystokinin (CCK), gastric inhibitory polypeptide, glucagon and glucagon-like peptide1 are thought to bring about insulin secretion by activation of phospholipase C [Rasmussen et al,1990], which converts phosphatidylinositol 4,5-bisphosphate (PIP₂) to inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG) (See Figure 1.8). IP₃ mobilises intracellular calcium thereby activating calmodulin dependent protein kinases whereas DAG activates protein kinase C (PKC). The net result is phosphorylation of cell membrane and other key proteins with triggering of the granule recruitment and extrusion process.

At least one study has shown that the failure of islets to sustain insulin release in the face of chronic hyperglycaemia was associated with impaired membrane phosphoinositide hydrolysis, with a resultant fall in DAG and IP₃ levels and failure of activation of PKC and other kinases [Zawalich et al,1990].

Glucose is also known to stimulate Prostaglandin E₂ (PGE₂) synthesis. PGE₂ is known to inhibit insulin secretion when plasma glucose levels are elevated, but has mild insulin stimulatory effects if the glucose levels are held at a basal level [Robertson,1988]. The insulin responses to other secretagogues however, appear to be unaffected by PGE₂ thus its inhibitory effect would appear to be glucose specific [Robertson,1986]. Treatment of subjects with NIDDM even for short periods with non-steroidal anti-inflammatory agents may result in partial restoration of first phase insulin responses [Robertson et al,1977], but this is not seen in IDDM near to diagnosis when endogenous secretion can still be measured [Robertson,1989]. The role of prostaglandins in the pathophysiology of β -cell dysfunction would appear, therefore, to be specific for NIDDM.

In summary (see Figure 1.17), both animal models of NIDDM and results from human studies suggest an important role for glucose per se in the pathophysiology of NIDDM. The mechanism by which 'glucose toxicity' occurs is not clear but it would appear to operate beyond the simple transport of glucose across the β -cell membrane and after metabolism of glucose to three carbon moieties. There is evidence of abnormal functioning of mitochondrial oxidative processes, cell membrane hydrolysis and activation of protein phosphorylation leading to recruitment and exocytosis of insulin secretory granules. The relative contribution of each of these abnormal processes to the failure of glucose-induced insulin secretion remains unclear.

Figure 1.17. Potential mechanisms for glucose toxicity



1.9 Insulin resistance

The observation of delayed hyperinsulinaemia in response to oral glucose seen in the patients studied by Yalow and Berson [Yalow et al,1960b] and subsequent studies [Bagdade et al,1967] suggested to many investigators that insulin resistance was a potential factor in the pathophysiology of NIDDM. A number of techniques were subsequently devised to allow assessment of insulin action documenting reduced insulin sensitivity in subjects with NIDDM compared to matched controls. Consequently, insulin resistance has become widely regarded as the principle underlying cause for NIDDM by many influential investigators [DeFronzo et al,1992; Kahn,1994].

1.9.1 Sites of insulin resistance

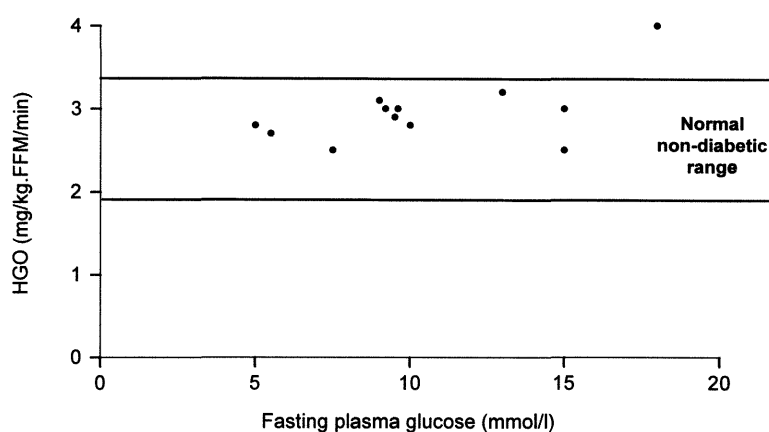
Restoration of normoglycaemia in normal subjects following intravenous or oral glucose relies on the uptake of glucose by peripheral insulin-dependent and insulin independent tissues and the co-ordinated suppression of HGO. These processes are aberrant in subjects with NIDDM.

Hepatic Glucose Output

In the basal (fasting) state, the liver of normal subjects produces glucose at a rate of $\approx 1.8\text{--}2.2$ mg/kg/min [DeFronzo,1988]. Using isotope dilution methods [Steele et al,1956] subjects with NIDDM have been calculated to produce in addition 0.5 mg/kg/min of glucose in the basal state equivalent to an extra 50g of glucose per day [DeFronzo,1992]. Basal hepatic glucose output has also been shown to correlate closely with fasting hyperglycaemia [DeFronzo,1988]. In normal subjects, HGO is exquisitely sensitive to increases in portal vein insulin concentrations and as NIDDM subjects had been shown to demonstrate 'hyperinsulinaemia' following glucose stimulation, the conclusion was that hepatic insulin resistance was a major factor leading to fasting hyperglycaemia.

This conclusion is increasingly challenged however, as a result of reappraisal of both the original data and the technique of isotope dilution. DeFronzo's original study [DeFronzo,1988] found normal HGO in subjects with fasting plasma glucose <7.8 mmol/l. becoming elevated in only 9 of 27 individuals with fasting plasma glucose between 7.9 and 10 mmol/l.

Figure 1.18. Correlation between HGO and FPG in NIDDM subjects



Adapted from Beck-Nielsen, H. *Diabetologia* 37:217-221, 1994

More recent studies have failed to reproduce these findings in diabetic subjects with FPG up 15 mmol/l [Hother-Nielsen et al,1990] (see Figure 1.18) and the detection of significant flaws in the isotope dilution technique have lead to the conclusion that many previous studies may have overestimated HGO in both lean and obese NIDDM subjects [Beck-Nielsen et al,1994]. As a result of these concerns, the role of impaired hepatic sensitivity to insulin in the pathophysiology of NIDDM is currently being reassessed.

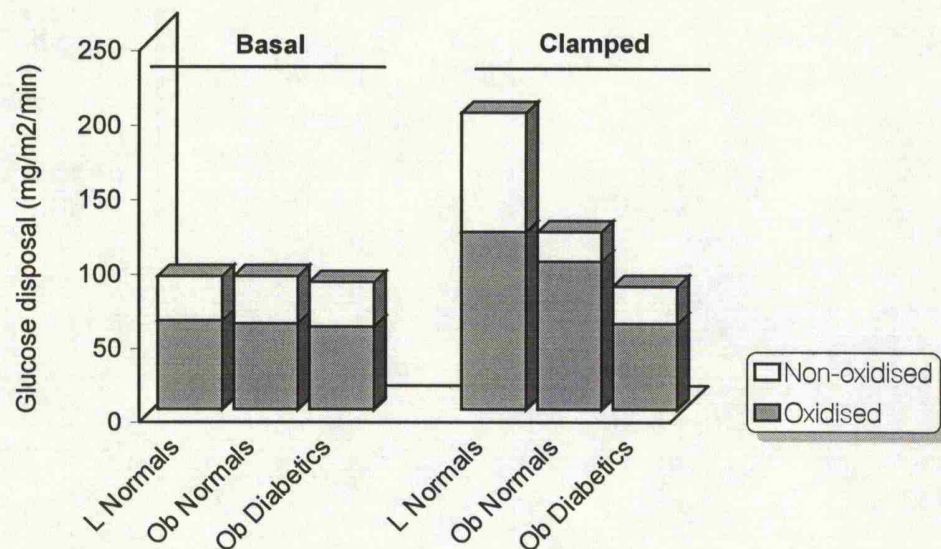
Peripheral (muscle) glucose uptake

Glucose uptake by muscles is mainly dependent on insulin-dependent GLUT4 transporters although a small number of insulin-independent GLUT1

transporters are also present [Joost et al,1989]. Once transported into the muscle cell, glucose is phosphorylated by glucokinase to glucose-6-phosphate which is then metabolised via the oxidative pathway (Krebs cycle), or the nonoxidative pathway to glycogen, triglyceride or lactate. After insulin stimulation, the majority of glucose is converted into glycogen [Beck-Nielsen et al,1992].

Isoglycaemic hyperinsulinaemic clamp studies of NIDDM subjects, when compared to matched obese and lean controls have repeatedly shown reductions in insulin-stimulated glucose disposal [Bogardus et al,1984a; Felber et al,1987; DeFronzo,1988] (see Figure 1.19).

Figure 1.19. Insulin-stimulated glucose disposal in lean and obese normal and NIDDM subjects



Adapted from Bogardus, C. J Clin Invest:74, 1238-1246, 1984

Studies employing techniques to assess arterio-venous plasma glucose differences across the leg [Kelley et al,1990] and forearm [Mitrakou et al,1990] have demonstrated that the reduced glucose disposal is predominantly due to reduced insulin-stimulated glucose disposal in skeletal muscle. It is estimated that this reduction in muscle glucose disposal accounts for up to 90% of the reduction in total body glucose disposal in NIDDM subjects in these studies [DeFronzo,1992]. The reduced glucose disposal in skeletal muscle has been shown using nuclear magnetic resonance techniques to be secondary to defects primarily in nonoxidative disposal i.e. glycogen synthesis [Schulman et al,1990].

1.10 Mechanisms of insulin resistance

1.10.1 The Insulin receptor

The insulin receptor gene from subjects with NIDDM appears to be normal [Kusari et al,1991]. Reduced insulin receptor expression has been demonstrated on monocytes and adipocytes from some NIDDM subjects [Olefsky,1981] but this is not thought to apply in the majority of NIDDM subjects [Granner et al,1992]. Binding of insulin to its receptor in adipocyte preparations has been shown to be reduced by 50% in obese NIDDM subjects compared to lean controls [Friedenberg et al,1987]. Consequently subsequent tyrosine kinase activity is also reduced [Caro et al,1986] but these changes are readily reversible by dietary treatment [Friedenberg et al,1988]. It is of interest to note that individuals with genetic syndromes of severe insulin resistance associated with a variety of insulin receptor mutations e.g. leprechaunism and the Rabson-Mendenhall syndrome, are not always diabetic. The presumed explanation for this is that these individuals

have normal peripheral glucose uptake as their β -cells are capable of huge increases in insulin secretion [Granner et al,1992].

1.10.2 Post-receptor events

(a) Second messenger generation

Most authorities agree that the site of insulin resistance in NIDDM is beyond the interaction of the insulin molecule with its receptor [DeFronzo et al,1992; Kahn,1994]. Unfortunately, when cells are cultured from individuals with NIDDM, it is very difficult to demonstrate any signalling abnormalities [Prince et al,1981]. In the *ob/ob* mouse, one model for human diabetes, an 80% reduction in IRS-1 phosphorylation and a 90% decrease in insulin stimulated PI 3-Kinase activity have been reported [Folli et al,1993]. There are currently no reports of similar changes in signalling documented in NIDDM subjects.

(b) Enzyme activation

Nuclear magnetic resonance imaging in NIDDM subjects and individuals at risk from the syndrome have documented reduced muscle glucose-6-phosphate generation and reduced glycogen synthase activity [Shulman et al,1990; Rothman et al,1992]. Clearly, therefore, both glucose uptake and phosphorylation and especially non-oxidative metabolism are defective in NIDDM subjects and prediabetic individuals. Following treatment of NIDDM, glycogen synthase activity improves, but still remains subnormal [Damsbo et al,1990; Johnson et al,1990]. The documentation of abnormal glycogen synthase activity in prediabetic individuals and the failure of its complete restoration in near normoglycaemic NIDDM subjects may therefore suggest that this represents an underlying genetic element to insulin resistance

(c) Glucose transport

Reduced glucose-6-phosphate generation in muscle from NIDDM subjects may be secondary to impaired enzyme activation, as suggested above, however, reduced glucose transport could also be causative [Rothman et al,1992]. Data on glucose transport abnormalities in NIDDM is conflicting. GLUT4 transporters from NIDDM subjects have been sequenced and shown to be structurally normal [Kusari et al,1991], however both normal and decreased numbers of GLUT4 receptors on skeletal muscle have been reported [Pederson et al,1990; Garvey,1992]. In rat models of NIDDM, treatment with phlorizin results in restoration of normal GLUT4 numbers and function suggesting that the observed GLUT4 abnormalities are more likely to be epiphenomena as distinct from being aetiologically important.

1.10.3 Extracellular mechanisms of Insulin resistance

Increased fatty acid oxidation

Competition for metabolic disposal between FFAs and glucose in rat myocytes was demonstrated by Randle and colleagues [Randle et al,1963] in the early 1960s. The plasma insulin concentration required to half-maximally suppress FFA concentrations in normal subjects is estimated to be three times less than that stimulating half-maximal glucose uptake by muscle [Reaven,1988], suggesting exquisite sensitivity of NEFA metabolism to ambient insulin concentrations.

Experimentally increasing plasma FFA concentrations in normal subjects results in increased lipid oxidation and reduced oxidative and non-oxidative glucose disposal [Ferrannini et al,1983; Bonadonna et al,1989]. This is

brought about by increased activity of the Randle cycle. Excessive FFA oxidation produces an intracellular build up of acetyl coA and subsequent slowing of Krebs cycle. Citrate accumulation inhibits phosphofructokinase with resultant build up of glucose-6-phosphate [Randle et al,1963]. High intracellular concentrations of glucose-6-phosphate inhibits both glucokinase with reduced glucose entry into the cell, and the activity of glycogen synthase.

Previous work has documented that obese NIDDM subjects have elevated basal FFA concentrations and rates of lipid oxidation compared to lean controls [Felber et al,1987] and that suppression of these concentrations by insulin is also impaired [Chen et al,1987]. In these studies a strong inverse relationship was found between lipid oxidation and glucose oxidation and a weaker but still significant correlation between lipid oxidation and glucose storage. Thus elevated rates of lipid oxidation may contribute to the defects in glucose oxidation and to a lesser extent in non-oxidative glucose disposal in this group. In normal weight NIDDM subjects with mild fasting hyperglycaemia however, basal rates of lipid oxidation are normal and suppress normally to insulin. Increased activity of the Randle cycle resulting in insulin resistance is therefore difficult to support in this latter group [Golay et al,1988a].

Factors relating to muscle

(a) Capillary density/Muscle fibre type

At least one study in obese non-diabetic subjects has demonstrated a significant correlation between reductions in muscle capillary density and genetically determined, insulin-sensitive Type 1 oxidative slow-twitch muscle fibres and insulin resistance measured by the euglycaemic hyperinsulinaemic clamp [Lillioja et al,1987]. In addition, the appearance in and concentration of

insulin in lymph (presumed to most closely represent the interstitial fluid to which tissues are exposed) is abnormal. The rate of appearance of radiolabelled insulin in thoracic duct lymph is reduced, as is its concentration [Bergman,1989]. These findings do not necessarily help to explain the decreased non-oxidative glucose metabolism in obese NIDDM subjects, however, they represent interesting observations in the context of the previously discussed 'thrifty phenotype' hypothesis [Hales et al,1992].

(b) Muscle blood flow

Muscle blood flow is an independent regulator of glucose uptake and exercise is known to contribute to improved glycaemic control in NIDDM subjects. Isoglycaemic hyperinsulinaemic clamp studies combined with exercise in normals demonstrate a close correlation between blood flow and insulin-dependent glucose uptake [DeFronzo et al,1981]. Muscle blood flow has been shown to be abnormal in NIDDM subjects, with impaired responses to factors normally resulting in vasodilatation i.e. exercise, acetylcholine or glyceryl trinitrate infusions [McVeigh et al,1992; Menon et al,1992]. In addition, the vasodilatory responses to insulin are reduced compared to those of normal subjects [Laakso et al,1992].

The link between insulin resistance and muscle blood flow is strengthened by the documentation of improved insulin sensitivity brought about by treatment of individuals with certain vasodilator drugs e.g. α -blockers, angiotensin converting enzyme inhibitors [Ganrot,1993].

Whether impaired muscle blood flow in NIDDM subjects is secondary to endothelial dysfunction, as suggested by the failure to respond to known modulators of endothelial vasodilatation, or a reduction in the absolute

numbers of capillaries within the muscle bed is unclear. These findings however suggest a significant vascular component to the insulin resistance in NIDDM subjects.

1.10.4 Environmental factors causing insulin resistance

Anthropometric factors

Obesity is associated with insulin resistance but is itself an independent major risk factor for the development of NIDDM [Skarfors et al,1991]. Its impact on the development of NIDDM does however depend on family history of NIDDM, the duration of obesity [Bennett,1990] and the distribution of body fat.

Studies in Pima Indians (who may not represent 'typical' NIDDM) suggest that the risk of an obese individual developing NIDDM is increased 10 fold if one of their parents also has NIDDM. Also in this population, severe obesity is not predictive of the development of NIDDM if the parents of the individual are not themselves diabetic [Bennett,1990].

The distribution of adipose tissue is also of major importance. Android (upper body) fat distribution is associated with an increase in the ratio of intra-abdominal to subcutaneous fat and is found more commonly in subjects with NIDDM. This distribution of body fat is also associated with increased insulin resistance, increased insulin-resistant muscle fibre types, reduced muscle capillary density and increased cardiovascular mortality - all features described in NIDDM subjects [Björntorp,1991].

Exercise

In addition to obesity, exercise levels are also significantly associated with the development of NIDDM in susceptible individuals. Normoglycaemic individuals who take part in regular exercise have lower fasting glucose and insulin concentrations than individuals who do not exercise [Cederholm et al,1985]. Regular physical activity has been shown to be associated with a reduction in the rate of development of NIDDM [Helmrich et al,1991; Manson et al,1991]. The benefit of a 500 kcal increase in energy expenditure per day is a 6% reduction in the risk of developing NIDDM which is independent of both body weight and family history of NIDDM [Helmrich et al,1991].

1.11 Insulin resistance and β -cell dysfunction - interactions leading to NIDDM

The fact that both of these pathophysiological processes are present in all patients with established NIDDM is not in doubt however which of the processes is primary continues to cause controversy [Kahn,1994; Taylor et al,1994]. The main reasons for this are the difficulty in selecting adequate 'at risk' populations along with suitable controls for study and the contradictory results obtained from previous 'definitive' studies. Previous attempts to circumvent the long, possibly unethical and hugely expensive study of large numbers of randomly selected individuals have been based around either the study of non-diabetic individuals from 'high risk' communities e.g. Pima Indians [Lillioja et al,1993], or the study of non-diabetic individuals first-degree relatives of NIDDM subjects, many of whom will go on to develop NIDDM [O'Rahilly et al,1986]. Both of these approaches are unsatisfactory. The study of 'high risk' communities presumes that the pathophysiology of NIDDM in

these groups is similar to that for other ethnic groups. The study of first-degree relatives presumes that the control group i.e. individuals without a family history of NIDDM contains no individuals destined to develop NIDDM anyway. More methodologically sound comparisons can be made between first-degree relatives of subjects with NIDDM who have either developed IGT or not [Eriksson et al,1989]. However, once IGT has developed it is impossible to ascertain whether a defect in insulin sensitivity or insulin secretion is the cause or result of the hyperglycaemia.

A typical example of the controversy resulting from these study designs is given by contrasting the results of the two studies above. In the first, the major factor predetermining the ultimate development of NIDDM in Pima Indians was found to be insulin resistance. In the second, β -cell dysfunction in first-degree relatives of subjects with NIDDM was found to be the major predictor. Similar conflicting results can be found by comparison of Martins 25 year follow-up of individuals with both parents affected by NIDDM [Martin et al,1992] with Johnstons study of similar individuals [Johnston et al,1990]. In the former, development of NIDDM was significantly associated with the presence of insulin resistance at the start of the study, and in the latter with impaired β -cell function.

These frankly contradictory results would appear to be impossible to reconcile. Some investigators feel that the available methods for assessing insulin resistance and insulin secretion may influence study outcomes [Groop et al,1993]. In the studies by Lillioja and Martin, the defects in insulin action were expressed early and the defects in insulin secretion detected only when glucose intolerance had developed. As a result of this, insulin resistance was felt to represent the earliest abnormality in those at risk individuals and therefore predicted the development of NIDDM. However, it is possible that at

the time that insulin action became mildly impaired, normal or slightly reduced β -cell function may have been adequate to compensate for this without glucose intolerance developing. Under these circumstances, tests which were able to maximally stimulate β -cell function would be better able to detect abnormalities and the emphasis of causation of NIDDM would shift to the β -cell. As tests of this nature do not exist, β -cell dysfunction may have been regarded artefactually as a secondary phenomenon [Groop,1995].

Others suggest that the diagnostic criteria for IGT or NIDDM themselves may be at fault [O'Rahilly et al,1994]. This stems from the observation that in pure insulin resistance syndromes e.g. insulin receptor mutations, the fasting plasma glucose is often normal, whilst after a glucose load severe hyperglycaemia ensues, whereas in conditions with pure β -cell defects e.g. MODY, the reverse is true. As IGT is diagnosed on the basis of an elevated plasma glucose 2 hours after an oral glucose tolerance test, this may result in subjects with insulin resistance being over-represented in studies.

Differences in the ethnic groups studied may also have influenced the conclusions from previous studies. In particular, African Americans, native Americans and Mexican Americans have been shown to have greater degrees of insulin resistance than Americans of European origin and Europeans in general [Banerji et al,1989; Gulli et al,1992; Lillioja et al,1993] in whom β -cell function appears to be more commonly a primary phenomenon [Groop,1995].

However, rather than being the results of methodological artefacts it is more probable that these opposing conclusions are correct and serve to confirm that for the majority of individuals, the disease results from the interaction

between the two proposed pathophysiological processes which are present to variable degrees.

Under these circumstances, individuals with a primary β -cell defect who subsequently develop mild insulin resistance, either as a result of environmental factors e.g. diet, lack of exercise, or simply as a consequence of chronic insulin deficiency, may subsequently become hyperglycaemic. Similarly, individuals who are primarily insulin resistant may develop a β -cell defect, either as a result of β -cell exhaustion, or as a result of glucose toxicity from mildly elevated plasma glucose concentrations.

Indirect experimental and other evidence for these proposed mechanisms exists. For example, subjects with IDDM have a primary secretory defect - autoimmune destruction of the β -cells - however they can also be shown to be insulin resistant [Yki-Järvinen, 1990] and treatment of NIDDM subjects with insulin itself has been shown to result in dramatic improvements in insulin sensitivity [Garvey et al, 1985]. Additionally, mutations in the glucokinase gene result in impaired β -cell glucose sensing and ultimately mild hyperglycaemia (MODY). In contrast, the effects of insulin resistance leading to persistent hyperglycaemia, glucose toxicity and subsequent β -cell dysfunction are well documented [Unger et al, 1985; Leahy et al, 1992a] and individuals with mutations of the insulin receptor gene leading to severe insulin resistance, often (but not always) develop diabetes, usually when their insulin secretory capacity becomes impaired [Accili et al, 1989].

In summary, the pathophysiology of the established syndrome of NIDDM is heterogeneous with insulin resistance predominating in some individuals and β -cell dysfunction in others. The relative contributions of these pathological processes may appear to vary depending on the method of investigation,

ethnicity, presence or absence of obesity, exercise status and the point during the natural history when the patient is first investigated. Current attempts to further define initiating events at a molecular level may result in a clearer description of the aetiology of the syndrome. It is also of importance to study subjects with established NIDDM in terms of how treatment modalities may influence these pathophysiological processes in an attempt to understand the evolution of established NIDDM. With the availability of new methods of assessing both β -cell function and insulin sensitivity this can be carried out on a longitudinal basis and work of this nature will be presented later in this thesis.

Chapter 2 - Aims, Subjects and Methods

2.1 Aims

Non-insulin dependent diabetes mellitus usually appears to develop as a result of the interaction of pancreatic β -cell dysfunction and insulin resistance in susceptible individuals. Much research energy has been devoted to the investigation of the interaction of these two pathophysiological processes prior to the development of the syndrome, but relatively little data exists on the measurement of both these abnormalities at the time of presentation with NIDDM or following treatment in the medium term (one to two years).

The broad aims of this thesis were both methodological and investigational.

2.1.1 Methodological

This thesis sets out to:-

(1) Validate the use of minimal model analysis of the insulin-modified frequently sampled intravenous glucose tolerance test (FSIVGTT) as a means of estimating insulin sensitivity in NIDDM subjects by:

(a) comparing estimates of insulin sensitivity from the insulin-modified FSIVGTT to estimates derived from the more widely exploited isoglycaemic hyperinsulinaemic clamp

(b) examining the effects of changes in blood sampling protocol suggested by other investigators on estimates of insulin sensitivity from the insulin-modified FSIVGTT, and

(c) examine the effects of insulin assay method on estimates of insulin sensitivity from the insulin-modified FSIVGTT.

[(a) and (b) are the subject of two publications in 'Diabetes' (Coates et al 1993, Coates et al 1995).]

(2) To use these studies to suggest a standardised protocol for the exploitation of the insulin-modified FSIVGTT technique to derive an estimate of insulin sensitivity in NIDDM subjects.

2.1.2. Investigational

(1) To use an established investigational technique (the mixed meal tolerance test) to define the spectrum of metabolic abnormalities present in NIDDM subjects at the time of presentation (Coates et al, 1994).

(2) To combine measurement of β -cell function during a mixed meal test using the newly available specific assays for insulin and proinsulin with estimation of insulin sensitivity from minimal model analysis of the standardised insulin-modified FSIVGTT in newly presenting NIDDM subjects.

(3) To derive comparative age, sex and BMI matched data from healthy subjects using the same tests for the purpose of comparison.

(4) To repeat the investigations in the NIDDM subjects at intervals over the following two year period to assess changes in these parameters occurring as a result of treatment either with diet alone or a combination of diet and sulphonylurea agents.

2.2 Subjects

All studies took place with Local Ethical Committee approval and informed consent on behalf of the participants i.e. both NIDDM and healthy subjects.

2.2.1 Diabetic Subjects

All diabetic subjects were recruited from the Diabetic Out-patient Clinic of the University Hospital of Wales run by a single Consultant Physician. Unless otherwise stated three main groups of NIDDM subjects were recruited and studied.

(1) A large cohort of newly diagnosed NIDDM subjects who were subjected to the MTT alone at the time of diagnosis and prior to any therapeutic intervention.

(2) A smaller number of NIDDM subjects were recruited either at the time of diagnosis or when they had been stabilised on diet or SFU therapy to take part in the studies validating the insulin-modified FSIVGTT.

(3) A group of NIDDM subjects were specifically recruited from all new subjects presenting to the clinic with NIDDM between January 1992 and December 1993 to take part in the detailed study of β -cell function and insulin

sensitivity. All eligible subjects (see entry criteria below) were invited to take part in the studies having been given a full explanation of the investigations and requirements of the studies. Thirty-five subjects gave verbal consent to take part.

Inclusion criteria for study

- aged between 35 and 70 years
- newly diagnosed (i.e. within the previous two weeks)
- on no treatment for NIDDM prior to study
- having no clinically significant co-existing pathology
- on no medical treatment likely to interfere with carbohydrate metabolism

Regardless of study design, all subjects studied fulfilled WHO diagnostic criteria for diabetes [WHO,1985] and were islet cell antibody negative.

2.2.2 Normal healthy subjects

Healthy (non-diabetic) volunteers (subsequently referred to as 'normals') were sought by advertisement in the local community. Eligibility criteria were otherwise as for the NIDDM subjects although volunteers with a family history of NIDDM were excluded. Each subject underwent a mixed meal tolerance test and an insulin modified FSIVGTT in random order.

2.3 Methods

2.3.1 General

All tests took place in an investigation ward following a 10 hour overnight fast. NIDDM and normal subjects were treated identically. Prior to the commencement of each investigation, subjects had their height (m), weight (kg) and sitting blood pressure (Korotkov V) recorded. Following these procedures intravenous cannulation took place with the subject lying semi-recumbent on a bed. Investigations began at least 15 minutes after the placement of intravenous cannulae.

2.3.2 Mixed meal test (MTT)

One antecubital vein was cannulated for blood sampling and maintained patent by a slow infusion of N saline. Basal blood sampling for plasma glucose, insulin, total proinsulin, glycosylated haemoglobin (HbA_{1c}) and plasma lipids were taken at times designated -30 and 0 minutes. Following the 0 minute sample, the subjects consumed over a 10 minute period a 500 kcal mixed meal (cereal/milk/chicken/bread/fruit juice) the calorific contribution being 55% carbohydrate, 25% fat, 20% protein. Sampling for plasma glucose, insulin and proinsulin continued every 30 minutes during the 4 hour post-prandial period. Plasma was separated in a centrifuge and stored at -20 °C until assay.

2.3.3 Insulin modified FSIVGTT

Antecubital veins were cannulated in both arms, one for sampling and the other for the administration of glucose and insulin. The cannula for sampling

was kept patent as before. After basal sampling for plasma glucose and immunoreactive insulin (IRI) at times designated -30 and -15 minutes, glucose (300mg/kg) was administered at a constant rate over 2 minutes at time designated 0 minutes. At 20 minutes 0.05U/kg (NIDDM subjects) or 0.03U/kg (normal subjects) human Actrapid (NovoNordisk, Bagsvaerd, Denmark) was given as a bolus injection. Blood samples for plasma glucose and IRI were taken at times 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 minutes [Bergman et al,1979]. An additional sample was taken at 240 minutes in the comparative study between the insulin modified FSIVGTT and the isoglycaemic hyperinsulinaemic clamp as the clamp protocol continued for 4 hours. Plasma glucose and IRI samples were treated as previously.

2.3.4 Isoglycaemic hyperinsulinaemic clamp

An antecubital vein was cannulated and used for glucose and insulin infusions. A contralateral hand vein was cannulated retrogradely and the hand warmed in a heated box for sampling of arterialised blood as venous sampling leads to overestimation of glucose disposal [Bergman et al,1985]. After three basal samples for plasma glucose, an infusion of human Actrapid at a rate of 160mU/min/m² was commenced for 4 minutes as a priming dose and then reduced to 40mU/min/m² for the remainder of the study. The plasma glucose was clamped at basal (mean of the three basal plasma glucose values) by means of a variable infusion rate of 20% D-glucose changed on the basis of plasma glucose concentrations measured every 10 minutes on a Yellow Springs Y2300 glucose analyser (Yellow Springs Instruments Co. Inc., Ohio, USA). In addition to samples for plasma glucose, blood was drawn for the measurement of IRI in the basal period and at regular intervals during the procedure. IRI samples were treated as previously.

2.3.5 Laboratory methods

Glucose assay

Unless otherwise stated, glucose was measured on an autoanalyser (Chemlab Instruments Ltd, Hornchurch, Essex), using an enzymatic colorimetric method [Trinder,1969] obtained from BCL Ltd, Lewes, Sussex. Briefly, glucose and oxygen are converted by the enzyme glucose oxidase to hydrogen peroxide which subsequently generates phenazone from phenol and 4-aminophenazone. The optical density of the generated phenazone is proportional to the glucose concentration. Standards and quality control samples were run regularly and the inter and intra assay coefficients of variation were <2%.

Glycosylated haemoglobin assay

Glycosylated haemoglobin was measured by column chromatography using the test-combination Haemoglobin A1 kit (BCL Ltd, Lewes, Sussex). Normal range for this assay was 5.4-7.8%.

Immunoreactive insulin assay

A modification of the method described by Heding [Heding,1972] was used to measure immunoreactive insulin. The polyclonal antibody M8309 (Novo Research Institute, Copenhagen, Denmark) was incubated with plasma for 6 hours before the addition of ¹²⁵I labelled insulin. A second antibody was subsequently added to separate free and bound insulin. Following centrifugation, the precipitate was counted using a gamma counter. All samples were measured in duplicate. Standard solutions of human insulin

(Novo Research Institute, Copenhagen, Denmark) were included in every assay to calculate a standard curve from which IRI concentrations could be read. The intra-assay and inter-assay CVs of the IRI assay were 4.6% and 7.3% respectively. Cross reactivity of M8309 with human intact proinsulin was of the order of 60%.

Specific insulin assay

True insulin was measured by enzyme linked two site immunoassay using the method of Andersen [Andersen et al,1993]. This employs two murine monoclonal antibodies, OXI-005 directed against an epitope near the C-terminal end of the B-chain, and HUI-018 which has an epitope on the A-loop of the insulin molecule. The major circulating insulin-like components, intact proinsulin, split (32-33) and des (31,32) proinsulin do not cross react in this assay [Andersen et al,1993]. All assays were performed in duplicate and the intra-assay and inter-assay CVs were 6.9% and 8.3% respectively.

Total proinsulin assay

Total proinsulin was also measured by measured by enzyme linked two site immunoassay as described by Kjems [Kjems et al,1993]. This employs two murine monoclonal antibodies, PEP 001 directed against human C-peptide and HUI-001 directed against the C terminal third of the human insulin B chain. This assay does not cross react with human insulin. All assays were performed in duplicate with the intra-assay CV <4.5% at concentrations between 0.78 and 50 pmol/l and the inter-assay CV <4.3% at concentrations between 5 and 29 pmol/l.

Plasma lipid assays

These were measured in the Department of Medical Biochemistry Department at UHW on a Hitachi 747 analyser using standard enzymatic techniques and reagents commercially available from Boehringer-Mannheim Inc.

2.3.6 Statistical methods

Unless otherwise indicated, all statistical analyses were carried out either using SPSS for Windows or Statsgraphics for Windows software running on an personal computer. Minimal model analysis of the data from the insulin modified FSIVGTT was carried out using the MINMOD computer programme (courtesy of Professor Richard Bergman) using weighting of variables as suggested in the original description of the software [Pacini et al,1986]. Certain specific statistical techniques are described in the relevant chapters

Normally distributed variables are given as mean \pm standard error of mean (SEM) and matched groups were compared by the paired t-test. For skewed variables the median and interquartile range are given and the Wilcoxon signed rank test was used to compare the groups. For unmatched groups e.g. diet and sulphonylurea treated NIDDM subjects, the unpaired t-test and the Mann-Whitney U test were used to compare groups. Correlation coefficients were calculated using simple linear regression analysis after log transformation of skewed variables. Area under the curve (AUC) provides a useful summary of individual time series data [Matthews et al,1990] and provides an estimate of the amount of 'substance' e.g. glucose, insulin etc., to which a subject was exposed during a test. AUC was calculated using the trapezoidal rule. AUC data from different subjects groups e.g. normals,

NIDDM subjects with a FPG <9 mmol/l etc. were compared using analysis of variance. Trends in this data were examined by the Kruskal-Wallis test.

2.4 A review of methods for the assessment of β -cell function and insulin resistance

Himsworth provided the first systematic approach to assessing insulin sensitivity with his combined glucose/insulin test [Himsworth,1936]. The ratio of the areas under the glucose curves from two 50g oral glucose tolerance tests, one with and one without an intravenous insulin bolus was used as a measure of insulin action. With this technique, he was able to detect clear differences in insulin sensitivity between young ketosis prone diabetics (sensitive) and older more obese, non-ketotic patients (insensitive). He also reported reduced insulin sensitivity in both the elderly [Himsworth et al,1939] and non-diabetic obese individuals [Himsworth,1949].

The major criticism of Himsworths elegant method is that it was not able to measure the relationship between a measurable insulin dependent metabolic process and plasma insulin concentrations, due to the absence of a suitable assay at the time. In addition, a number of factors could contribute to the differences in insulin sensitivity derived from the test. For example, reproducibility of the OGTT is poor with a coefficient of variation approaching 20% [Yudkin et al,1990] and as the rate of gastric emptying may vary from day to day, stimulation to insulin secretion would also vary [Turner et al,1995].

Later, with the development of an insulin radioimmunoassay by Yalow and Berson [Yalow et al,1960a], a variety of techniques have been described

linking the measure of circulating insulin concentrations and relating this to an insulin dependent metabolic process i.e. glucose disposal.

2.4.1 Fasting plasma insulin concentrations

Fasting plasma insulin concentrations (the higher the insulin concentration, the greater the insulin resistance) are not generally exploited as a measure of insulin resistance although there have been recent suggestions that it may be used as such [Turner et al,1995]. The relationship between fasting plasma insulin concentrations as measured by IRI and fasting plasma glucose concentrations is described in Starlings curve of the pancreas(see Fig 1.15) There are at least two problems with using the fasting plasma insulin concentrations as a measure of insulin resistance apparent from this relationship. Firstly, severely hyperglycaemic NIDDM subjects appear to have equivalent fasting IRI concentrations to normals and secondly the more recent use of highly specific insulin assays suggests that newly diagnosed NIDDM subjects have significantly lower fasting insulin concentrations than normal subjects [Davies et al,1993b]. These relationships do not encourage the use of fasting insulin concentrations as a meaningful measure of insulin resistance in NIDDM subjects although it may be an appropriate measure in healthy individuals [Kahn et al,1993].

2.4.2 Oral glucose tolerance tests with measurement of circulating insulin

Yalow and Berson [Yalow et al,1960b], documented supranormal insulin concentrations during OGTTs in moderately hyperglycaemic, overweight diabetics. Elevated total immunoreactive insulin concentrations in the presence of hyperglycaemia suggested a state of insulin insensitivity. Similarly, supranormal IRI concentrations accompanied by normal plasma

glucose responses during an OGTT in obese, non-diabetic subjects were also reported again equating to the presence of insulin insensitivity [Karam et al,1963]. These early observations of supranormal IRI concentrations in the presence of normal or elevated plasma glucose concentrations was accepted as indirect and compelling evidence of insulin resistance in these population groups.

Criticisms of this interpretation in NIDDM include the observation that insulin secretion in response to glucose is often heterogeneous and dependent on degree of obesity and prevailing glycaemia [Perley et al,1967; Reaven et al,1976]. The finding of hyperinsulinaemia in response to the OGTT in mildly hyperglycaemic NIDDM subjects but hypoinsulinaemia in more severely hyperglycaemic NIDDM subjects makes it difficult to assess the role of insulin resistance in the pathogenesis of the syndrome. The previously discussed development of specific assays for insulin and other insulin-like peptides has also made the validity of these historical interpretations of OGTT data in NIDDM subjects difficult.

2.4.3 Direct measures of insulin/glucose dynamics

(a) The glucose clamp

This technique was initially introduced in 1966 [Andres et al,1966] but modified to its current form by DeFronzo and colleagues [DeFronzo et al,1979]. The method exploits the negative feedback principle with plasma glucose being 'clamped' by the combination of a constant infusion of insulin along with variable glucose infusion rates to ensure isoglycaemic, hyperglycaemic or hypoglycaemic steady states.

The principle of the clamp relies on the fact that in postabsorptive (fasting) steady state, glucose production by the body (R_a) equals glucose utilisation (R_d). The unopposed administration of insulin will result in hypoglycaemia from enhanced glucose utilisation and inhibition of glucose production. Administration of glucose to maintain the plasma glucose at a predetermined level (usually fasting) gives a measure of the action of insulin on production and utilisation of glucose. The independent measure of glucose production by the liver allows the specific effect of insulin on glucose utilisation to be estimated. Hepatic glucose production i.e. R_a is however almost completely suppressed by the supraphysiological insulin concentrations established during the standard clamp technique [Bergman et al,1985; Ng,1988].

The glucose clamp has become the standard technique against which others are compared. It remains a labour intensive procedure requiring relatively sophisticated equipment, highly trained staff and significant data manipulation following the procedure. Equations for calculating insulin sensitivity from the clamp are contained within Appendix 1.

As with other methods of assessing insulin sensitivity, the clamp has certain scientific and practical drawbacks. Common criticisms include the imposition of a continuous hyperinsulinaemic state rarely if ever present under normal physiological conditions and the varying times to reach steady state in different patient groups [Prager et al,1986]. There is also a tendency for observed insulin sensitivity to increase with prolongation of the clamp [Ng,1988]. The glucose clamp is not a technique easily applicable to large epidemiological studies and has therefore been restricted to small scale pathophysiological investigations.

(b) Model based techniques

Continuous infusion of glucose with model assessment (CIGMA)

CIGMA was introduced in 1985 [Hosker et al,1985] following the previous development of a mathematical model relating β -cell dysfunction insulin resistance and hyperglycaemia [Turner et al,1979]. An array of plasma insulin and glucose concentrations expected on the basis of varying degrees of β -cell and insulin resistance was constructed by computer model-solving programs. β -cell function and insulin resistance can be derived from the plasma glucose and insulin concentrations measured at the end of a 60 minute intravenous glucose infusion.

CIGMA estimates of insulin resistance and β -cell function correlate well with those from euglycaemic and hyperglycaemic hyperinsulinaemic clamps respectively. The coefficients of variability for the technique are in the region of 20%. Although offering simple measures of insulin resistance, β -cell function and glucose tolerance, the technique has not been widely exploited since its introduction.

Homeostasis model assessment (HOMA)

This technique was also introduced in 1985 in attempt to simplify CIGMA analysis by requiring only the fasting plasma glucose and insulin concentrations from the subject [Matthews et al,1985]. The technique produces estimates of insulin resistance which correlate well with those from the euglycaemic hyperinsulinaemic clamp. The estimates of β -cell function also correlate well with those from the hyperglycaemic clamp and the intravenous glucose tolerance test [Matthews et al,1985], however the

estimates from the model have low precision (coefficient of variation $\approx 30\%$) making it mainly suitable for population studies.

Other theoretical drawbacks of these two techniques are that they assume that glucose metabolism in different organs (liver, brain, muscle) is constant (although potentially abnormal) between subjects and in different disease states, and that other variables e.g. the glucose distribution space, are calculated from group averages rather than values specific to individuals [Ng,1988]. The availability of new insulin assays may also require the matrices to be recalculated.

The 'minimal' model of insulin sensitivity

'Minimal' modelling of the frequently sampled intravenous glucose tolerance test (FSIVGTT) was first described in 1979 [Bergman et al,1979]. The aim was to describe in the simplest mathematical terms the dynamic system regulating glucose disappearance following intravenous glucose, based on the time course of plasma insulin concentrations (the 'input') and the plasma glucose concentration (the 'output'). After several models were investigated one model closely predicted the time course of glucose concentrations after intravenous administration and possessed a number of parameters which could be precisely estimated. This model was incorporated into a computer program (MINMOD) which derives insulin sensitivity (S_I - a measure of the sensitivity of glucose elimination to insulin), glucose effectiveness (S_G - a measure of glucose-dependent glucose elimination at basal insulin concentrations), ϕ_1 (first phase pancreatic responsivity) and ϕ_2 (second phase pancreatic responsivity) from the plasma insulin and glucose concentrations submitted. The technique has since been extensively validated in a wide range of pathological states [Bergman,1989] and is

relatively simple to carry out. Importantly it is a dynamic test of the glucose regulatory mechanism and is independent of ambient glycaemia and insulinaemia.

In general terms, the potential disadvantages of the technique are that the MINMOD derived parameter S_i is a measure of insulin-mediated glucose uptake in all insulin sensitive tissues (i.e. an insulin sensitivity index), whereas that derived from the glucose clamp may predominantly reflect muscle uptake of glucose alone. Radiolabelled glucose infusions can be employed during the FSIVGTT in an attempt to separate out the different contributions of liver and skeletal muscle on glucose turnover if required [Avogaro et al,1989; Caumo et al,1991]. Additionally, the error in the measurement of plasma insulin concentrations needs to be low as systematic overestimation of insulin concentrations will lead to underestimation of S_i . This error can be reduced both by increasing the number of samples taken during the procedure and by including controls in studies to establish a 'normal' range for S_i .

The methodologies employed by investigators using the FSIVGTT have however varied considerably. Unmodified FSIVGTTs have generally been used to investigate non-diabetic subjects and subjects with impaired glucose tolerance in whom the insulin response is adequate for modelling [Godsland et al,1986; Godsland et al,1991; Bruce et al,1992; Walton et al,1992]. However, the sampling regimens have varied from 10 time-points [Godsland et al,1991] to 19 time-points [Bruce et al,1992]. Additionally, a recently reported study using data collected 25 years ago in individuals at risk of NIDDM, used an unmodified IVGTT with a sampling regimen of 13 time-points [Martin et al,1992]. Modifications of the FSIVGTT have been introduced to augment the insulin response in normals following the glucose bolus using a single intravenous dose of tolbutamide (300mg) regardless of

subject size [Alleman et al,1993], or varying the dose according to body surface area [Bergman et al,1987; Steil et al,1993]. An insulin modified FSIVGTT protocol was introduced for subjects with NIDDM with insulin administered either as an intravenous bolus [Finegood et al,1990; Coates et al,1993] or an infusion over five minutes [Finegood et al,1990; Taniguchi et al,1992]. This modification of the technique for NIDDM subjects has recently been validated against the isoglycaemic hyperinsulinaemic clamp [Saad et al,1994; Coates et al,1995] with varying degrees of success ((see Chapter 3).

'Minimal' modelling analysis of the unmodified and modified FSIVGTT remains a useful and flexible technique for the investigation of insulin sensitivity but guidelines may be required for the appropriateness of protocol modifications depending on the questions to be answered by the investigation and the subjects to be investigated.

(c) The short insulin tolerance test (SITT)

This test has become increasingly used, especially in the UK, for epidemiological research. It uses the rate of fall in fasting plasma glucose concentrations over 15 minutes following an intravenous bolus of insulin (0.1 U/kg) as the basis for the measurement of insulin sensitivity. Arterialised samples are taken every minute from a retrogradely cannulated hand vein heated in a hand-box. Several studies have shown estimates of insulin sensitivity from the SITT to compare favourably with those from the euglycaemic hyperinsulinaemic clamp provided arterialised blood samples are employed [Bonora et al,1989; Akinmokun et al,1992] and more recently the technique has been shown to be reasonably reproducible [Hirst et al,1993].

The advantages of the technique are its simplicity and short duration (15 - 30 minutes). Potential problems are those of hypoglycaemia and the effect on insulin sensitivity of counterregulatory hormone secretion although these do not appear to be troublesome in the shorter protocols (15 minutes) [Akinmokun et al,1992]. Of greater importance is the fact that the test only allows ranking of individuals with respect to insulin sensitivity. Nevertheless, the test is regarded by many as a useful epidemiological and screening tool.

2.5 Assessment of β -cell function

2.5.1 Fasting plasma glucose

FPG provides a fairly precise measure of the severity of diabetes and has a low day-to-day variability [Turner et al,1977] however although it has been suggested to be an appropriate surrogate marker for β -cell dysfunction [Turner et al,1995] few if any modern studies employ it as such.

2.5.2 Fasting plasma insulin concentrations

Fasting plasma insulin concentrations are also rarely used as measures of β -cell dysfunction because of the difficulties in separating out the effects of other influences on insulin secretion e.g. glycaemia or obesity. Of more value perhaps is the plasma 32/33 split proinsulin concentration which has been shown to be useful as an early indicator of β -cell dysfunction [Williams et al,1991; Davies et al,1993b]. Unfortunately, the assay technology for reliable proinsulin split-product measurement is not widely available for routine purposes.

2.5.3 The mixed meal tolerance test (MTT)

Dynamic assessment of the pancreatic β -cell insulin response has traditionally been measured via a glucose challenge in NIDDM. Intravenous glucose challenge avoids the variability of gastric emptying and transit time but delivers the stimulus to insulin secretion to the systemic rather than the portal circulation. Oral glucose delivers glucose to the portal circulation but is unphysiological in the sense that it does not represent the typical everyday challenge to the β -cell. A potential compromise between these two methods exists in the mixed meal tolerance test.

The mixed meal challenge has been used infrequently in the study of NIDDM, mainly because of the greater uniformity of stimulus which a known amount of glucose provides, allowing comparison of results from different studies. Despite this the mixed meal remains a more physiological challenge to the β -cell [Lefebvre et al,1976] and typically contains glucose and the non-glucose secretagogues e.g. amino acids which are part of the everyday diet of most individuals. As such the MTT represents a more relevant test of β -cell function in NIDDM subjects than either oral or intravenous glucose. Previous studies using mixed meals in NIDDM subjects have shown that whereas the plasma glucose profiles may differ to those studies in which glucose alone is used, total insulin responses are usually similar [Jackson et al,1983; McMahon et al,1989; Marena et al,1992].

The typical content of the meals employed in previous studies have been carbohydrate 50-60%, fat 20-25% and protein 15-20%, reflecting the normal dietary recommendations for subjects with NIDDM. In the studies reported here, a specially prepared, highly standardised mixed meal was employed throughout. The meal consisted of breakfast cereal and milk, a chicken

sandwich and a fruit juice drink. The calorific composition was of 55% carbohydrate, 25% fat and 20% protein and all meal were consumed within 10 minutes.

Chapter 3 - A comparison of estimates of insulin sensitivity from MINMOD analysis of the insulin-modified frequently sampled IVGTT (FSIVGTT) and the isoglycaemic hyperinsulinaemic clamp in subjects with NIDDM.

3.1 Introduction

Although a wide range of techniques of varying degrees of complexity now exist for the estimation of insulin resistance (see Chapter 2), the glucose 'clamp' introduced by DeFronzo and colleagues [DeFronzo et al,1979] is generally accepted as the standard against which others are compared. This procedure is labour intensive, can involve the use of isotopic glucose solutions to estimate hepatic glucose output and requires considerable expertise and equipment. The use of computer modelling of glucose and insulin dynamics during a frequently sampled intravenous glucose tolerance test (FSIVGTT) - the minimal model (MINMOD) technique - to derive both an insulin sensitivity index (S_i) and glucose effectiveness (S_G) was introduced by Bergman and colleagues [Bergman et al,1979]. This method offers advantages in terms of relative simplicity of the technique with the only equipment requirements being a personal computer and the MINMOD program.

Early comparisons of the minimal model technique with the glucose clamp in humans were disappointing, with only weak agreement between estimates from the two techniques [Donner et al,1985; Foley et al,1985]. The poor agreement was shown to be related to the magnitude of the endogenous insulin response to glucose during the FSIVGTT [Yang et al,1987]. Subsequent modifications of the FSIVGTT with administration of tolbutamide to enhance endogenous insulin secretion to an intravenous glucose bolus in normal subjects [Beard et al,1986; Bergman et al,1987], or the use of a

higher dose of glucose (500mg/kg vs 300mg/kg) in both normal subjects and subjects with heart failure [Swan et al,1994] have produced close correlations between clamp and FSIVGTT derived estimates of insulin resistance. Estimation of insulin resistance by the minimal model technique has also been shown to be reproducible when repeated in the same individual [Ferrari et al,1991; Duysinx et al,1994; Krempf et al,1994; Steil et al,1994].

The study of insulin resistance in subjects with NIDDM using minimal model analysis of the FSIVGTT is limited by the characteristically poor early insulin responses of these subjects to intravenous glucose alone [Cerasi et al,1967] and the variable response of the pancreatic β -cell response to sulphonylureas. These difficulties have led many investigators to use exogenous insulin following the glucose bolus to facilitate glucose disposal and aid modelling [Welch et al,1990; Taniguchi et al,1992]. Validation of this modification of the technique has been limited and largely dependent on either comparisons with the tolbutamide modified FSIVGTT or studies involving subjects with Type 1 diabetes [Finegood et al,1990; Welch et al,1990]. A previous study in NIDDM subjects has however shown a moderate correlation between the two techniques [Pedrosa et al,1990].

This study examines the agreement between estimates of insulin sensitivity from the insulin-modified FSIVGTT ($SI_{(ivgtt)}$) and the glucose clamp ($SI_{(clamp)}$) in subjects with established, well controlled NIDDM. The clamp studies were isoglycaemic i.e. the subjects were clamped at their fasting glucose levels, to allow comparison with minimal model estimates at comparable glycaemia.

3.2 Subjects and methods

Twelve male subjects with established NIDDM agreed to take part and their clinical characteristics are given in Table 3.1.

Table 3.1. Clinical characteristics (n=12) - median (interquartile range (IQR))

	Median (IQR)
Age (years)	59.5 (16.75)
Time since diagnosis (years)	7.0 (3.5)
Weight (kg)	80.2 (23.1)
Body mass index (kg/m ²)	28.4 (6.0)

The subjects had no other medical condition than NIDDM and were taking no drugs other than sulphonylureas. Each subject underwent an insulin-modified FSIVGTT and an isoglycaemic hyperinsulinaemic clamp in random order 2-4 weeks apart during which time study participants maintained their normal isocaloric diets. Sulphonylurea therapy was omitted on the study days

(A) Procedures

Isoglycaemic hyperinsulinaemic clamp. These studies took place as described in Chapter 2. In the current study, without the use of radiolabelled glucose to assess hepatic glucose output, $SI_{(clamp)}$ was derived, reflecting the glucose infusion rate required to maintain isoglycaemia at steady state during the third hour of the clamp (M value) corrected for the change in insulin concentration from basal and the ambient glucose concentration ($M/\Delta I \times G$ - where ΔI is the increment in insulin concentration from basal, and G is the

clamped glucose concentration [Bergman et al,1985]). During this steady state period the mean (\pm SEM) plasma glucose concentration was 8.34 (0.67) mmol/l and the mean plasma insulin concentration 575 (40) pmol/l. The mean coefficients of variation of steady state plasma glucose and insulin concentrations were 3 ± 1 and $8 \pm 3\%$ respectively

Insulin-modified FSIVGTT. These studies were also carried out as described in Chapter 2. Plasma glucose concentrations from the FSIVGTT were measured on the Yellow Springs analyser in this study. The insulin and glucose dynamics were modelled using the minimal model [Bergman et al,1979] facilitated by the MINMOD computer program, which provides estimates of insulin sensitivity $SI_{(ivgtt)}$ and glucose effectiveness (S_G) [Pacini et al,1986].

3.3 Results

Table 3.1 includes data derived from the insulin-modified FSIVGTT and Table 3.2 the data from the isoglycaemic hyperinsulinaemic clamp.

Table 3.1. Insulin-modified FSIVGTT data

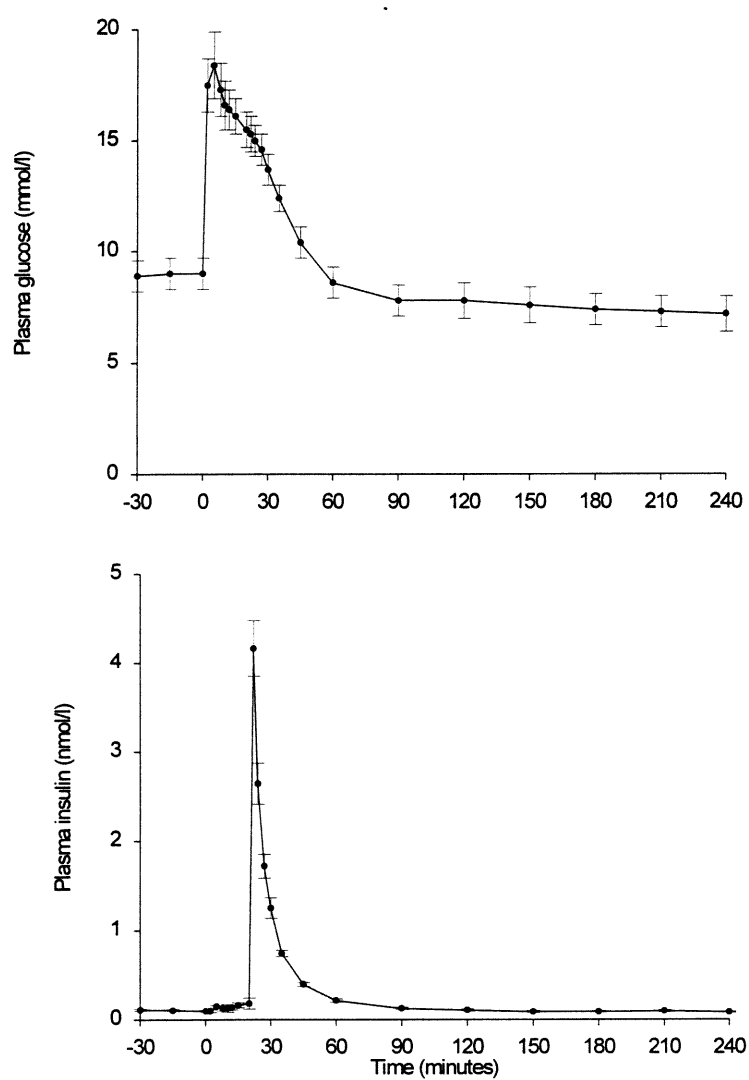
Subject number	Basal insulin (pmol/l)	Basal glucose (mmol/l)	$SI_{(ivgtt)}$ $\text{min}^{-1}/\text{mU}/\text{ml} \times 10^4$
1	64	9.3	0.95
2	132	7.9	0.31
3	48	6.0	2.30
4	80	11.5	0.62
5	132	8.8	0.59
6	152	11.0	0.17
7	60	7.6	2.83
8	78	12.6	1.26
9	132	8.0	1.10
10	42	8.1	1.78
11	116	7.2	0.92
12	182	9.3	0.94
Mean (\pmSEM)	101.5\pm13.1	8.9\pm0.6	1.06\pm0.18

Table 3.2. Isoglycaemic clamp data

Subject number	Basal insulin (pmol/l)	Clamped insulin (pmol/l)	Basal glucose (mmol/l)	Clamped glucose (mmol/l)	M (mmol/min)	$S_{I(\text{clamp})}$ l.min ⁻¹ /pmol.l ⁻¹ ×10 ⁴
1	72	384	8.5	8.4	1.79	6.9
2	141	564	9.0	9.0	2.02	5.3
3	48	438	5.1	5.1	1.62	8.1
4	87	630	9.3	9.2	1.42	2.8
5	90	756	7.7	7.6	1.81	3.6
6	156	528	12.7	12.6	0.90	1.9
7	72	462	6.3	6.2	1.64	6.8
8	75	654	11.6	11.3	2.26	3.5
9	138	492	7.6	7.5	1.70	6.4
10	60	480	7.1	7.0	2.62	8.9
11	162	846	5.7	5.7	1.32	3.4
12	153	630	10.1	10.0	0.98	2.1
Mean	104.5	572	8.4	8.3	1.67	5.0
SEM	12.1	39.4	0.7	0.7	0.14	0.7

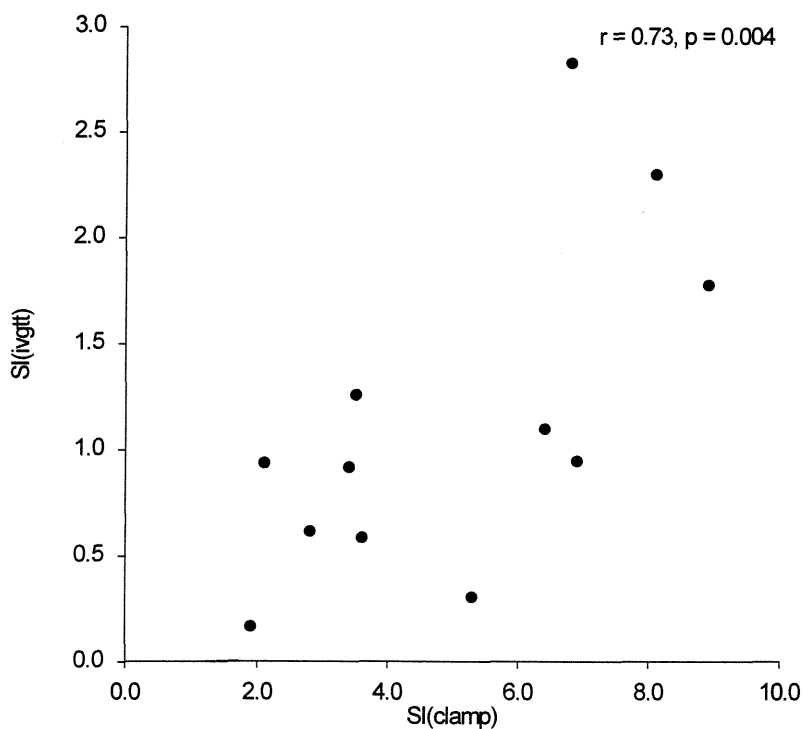
Fasting plasma glucose and insulin concentrations were not different between the two study days ($p = 0.12$ and $p = 0.64$ respectively). Figure 3.1 shows the mean plasma glucose and plasma insulin concentrations during the FSIVGTT.

Figure 3.1. Mean plasma glucose and insulin concentrations during the FSIVGTT



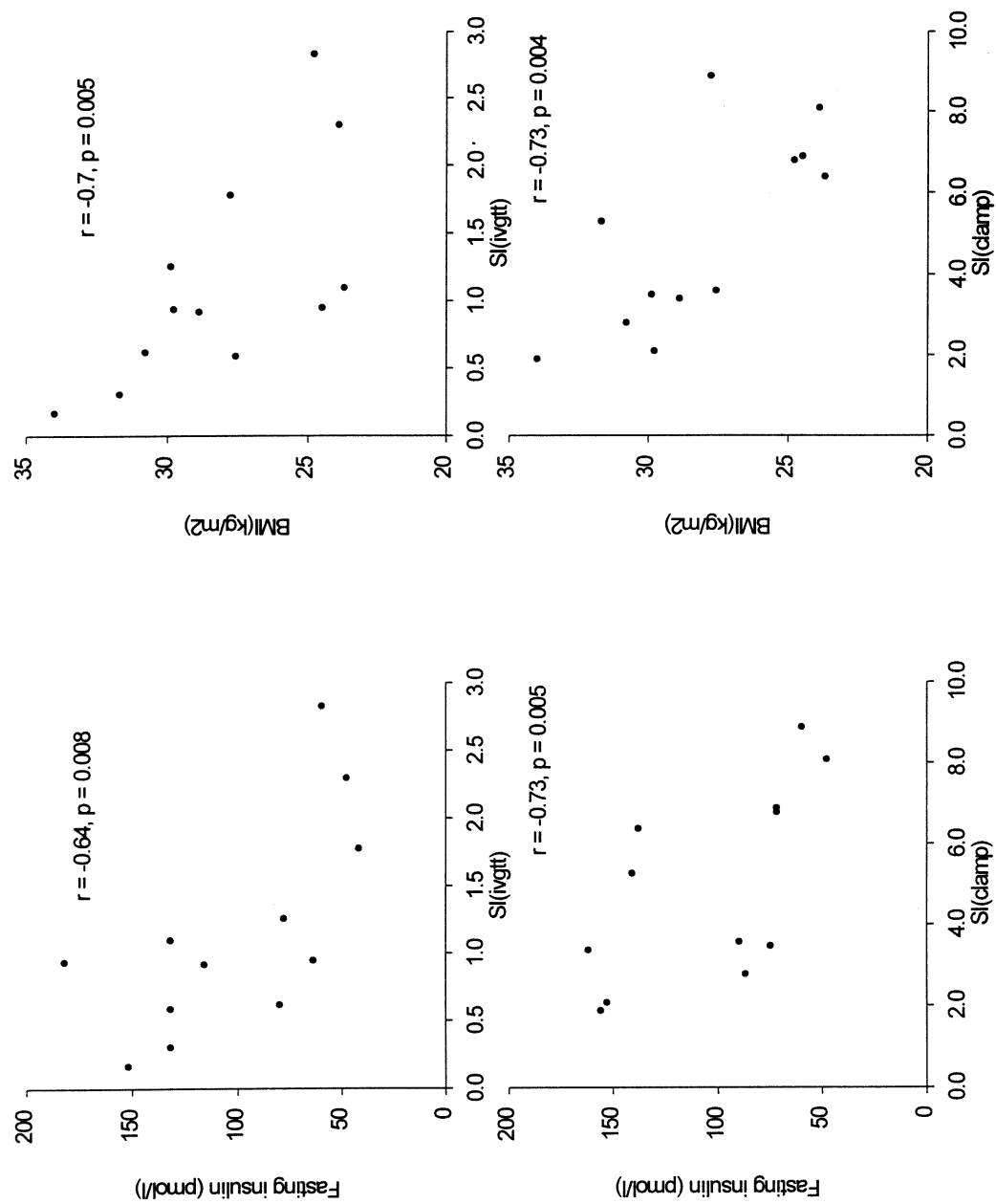
$SI_{(ivgtt)}$ and $SI_{(clamp)}$ were highly correlated ($r = 0.73$, $p = 0.004$) (Figure 3.2).

Figure 3.2. Correlation between $SI_{(ivgtt)}$ and $SI_{(clamp)}$



$SI_{(ivgtt)}$ also correlated significantly with fasting insulin concentrations ($r = -0.64$, $p = 0.008$) and body mass index ($r = -0.7$, $p = 0.005$) (Figure 3.3 upper panels). $SI_{(clamp)}$ was significantly correlated with both fasting insulin concentrations ($r = -0.73$, $p = 0.005$) and body mass index ($r = -0.73$, $p = 0.004$) (Figure 3.3, lower panels).

Figure 3.3. Correlations between SI(ivgtt), SI(clamp), fasting IRI and BMI



$SI_{(ivgtt)}$ was also calculated using the more commonly employed 180 minute sampling time frame. Again, all plasma insulin and glucose profiles were successfully modelled and derived a mean $SI_{(ivgtt\ 0-180)}$ of 1.03 (0.16) $\text{min}^{-1}/\text{mU}/\text{ml}\times 10^4$. This was not significantly different from $SI_{(ivgtt\ 0-240)}$ ($p = 0.87$) but correlated less well with $SI_{(clamp)}$ ($r = 0.52$, $p = 0.04$)

3.4 Discussion

It is clear from earlier studies that the techniques available for the assessment of insulin action i.e. the glucose clamp [DeFronzo et al,1979], continuous infusion of glucose with model assessment (CIGMA) [Hosker et al,1985], the short insulin tolerance test [Akinmokun et al,1992] and minimal modelling of the FSIVGTT [Bergman et al,1979] are all intended to measure a quantity felt to represent the individuals state of insulin sensitivity [Ng,1988]. Consequently, the choice of method depends on the aims and scope of the study and the investigators preference. The glucose clamp has been widely exploited for the study of insulin resistance in subjects with NIDDM, but is time consuming and labour intensive to conduct and therefore suitable mainly when small numbers of subjects are involved. Minimal model analysis of glucose and insulin data from the FSIVGTT is also labour intensive but relatively simpler to carry out providing estimates of both insulin sensitivity and glucose effectiveness either or both of which may be pathophysiologically important in NIDDM [Taniguchi et al,1992]. The insulin modification of the FSIVGTT also avoids the potential unpredictability of the quantitative and qualitative β -cell response to sulphonylureas in NIDDM subjects implicit in the tolbutamide modified FSIVGTT.

This study demonstrates that estimates of insulin sensitivity derived from the insulin-modified FSIVGTT correlate well with estimates of insulin sensitivity derived from the isoglycaemic hyperinsulinaemic clamp in the same individuals. It is also encouraging that both sets of estimates provide equally strong correlations with other factors known to be associated with insulin resistance, namely fasting plasma insulin concentration [DeFronzo et al,1992] and obesity [Björntorp,1991; Duysinx et al,1994; Krempf et al,1994].

These findings merit comparison to those of the Insulin Resistance Atherosclerosis Study (IRAS) group [Saad et al,1994]. The IRAS group recently reported good agreement between clamp and extended sampling protocol insulin-modified FSIVGTT derived estimates of insulin sensitivity in normal healthy subjects ($r=0.53$) although correlations were weaker in subjects with impaired glucose tolerance ($r=0.48$) and NIDDM ($r=0.41$). Importantly, in up to 50% of NIDDM subjects studied by the IRAS group, $SI_{(ivgtt)}$ could not be estimated with extended sampling protocol and was therefore set to zero. As a result of these findings, the authors recommended that the insulin-modified FSIVGTT should be used in population studies involving non-diabetic populations only and that additional studies were needed before advocating the routine use of this test in subjects with NIDDM

The current study demonstrated a greater correlation ($r = 0.73$, $p = 0.004$) than that observed by the IRAS study group between estimates of insulin sensitivity from the insulin-modified FSIVGTT and the isoglycaemic clamp in NIDDM subjects. The explanation for the discrepancy between these two studies investigating similar subjects must reside in the dose of insulin and the sampling schedule used in the insulin-modified FSIVGTT. This study employed a higher dose of insulin than that used in the IRAS study (0.05 vs 0.03U/kg) mainly because of concern over the marked insulin resistance of

NIDDM subjects and the absence of any published data comparing insulin doses. Indeed, the insulin dose may be more important than the sampling schedule, as when the FSIVGTT data was modelled using the more traditional 180 minute sampling schedule then a slightly reduced but still significant agreement ($r = 0.52$, $p = 0.04$) for the estimates of insulin sensitivity between the two techniques was derived. This level of agreement was still greater however than that achieved in the IRAS study with all $SI_{(ivgtt\ 0-180)}$ values distinguishable from zero.

These results demonstrate that the insulin-modified full sampling protocol intravenous glucose tolerance test with minimal model analysis provides a valid measure of insulin sensitivity justifying its continued use in the investigation of subjects with or at risk of NIDDM. For studies involving large numbers of NIDDM subjects, or studies in which major changes in insulin sensitivity may be expected, the 180 minute sampling schedule with the high dose insulin bolus should suffice. However for studies with small subject numbers or uncertainty over changes in insulin sensitivity, consideration to the full 240 minute sampling schedule should be given.

Chapter 4 - Reduced sampling protocols in the estimation of insulin sensitivity and glucose effectiveness using the "minimal" model in non-insulin dependent diabetes mellitus

4.1 Introduction

The use of the FSIVGTT in its original form as a means of estimating insulin sensitivity requires a large number of blood samples. This may reduce the usefulness of the method for large scale epidemiological studies. Recently Steil and co-workers [Steil et al,1993] suggested that the number of blood samples required for estimation of S_I and S_G in healthy subjects, the aged and subjects with gestational diabetes or drug-induced insulin resistance could be reduced to 12 with minor loss of precision.

As a result of this suggestion, data from 26 insulin-modified FSIVGTTs utilising the full sampling schedule carried out in subjects with established NIDDM was studied. The aim of this study was to determine whether the suggested reduced sampling regimen could produce estimates of S_I and S_G which satisfactorily approximate those estimates derived from the full sampling schedule or whether, as intimated by Steil [Steil et al,1991] and Bergman [Bergman et al,1992], further modification of the timings of the reduced sampling regimen could reduce bias and improve upon this precision.

4.2 Subjects and methods

Twenty-one normotensive NIDDM subjects (16 men, 5 women) were recruited from the Diabetic Clinic at the University Hospital of Wales, UK. All subjects were either newly presenting previously untreated (9 men, 4 women) or were treated by diet alone (7 men, 1 woman). All were free of specific diabetes related complications. Five subjects (4 men, 1 woman) had repeat tests 3 or 6 months later and their indicators of metabolic control were sufficiently different to regard the repeat tests as independent of the original tests, thus making a total data set of 26 full sampling schedule insulin-modified FSIVGTTs. Table 4.1 gives a summary of subject characteristics.

Table 4.1. Subject characteristics at initial FSIVGTT (16 men, 5 women)
(Median (IQR))

Age	57.0 (16.5)
HbA _{1c} (%)	10.1 (2.0)
Body mass index (kg/m ²)	28.8 (6.5)
Fasting plasma glucose (mmol/l)	11.5 (2.2)

The insulin-modified FSIVGTT took place in the manner previously described.

'Minimal' model analysis

S_i and S_e (full and reduced sampling schedules) with corresponding CVs were calculated from the FSIVGTT data using the MINMOD program [Pacini et al,1986]. Sample times used were 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 and 180

minutes to give $S_{I(30)}$ and $S_{G(30)}$, and as suggested by Steil [Steil et al,1993], 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180 minutes to give $S_{I(12)}$ and $S_{G(12)}$. A third pair of estimates, $S_{I(13)}$ and $S_{G(13)}$, was determined following the inclusion of the 25th minute time-point. In all cases the zero value was calculated as the mean of the basal samples.

Statistical Analysis

Percentage relative errors of S_I and S_G for the reduced sampling regimens versus the full sampling schedule were calculated according to the formula $100(\text{full-reduced})/(\text{full})$. The reduced sampling protocols were assessed in terms of bias (the presence of systematic error), precision of estimation (the level of agreement with the full sampling protocol values) and precision of determination (the size of the CVs).

The Wilcoxon signed rank test was used to evaluate the statistical support for bias in the estimation of $S_{I(30)}$ by $S_{I(12)}$ and $S_{I(13)}$, and of $S_{G(30)}$ by $S_{G(12)}$ and $S_{G(13)}$, p values are two-sided. Confidence intervals of the medians were calculated according to the nonparametric method of Gardner [Gardner et al,1989]. The interquartile ranges (IQRs) of %RE data were used to gauge the precision of the reduced sampling protocol estimates. The CVs calculated by MINMOD were used to assess the precision of determination. Data analysis was carried out using S-Plus software [Becker et al,1988].

4.3 Results

The estimates of insulin sensitivity and glucose effectiveness are given in Tables 4.2 and 4.3 respectively, together with their corresponding CVs and %REs. Median and IQR data are also given.

Table 4.2. Insulin sensitivity data ($\text{min}^{-1}/\text{mU/ml}$) $\times 10^4$

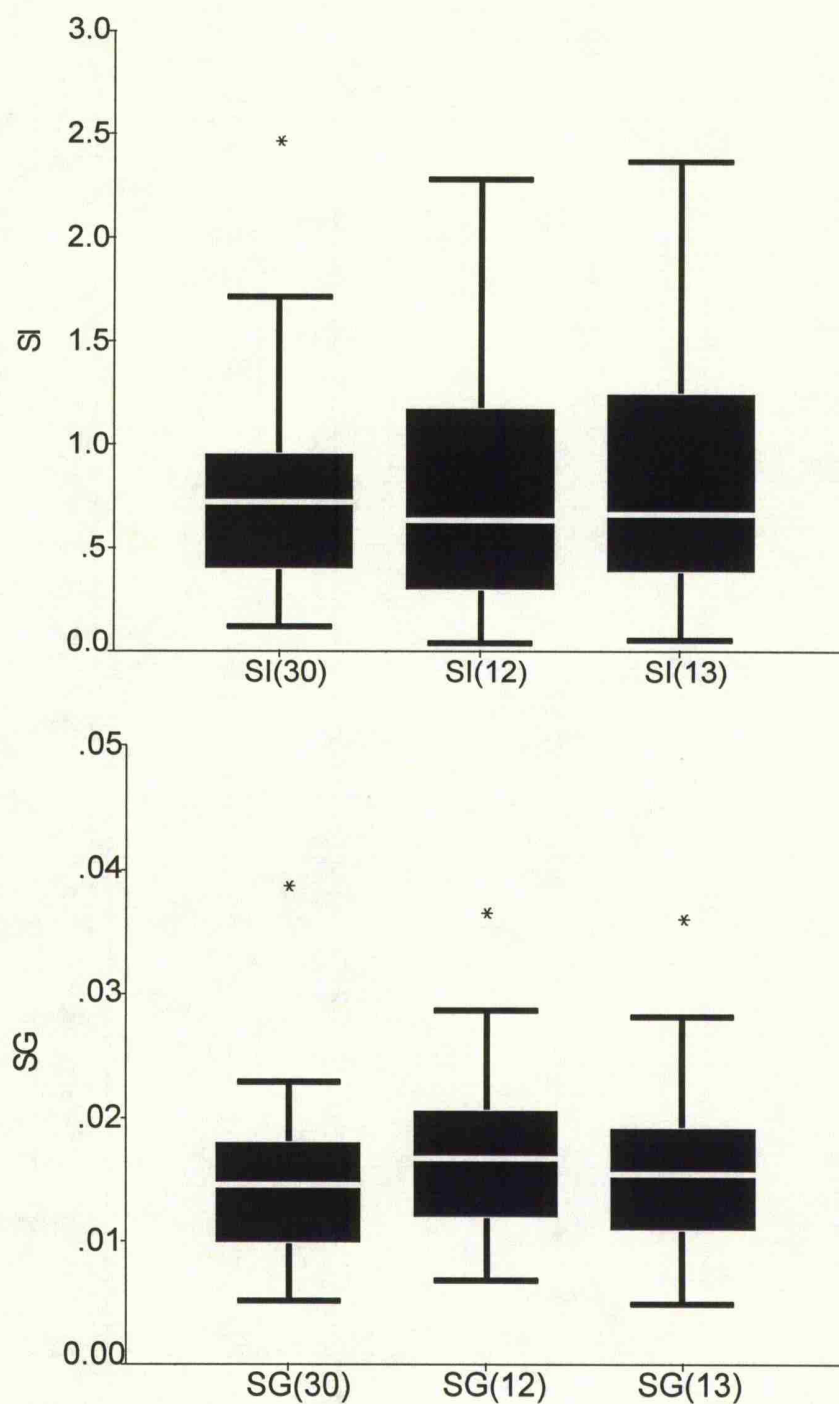
	Full		Reduced					
	SI(30)	CV(%)	SI(12)	CV(%)	%RE	SI(13)	CV(%)	%RE
	0.96	17	1.22	28	-27	1.25	27	-30
	0.40	54	0.42	66	-5	0.44	51	-10
	0.17	55	0.13	60	24	0.18	58	-6
	0.64	11	0.51	14	20	0.70	14	-9
	0.94	11	0.87	18	7	1.04	17	-11
	0.77	16	0.69	25	10	0.82	23	-6
	1.65	10	1.29	14	22	1.59	13	4
	1.35	10	1.18	18	13	1.37	17	-1
	0.43	24	0.32	52	26	0.39	47	9
	0.44	28	0.30	72	32	0.39	50	11
	0.67	15	0.46	28	31	0.56	25	16
	0.73	40	0.73	58	0	0.72	52	1
	0.16	117	0.28	117	-75	0.36	97	-125
	0.12	20	0.15	30	-25	0.21	29	-75
	1.72	10	1.21	13	30	1.52	14	12
	0.72	22	0.72	30	0	0.61	28	15
	0.82	18	0.69	28	16	0.70	24	15
	0.91	15	0.78	27	14	0.90	25	1
	0.12	56	0.04	236	67	0.06	243	50
	0.29	23	0.22	33	24	0.30	31	-3
	0.80	28	0.58	37	28	0.63	38	21
	1.32	18	1.40	63	-6	1.46	57	-11
	2.47	10	2.28	16	8	2.37	14	4
	1.58	11	1.46	16	8	1.75	15	-11
	0.49	22	0.32	30	35	0.46	27	6
	0.34	13	0.24	21	29	0.32	20	6
Median	0.725	18.4	0.635	29.0	15.1	0.665	27.0	1.2
IQR	0.548	15.3	0.798	37.5	25.2	0.808	31.2	20.7

Table 4.3. Glucose effectiveness data (min^{-1})

	Full		Reduced					
	SG(30)	CV(%)	SG(12)	CV(%)	%RE	SG(13)	CV(%)	%RE
	0.01898	6	0.01880	9	1	0.01952	8	-3
	0.01820	6	0.01770	10	3	0.01623	10	10
	0.01668	9	0.01610	14	3	0.01528	14	8
	0.00709	15	0.00690	24	3	0.00499	33	22
	0.00630	17	0.00690	25	-9	0.00627	26	-2
	0.01537	7	0.01530	11	0	0.01416	12	8
	0.00528	22	0.00900	18	-70	0.00695	22	-33
	0.01519	6	0.01860	8	-22	0.01779	8	-18
	0.01564	9	0.02060	11	-32	0.01921	11	-23
	0.01810	8	0.02660	9	-47	0.02311	9	-28
	0.01270	6	0.01560	8	-23	0.01454	8	-14
	0.02285	7	0.02369	10	-4	0.02308	10	-1
	0.03874	3	0.03650	5	6	0.03599	4	7
	0.01369	8	0.01200	15	12	0.01215	14	10
	0.00721	16	0.01070	16	-48	0.00995	16	-40
	0.00992	13	0.00980	21	1	0.01019	19	-2
	0.01088	12	0.01000	23	8	0.00910	24	18
	0.01404	9	0.02060	9	-47	0.01935	9	-38
	0.01903	6	0.02860	7	-50	0.02810	7	-45
	0.00878	14	0.01330	16	-52	0.01098	19	-30
	0.01972	12	0.01600	23	17	0.01533	23	20
	0.01080	18	0.01750	45	-62	0.01759	41	-63
	0.00935	7	0.01740	8	-86	0.01560	8	-67
	0.01690	6	0.01900	8	-12	0.01813	8	-7
	0.01623	11	0.02350	11	-45	0.01818	14	-13
	0.01214	7	0.01360	9	-12	0.01248	9	-3
Median	0.01462	8.6	0.01675	11.1	-12.2	0.01547	11.3	-4.8
IQR	0.00766	6.4	0.00767	9.0	49.2	0.00768	10.9	36.5

Boxplots showing the median (black line inside the box), IQR (height of the box) and outliers (dots outside the "whiskers") are presented for insulin sensitivity and glucose effectiveness data in Figure 4.1 (overleaf).

Figure 4.1. Distributions of the insulin sensitivity and glucose effectiveness data



Bias of estimates

Table 4.4 (overleaf) gives the statistics calculated to assess the bias introduced by the reduced sampling regimens.

Table 4.4. Bias of estimates

	median %RE	95% CI*	Z statistic	p value
$S_{I(12)}$	15.1	(7.4, 25.6)	2.706	0.0068
$S_{I(13)}$	1.2	(-9.4, 9.3)	0.1524	0.8789
$S_{G(12)}$	-12.2	(-46.7, 1.2)	-2.819	0.0048
$S_{G(13)}$	-4.8	(-27.8, 6.8)	-2.108	0.0350

*Actually 97% confidence intervals (nonparametric method of calculation)

Both confidence intervals and the test statistics suggest that $S_{I(12)}$ is a significantly biased estimate of $S_{I(30)}$ whereas this is not the case for $S_{I(13)}$. The bias introduced by the 12 point sampling regimen was of the order of 15% ($p = 0.0068$) while the corresponding value for $S_{I(13)}$ was 1% ($p = 0.8789$). Results for glucose effectiveness were equivocal. The Wilcoxon signed rank test suggested that $S_{G(12)}$, was a significantly biased estimate of $S_{G(30)}$ ($p = 0.0048$), however the confidence interval includes zero. This is unusual and may be due to a combination of the effects of bimodality in the $S_{G(12)}$ %RE data and the nonparametric method of calculating the confidence intervals. The median %RE was -12% for $S_{G(12)}$. The Wilcoxon signed rank test suggested that $S_{G(13)}$ was also a biased estimate of $S_{G(30)}$ at the 5% level. The median %RE for $S_{G(13)}$ was -5%.

Precision of estimation

Figure 4.2 shows boxplots of insulin sensitivity %REs and glucose effectiveness %REs respectively ($S_{I(13)}$ IQR 21% against $S_{I(12)}$ IQR 25%) ($S_{G(13)}$ IQR 37% against $S_{G(12)}$ IQR 49%). These, together with figures 4.3 (%REs versus full sampling schedule estimates), demonstrate the reduction in both the size and spread of %REs obtained by introducing the 25 minute time-point data.

Figure 4.2 Distributions of %REs of insulin sensitivity and glucose effectiveness estimates.

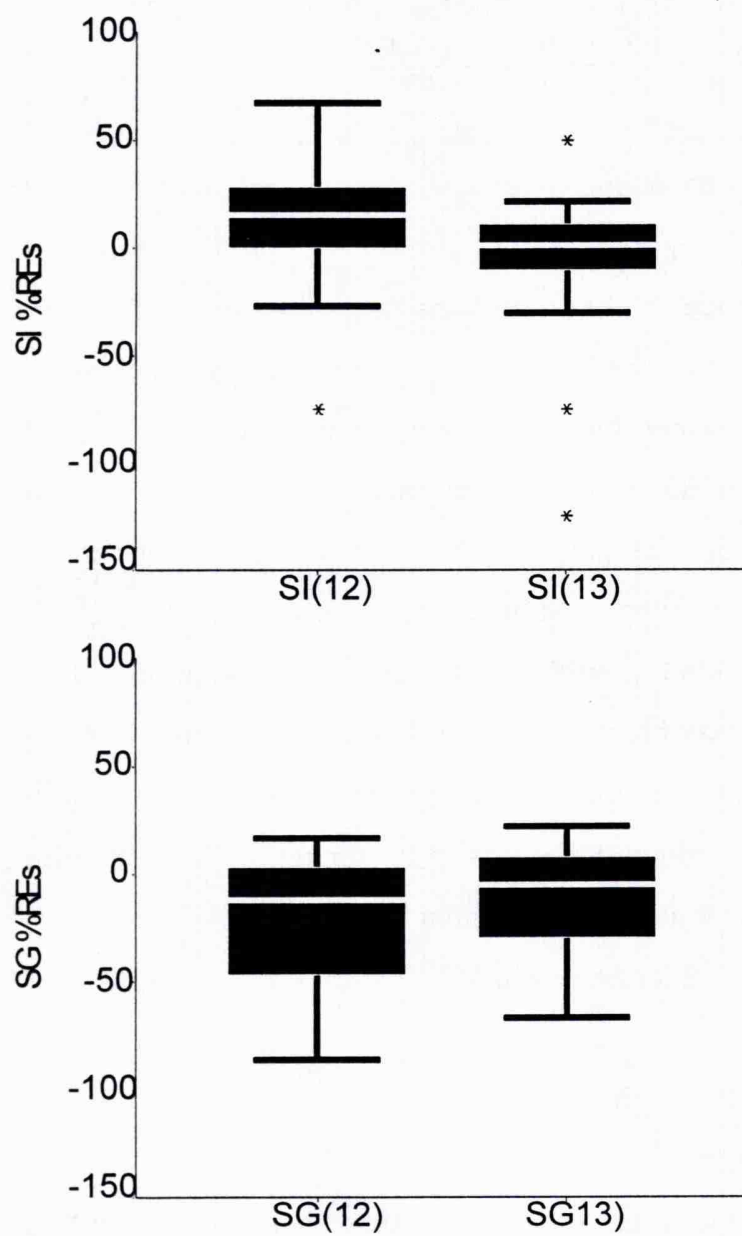
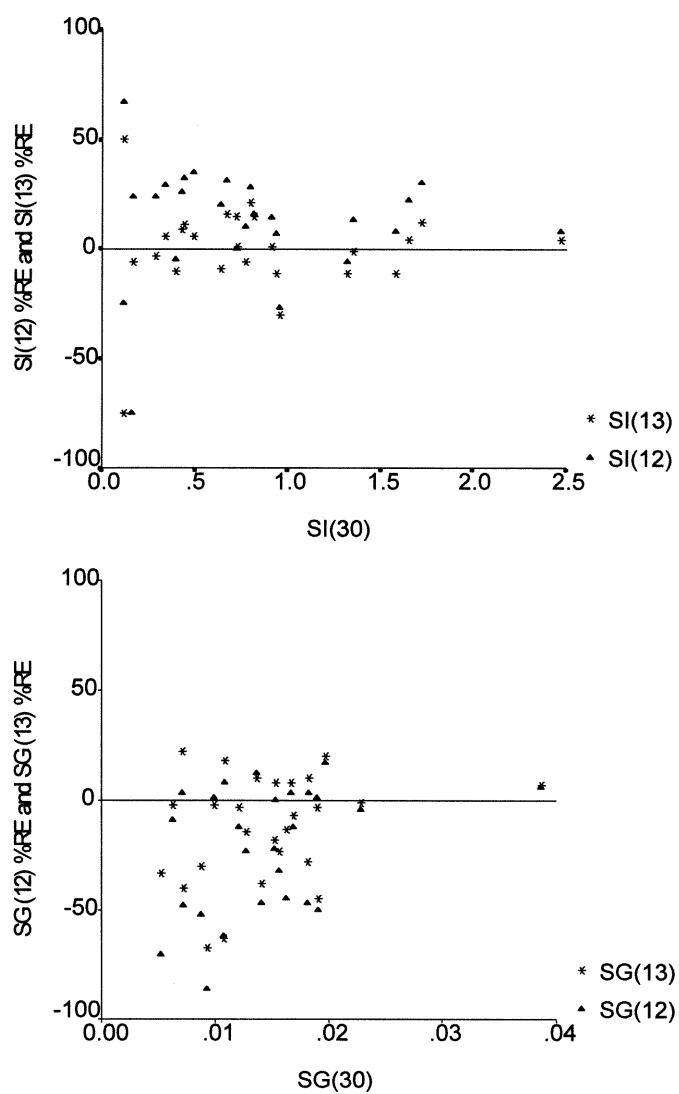


Figure 4.3. Scatterplots of %RE data from the reduced sampling schedules.
Insulin sensitivity data and glucose effectiveness data.



Precision of determination

CVs of the "minimal" model estimates were of the order of 18%, 29% and 27% for $S_{I(30)}$, $S_{I(12)}$ and $S_{I(13)}$; and of 9%, 11% and 11% for $S_{G(30)}$, $S_{G(12)}$ and $S_{G(13)}$ respectively. Median increases in CV for $S_{I(12)}$ and $S_{I(13)}$ compared to $S_{I(70)}$ were 9% and 7% respectively. Median increases in CV for $S_{G(12)}$ and $S_{G(13)}$ compared to $S_{G(30)}$ were both 2%.

4.4 Discussion

The advantages of the full sampling schedule FSIVGTT include the comparative simplicity of the technique and its ability to provide estimates of both S_I and S_G but in its original form it requires a large number of blood samples to be taken. Based on the study of a small number of normal subjects ($n=10$), Steil et al attempted to demonstrate that a reduced sampling regimen in a tolbutamide-modified IVGTT could adequately estimate S_I and S_G in a wider group ($n=87$, with a total of 118 tolbutamide modified FSIVGTTs) including healthy subjects, the aged and subjects with gestational diabetes or drug-induced insulin resistance. They suggested that this modification makes the technique suitable for use in certain population studies [Steil et al,1993]. The current study addresses the specific questions of the introduction of bias and the precision of estimation and determination of S_I and S_G from two reduced sampling regimens employed during an insulin-modified FSIVGTT in subjects with NIDDM treated by diet alone.

In the present study, statistically significant and clinically important bias was introduced by the use of reduced sampling regimens in the case of $S_{I(12)}$ but not $S_{I(13)}$. Results for $S_{G(12)}$ and $S_{G(13)}$ were statistically equivocal but the

introduction of bias cannot be discounted. Introduction of the 25 minute time-point data resulted in improvement in the accuracy and precision of estimation of the $S_{I(13)}$ values. The precision of estimation of the $S_{I(12)}$, $S_{G(12)}$ and $S_{G(13)}$ values were also substantially decreased. This 25-minute time-point was introduced empirically because of concerns over the reconstruction of the insulin profile in the critical period following the insulin bolus at +20 minutes. The precision of determination of S_I was markedly reduced for both $S_{I(12)}$ and $S_{I(13)}$ but this was not the case for $S_{G(12)}$ and $S_{G(13)}$. The CVs reported here appear larger than those reported originally by Bergman [Bergman et al,1979] and recently by Steil [Steil et al,1993] but this may be as a result of the low S_I values estimated in these NIDDM subjects. The major concern remains however that in the study of NIDDM subjects these effects on bias, accuracy and precision introduced by the reduced sampling schedule could accumulate, making the resulting values of little use even for large population studies.

Subsequent work by Steil, assessing the reproducibility of the tolbutamide-modified FSIVGTT in non-diabetic obese subjects [Steil et al,1994], failed to demonstrate the introduction of bias in the estimation of S_I using the 12 sample data set, however the precision of estimation was significantly reduced. The introduction of the 25-minute data point reduced this error in the precision of estimation and its inclusion in reduced sampling schedules is recommended. Also raised in the latter study is the increasingly relevant issue of the trade-off between blood sample numbers (and therefore cost) and statistical power in studies. Steil recommends the use of the reduced (13 sample) data set only in studies where there are a large number of subjects or where the predicted changes in S_I are large [Steil et al,1994].

It is also of additional interest that the IRAS group also investigated the use of the reduced sample protocol for the insulin-modified FSIVGTT in their comparative study with the isoglycaemic hyperinsulinaemic clamp. For the NIDDM subjects in their study they were unable to model 50% of the data and the remaining estimates of insulin sensitivity did not correlate at all with those from the isoglycaemic hyperinsulinaemic clamp. This lead Saad to conclude that the reduced sample protocols were unsuitable for use in the investigation of NIDDM subjects [Saad et al,1994].

The present study suggests that for the study of subjects with NIDDM the 12 time-point protocol for the IVGTT in conjunction with MINMOD analysis should not be used unless in large population studies where appropriate power calculations are carried out. A 13 time-point protocol as described here is clearly more acceptable for the estimation of insulin sensitivity in large population studies of NIDDM but still cannot be recommended for the assessment of glucose effectiveness, a fact appreciated and commented upon by Steil and colleagues [Steil et al,1993]. Therefore, for clinical research in subjects with NIDDM, the retention of the full sampling protocol would appear to be prudent at the present time.

Chapter 5 - Estimation of insulin sensitivity by minimal model analysis of the insulin-modified frequently sampled intravenous glucose tolerance test (FSIVGTT) in non-insulin dependent diabetes mellitus - effect of insulin assay method

5.1 Introduction

The minimal model technique was developed using plasma insulin concentrations from the FSIVGTT measured by radioimmunoassay of plasma [Bergman et al,1979]. It is now clear that these assays both cross-react with proinsulin and other insulin-like molecules and that subjects with NIDDM have elevated concentrations of proinsulin and its split products, both basally (fasting) and when stimulated by glucose or other insulin secretagogues [Yoshioka et al,1988; Temple et al,1989; Temple et al,1992; Davis et al,1993]. The more recent development of enzyme-linked immunoassays (ELISAs) with the use of monoclonal antibodies directed at different epitopes on the insulin molecule, has lead to the availability of a number of specific and sensitive assays for plasma insulin designed to eradicate cross-reactivity with other insulin-like molecules [Storch et al,1989; Andersen et al,1993]. As a result of these improvements in assay technology and the increasing requirement for standardisation of insulin assays it is likely that these assays will become used routinely in the future.

For the calculation of S_I in subjects with NIDDM using minimal model analysis of the FSIVGTT, submission of the typically lower (compared to RIA) ELISA-derived insulin concentrations [Temple et al,1990] to the MINMOD program may lead to an overestimation of S_I . The purpose of this study was to examine the error, if any, introduced into the estimation of S_I and S_G by such a submission. To achieve this, estimates derived from ELISA plasma insulin

concentrations were compared to those derived using plasma insulin concentrations from a standard radioimmunoassay.

5.2 Subjects and methods

This study involved the re-assay of 23 of the 26 plasma sets obtained from the previous study of reduced sample numbers in the accurate modelling of the insulin-modified FSIVGTT. The original IRI data was retained and the plasma samples were reassayed using the highly sensitive and specific enzyme-linked immunoassay described by Anderson [Andersen et al,1993]. Three of the insulin-modified FSIVGTT sample sets could not be reassayed for technical reasons - lack of plasma (one set), degradation of samples (two sets). Table 5.1 gives the revised study subject characteristics.

Table 5.1. Characteristics of the 20 NIDDM subjects at the time of the initial insulin-modified FSIVGTT (Median (IQR))

	Median (IQR)
Age	55.3(12.0)
Body mass index (kg/m ²)	28.2 (6.4)
Fasting plasma glucose (mmol/l)	11.4 (1.5)
HbA _{1c} (%)	9.9 (1.7)
Fasting ELISA insulin (pmol/l)	49.5 (27.0)*
Fasting RIA insulin (pmol/l)	106.8 (52.4)

*p<0.0001 versus fasting RIA insulin.

Minimal model analysis

Insulin sensitivity and glucose effectiveness with corresponding FSDs were calculated from the FSIVGTT data using the MINMOD program. Input data from the RIA was used to derive the estimates $S_{I(I)}$ and $S_{G(I)}$ and from the ELISA assay to derive the estimates $S_{I(E)}$ and $S_{G(E)}$.

Statistical analysis

The differences between the estimates of S_I and S_G derived by the use of RIA and ELISA data were assessed in terms of agreement of the estimates and precision of determination (the fractional standard deviations of the estimates calculated by MINMOD) as described in the previous Chapter. These data were not normally distributed, therefore nonparametric tests were used in the analysis.

5.3 Results

Agreement of estimates

Figure 5.1 gives the individual S_I and S_G estimates derived from the ELISA method (left side of figures) with the corresponding estimate derived from the RIA (right side of figures). Table 5.2 gives the median and interquartile range (IQR) values for $S_{I(I)}$, $S_{I(E)}$, $S_{G(I)}$ and $S_{G(E)}$.

Figure 5.1. S_I and S_G estimates by assay method

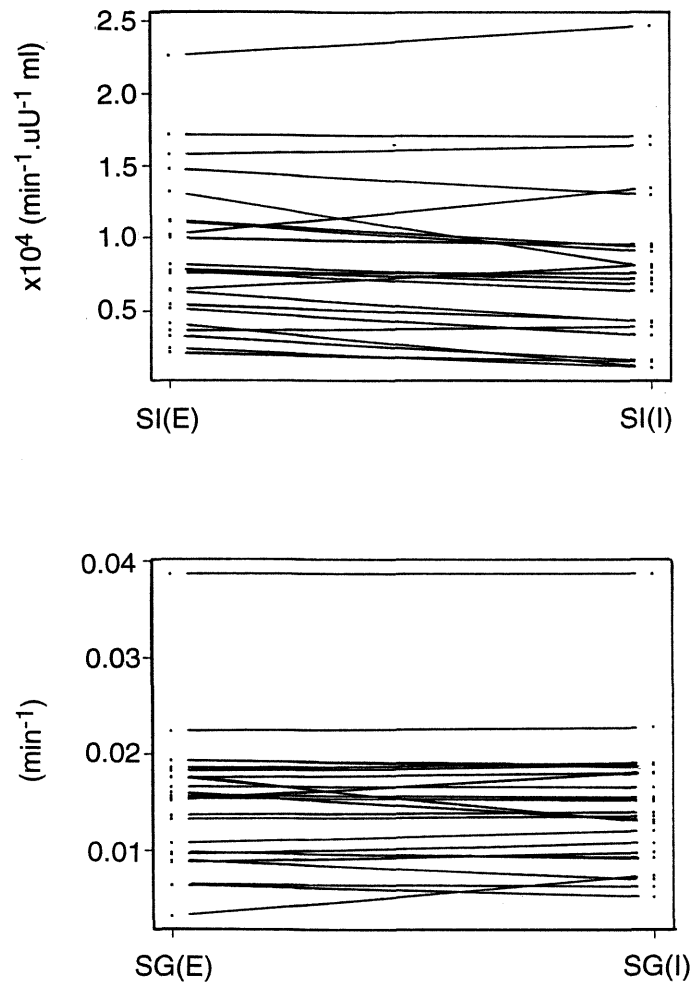


Table 2. Results of MINMOD S_I and S_G estimates from the 23 insulin-modified FSIVGTTs by insulin assay method

ELISA		RIA	
S _I $\times 10^4 \text{(min}^{-1} \text{/uU/ml)}$	S _G $\text{(min}^{-1})$	S _I $\times 10^4 \text{(min}^{-1} \text{/uU/ml)}$	S _G $\text{(min}^{-1})$
0.77 (0.58)	0.01539 (0.00793)	0.73 (0.535)	0.01404 (0.00775)

Figure 5.2 depicts boxplots of the paired differences between the $SI_{(E)}$ and $SI_{(I)}$ estimates (upper panel) and the $SG_{(E)}$ and $SG_{(I)}$ estimates (lower panel). Table 5.3 gives the statistics calculated to assess the agreement of the estimates of insulin sensitivity and glucose effectiveness using the RIA and ELISA data.

Figure 5.2.

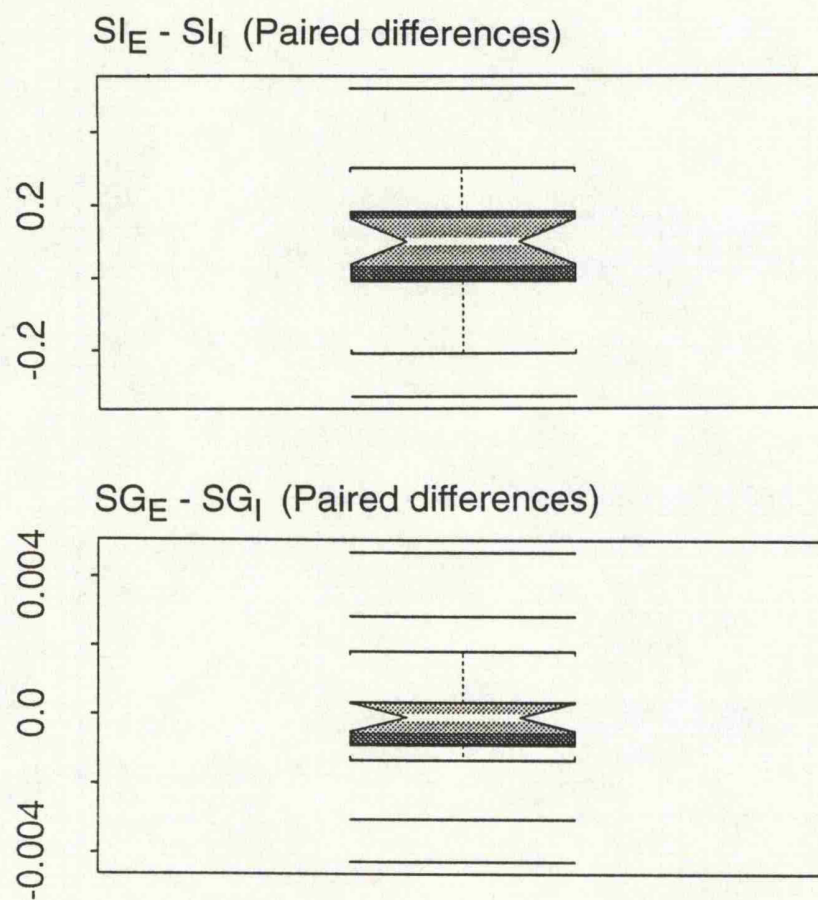


Table 5.3. Accuracy of estimation of S_I and S_G from the 23 insulin-modified FSIVGTTs by insulin assay method

Paired differences	95% CI	Z-statistic	p value
$S_{I(E)} - S_{I(I)}$	0.17, 0.04	2.1293	0.033
$S_{G(E)} - S_{G(I)}$	0.0004, -0.0004	-0.7452	0.456

Clearly, for the estimation of insulin sensitivity, there was a statistically significant ($p=0.033$) increase in the estimates, derived from the ELISA data compared to those derived from the RIA data. Similar differences did not apply to the estimation of glucose effectiveness.

Precision of determination

Figure 5.3 gives the sizes of individual FSDs of estimates of S_I and S_G for the ELISA method (left side of figure) and the RIA (right side figure). Table 5.4 gives the statistics calculated to assess the precision of determination of the estimates of insulin sensitivity and glucose effectiveness using the ELISA and RIA data.

Table 5.4. Precision of determination of S_I and S_G from the 23 insulin-modified FSIVGTTs by insulin assay method

Paired differences	95% CI	V-statistic	p value
$S_{ISD(E)} - S_{ISD(I)}$	0.005, 0.04	208	0.033
$S_{GSD(E)} - S_{GSD(I)}$	0.00003, -0.00003	137	0.998

Figure 5.3. FSDs for estimates of S_I and S_G for the ELISA (left side of figure) and th RIA (right side of figure)

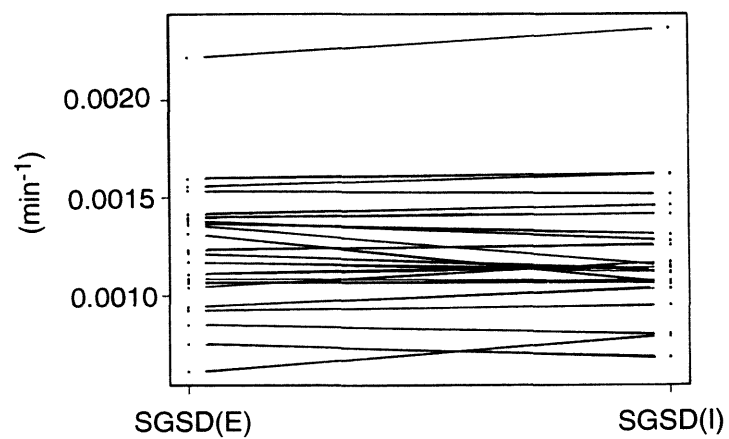
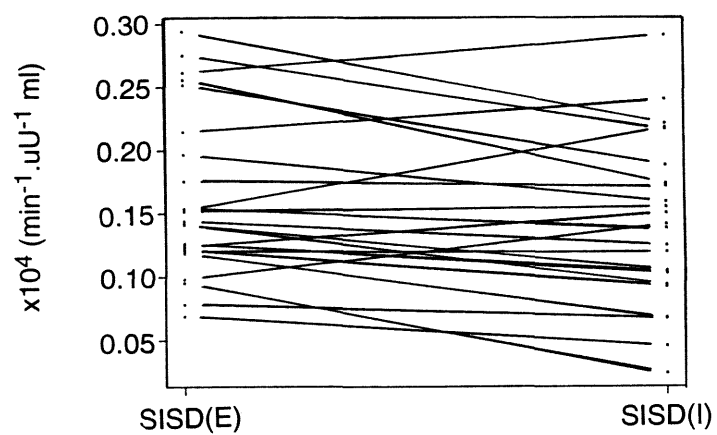
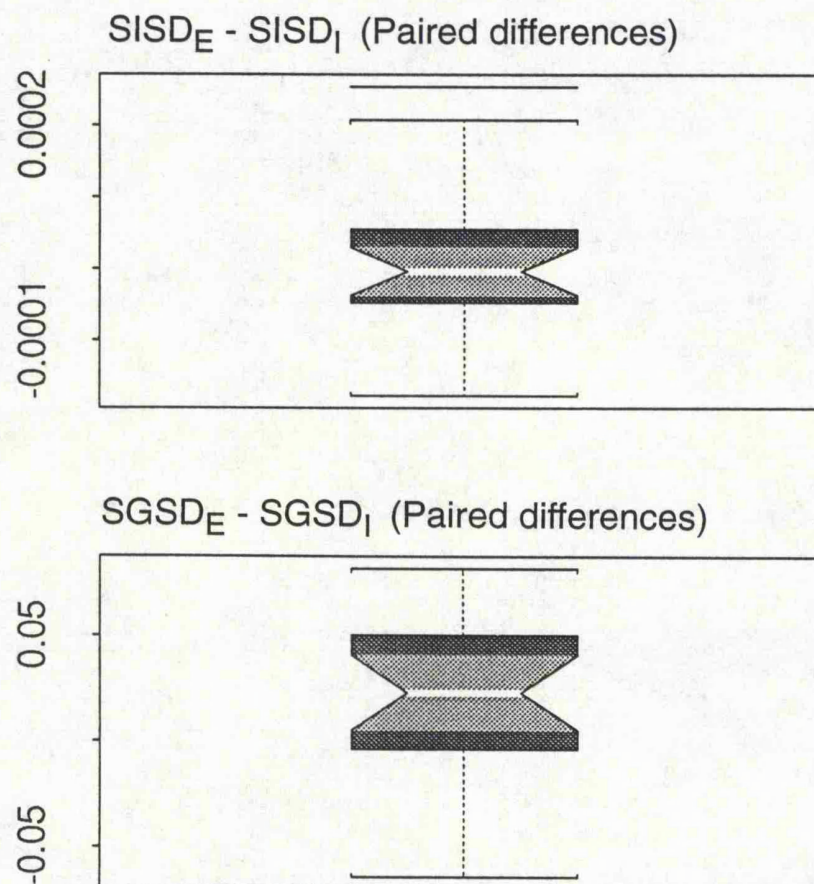


Figure 5.4 depicts boxplots of the paired differences of the $S_{ISD(E)}$ and $S_{ISD(I)}$ (upper panel) and $S_{GSD(I)}$ and $S_{GSD(I)}$ (lower panel).

Figure 5.4.



Again, the sizes of the SDs of individual estimates of insulin sensitivity from the MINMOD program were significantly different between the assay techniques ($p=0.033$), but this did not apply to the estimation of glucose effectiveness. In fact the narrow confidence interval including zero strongly supports the equality of $S_{GSD(E)}$ and $S_{GSD(I)}$.

5.4 Discussion

A potential source of error in the use of the insulin-modified FSIVGTT to assess insulin sensitivity is the increase in the use of highly specific ELISA assays for measuring plasma insulin concentrations.

In the current study, this problem has been addressed by comparing the results of MINMOD estimates of S_I and S_G using plasma insulin concentrations derived from both a conventional radioimmunoassay (RIA) [Heding,1972] and a highly specific and sensitive enzyme-linked immunoassay (ELISA) demonstrated not to cross react with the major circulating insulin-like components, intact proinsulin, split (32-33) and des (31,32) proinsulin [Andersen et al,1993]. As expected, estimates of S_G were not different between the two assay methods as the estimation of glucose effectiveness is independent of changes in insulin concentrations.

Estimates of S_I were significantly different between the two assay methods. The lower concentrations of 'true insulin' measured by the ELISA lead to a subsequent statistically significant overestimation of S_I by the MINMOD program. The clinical, pathophysiological or epidemiological significance of an approximately 4% higher median S_I estimate utilising ELISA insulin concentrations in the insulin-modified FSIVGTT in subjects with NIDDM remains unknown but it is unlikely to be great, especially as insulin sensitivity is characteristically low in this group of subjects,. Differences in S_I estimates have, however, recently been demonstrated in nondiabetic obese subjects with those from ELISA insulin concentrations up 20% higher than those derived from standard RIA insulin concentrations [Lovejoy et al,1994]. Perhaps of greater significance in subjects with NIDDM would be the difference in the estimates of S_I derived from the tolbutamide modified

FSIVGTT and the unmodified or tolbutamide modified FSIVGTT in subjects with conditions such as hypertension or obesity which are known to be associated with hyperinsulinaemia and insulin resistance [Sims et al,1982; Modan et al,1985; Björntorp,1991].

Therefore, insulin assay method does appear to influence the estimation of S_I by MINMOD, both in non-diabetic obese subjects and subjects with NIDDM. The ability to interpret and compare MINMOD estimates of S_I from either modified or unmodified FSIVGTTs in a variety of disease states will require caution depending on the method employed for the measurement of plasma insulin and the modification of the FSIVGTT.

In view of the above studies the estimation of insulin sensitivity in NIDDM subjects using 'minimal' model analysis of the insulin-modified FSIVGTT appears to require the retention of the full sampling schedule as originally described, the use of a high dose insulin bolus and the continuing use of the radioimmunoassay of insulin, at least until the standardisation of insulin assays. Studies reported in subsequent chapters all employ the above criteria.

Chapter 6 - The spectrum of metabolic and hormonal responses to a mixed meal test at the time of diagnosis in established NIDDM

6.1 Introduction

Patients presenting with fasting hyperglycaemia diagnostic of NIDDM have evidence of both β -cell dysfunction and insulin resistance of varying degrees. Evidence for the co-existence of these two pathological processes in NIDDM has been exhaustively presented [DeFronzo et al,1992] and these abnormalities are already detectable to some extent in glucose tolerant individuals considered 'at risk' of developing the syndrome [Cerasi et al,1972; O'Rahilly et al,1988; Eriksson et al,1989; Gulli et al,1992]. The pathophysiological picture of NIDDM is also complicated by the co-existence of obesity, itself associated with insulin resistance, in up to 60% of patients with this form of diabetes [UKPDS Group,1988]. An attempt to 'metabolically stage' these patients near to the time of diagnosis prior to any therapeutic intervention in terms of their responses to a mixed meal challenge may help to indicate the possible sequence of pathophysiological events in the 'natural history' of the disease which could have significant therapeutic and prognostic implications [Granner et al,1992]. Previous efforts to define the metabolic and hormonal responses of patients with a wide range of fasting plasma glucose (FPG) levels have clouded rather than clarified the issue. Studies in the last 30 years utilising both oral and intravenous glucose tolerance tests have observed either low, normal or high fasting immunoreactive insulin (IRI) concentrations in NIDDM subjects coupled with a reduced or absent 'early-phase' IRI secretory response in over 70% of the reports [Perley et al,1967; Kosaka,1978; DeFronzo et al,1982b; Bogardus et al,1984a; Efendic et al,1988; Temple et al,1992]. Similarly the 'late-phase' and total IRI responses to an oral glucose challenge have also been shown to be either reduced,

normal or increased when compared to healthy non-diabetic subjects [DeFronzo et al,1982b; Temple et al,1992]. The lack of homogeneity in these findings derives from the variety of test protocols applied, the study of subjects from different ethnic backgrounds, the failure to control for degree of obesity and treatment and the use of the radioimmunoassay of insulin which overestimates true insulin concentrations [Temple et al,1990].

The aim of this study was to 'metabolically stage' a large number of individuals with NIDDM at or near the time of diagnosis prior to any dietary or therapeutic intervention. The diabetic subjects were allocated to one of four sub-groups defined arbitrarily based on the fasting glucose and the level of obesity as defined by the body mass index (BMI). A group of healthy normal subjects were also recruited for the purpose of comparison.

6.2 Subjects and methods

With local ethical committee approval, newly diagnosed subjects with NIDDM were recruited at their first visit to the diabetic clinic. A total of 188 islet cell antibody negative Caucasian subjects gave informed consent to take part in the study. None had received dietary advice or oral hypoglycaemic agents prior to study and no patients had any evidence of clinically significant cardiac, hepatic, renal or other endocrine disease at the time of investigation. Additionally, a group of 38 non-diabetic subjects underwent identical investigation. All non-diabetic subjects were healthy volunteers (subsequently referred to as 'normals') obtained by advertisement within the local community with no family history of diabetes and taking no medication at the time of study. Each subject underwent a mixed meal tolerance test according to the protocol defined in Chapter 2. Insulin concentrations reported represent

measurements using the radioimmunoassay of insulin to allow comparison with previous studies.

Statistical Analysis

Subjects were classified according to a stratification based on (approximate) quartiles of the FPG data and tertiles of the BMI data. FPG categories were < 9, 9-12, 12-15 and >15 mmol/l, and BMI categories were <26.5, 26.5-30 and >30 kg/m². These categories were somewhat arbitrary, but they maintained a balance between sensitivity and group size.

The nonparametric method of Meddis [Meddis,1980] for the analysis of variance by ranks of unbalanced, multiple sample, multiple block designs was used to examine the statistical support for trends in sample medians. Analyses were blocked by BMI or FPG as appropriate and were undertaken using methods developed with S-Plus [Becker et al,1988] software.

6.3 Results

Tables 6.1 and 6.2 contain the characteristics of the diabetic subjects stratified according to FPG concentrations and body mass index, respectively. Figure 6.1 uses boxplots to show the plasma glucose and insulin data from the meal test in detail. Boxplots show the median (white line in box), interquartile range (height of box), range (whiskers) and extreme values (—).

Table 6.1. Subject characteristics (median (interquartile range)) by FPG (mmol/l).

Normals		FPG group			
n=38		<9 (n=39)	9-12 (n=66)	12-15(n=46)	≥15 (n=37)
Age (yrs)	55.5 (16.0)	52 (14.5)	57 (17)	48 (10.8)	58 (12.0)
BMI (kg/m ²)	24.4 (3.9)	27.9 (5.5)	28.8 (6.4)	27.5 (6.0)	27 (5.1)
FPG (mmol/l)	5.5 (0.4)	8.0 (1.0)	10.4 (1.7)	13.3 (1.5)	16.5 (2.2)
HbA _{1c} (%)	6.6 (1.2)	8.5 (1.1)	10.5 (1.9)	12.1 (2.0)	13.4 (2.9)
SEX (%m,%f)	42,58	87,13	74,26	63,37	68,32

Table 6.2 Diabetic subject characteristics (median(interquartile range)) by BMI (kg/m²)

	BMI group		
	<26.5 (n=65)	26.5-30 (n=60)	>30 (n=63)
Age (yrs)	56 (16.0)	57.5 (14.5)	49 (13.0)
BMI (kg/m ²)	24.5 (2.6)	27.9 (1.8)	33.0 (5.0)
FPG (mmol/l)	12.3 (4.9)	11.2 (5.1)	11.2 (4.3)
HbA _{1c} (%)	11.4 (3.5)	11.0 (3.7)	10.8 (2.8)
SEX (%m,%f)	88,12	70,30	60,40

Figure 6.1. Plasma glucose and insulin profiles from the MTT by FPG

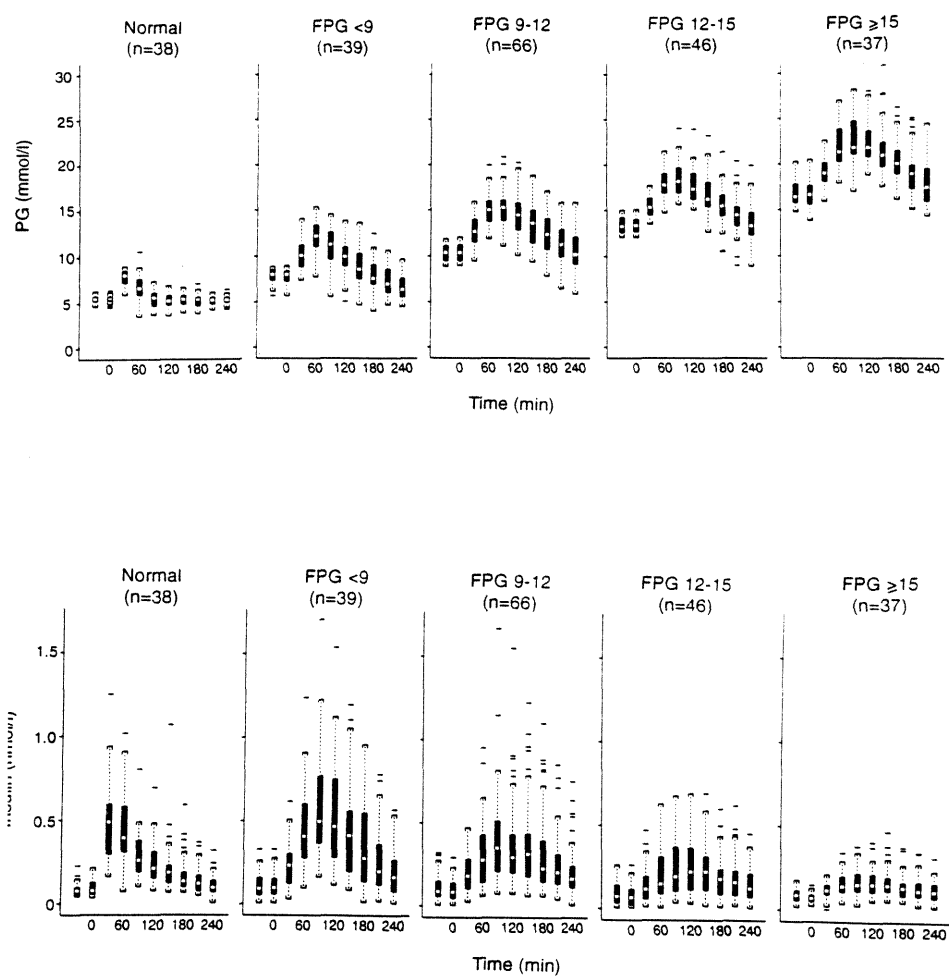


Table 6.1 suggests that BMI fell as FPG increased although this was not supported statistically. When stratified by BMI (Table 6.2) no difference could be shown for median FPG values. Table 6.3 illustrates the basal, maximum and 4 hour plasma glucose and IRI concentrations from the MTT. Figure 6.2 illustrates the AUC IRI data from the MTT stratified according to FPG.

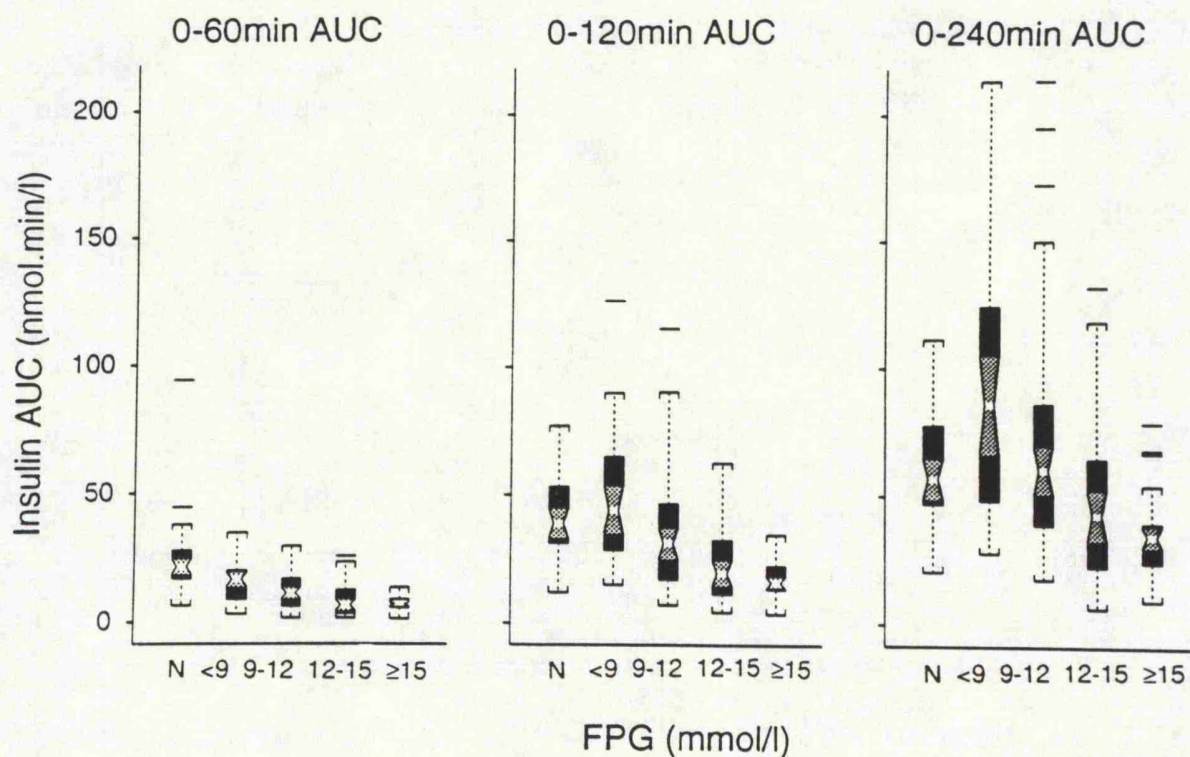
Table 6.3. MTT Plasma glucose and IRI data (median (semiquartile range))

FPG group	PG _{fasting} mmol/l	PG _{max} mmol/l	PG ₂₄₀ mmol/l	IRI _{fasting} pmol/l	IRI _{max} pmol/l	IRI ₂₄₀ nmol/l
normal	5.5 (0.2)	8.1 (0.5)	5.4 (0.2)	82.0 (43.0)	590 (180)	107 (32.0)
FPG <9	8.0 (0.5)	12.4 (1.1)	6.4 (0.9)	94.0 (37.0)	515 (210)	162 (91.0)
FPG 9-12	10.4 (1.7)	15.8 (1.0)	10.2 (1.4)	85.0 (46.0)	408 (115)	176 (62.0)
FPG 12-15	13.3 (1.5)	18.3 (1.2)	13.3 (1.1)	77.0 (35.0)	293 (140)	128 (65.0)
FPG ≥15	16.5 (2.2)	22.2 (1.8)	17.4 (1.8)	84.0 (23.0)	197 (57)	113 (47.0)

Figure 6.1 and Table 6.3 indicate that as fasting plasma glucose rose, the median maximum glucose excursion in response to the mixed meal increased from 2.6 mmol/l in normal subjects to between 4.4 and 5.7 mmol/l in the diabetic subjects. Additionally the fasting plasma glucose concentration in 82% of subjects with FPG < 9 mmol/l had returned to basal (fasting) levels at 4 h compared to 42%, 57% and 27% of those diabetic subjects with FPG 9-12, 12-15 and > 15 mmol/l, respectively. These changes occurred in conjunction with a generally reduced early-phase (0-60 min) IRI secretion, with particularly depressed insulin concentrations in the first 30-60 min of the test in all diabetic subjects (Fig. 3b), and an apparent augmentation of IRI response during the late-phase (60-240 min) in those subjects with FPG < 9

mmol/l. Thereafter a progressive decrease in the overall IRI response was observed with increasing FPG concentrations above this level.

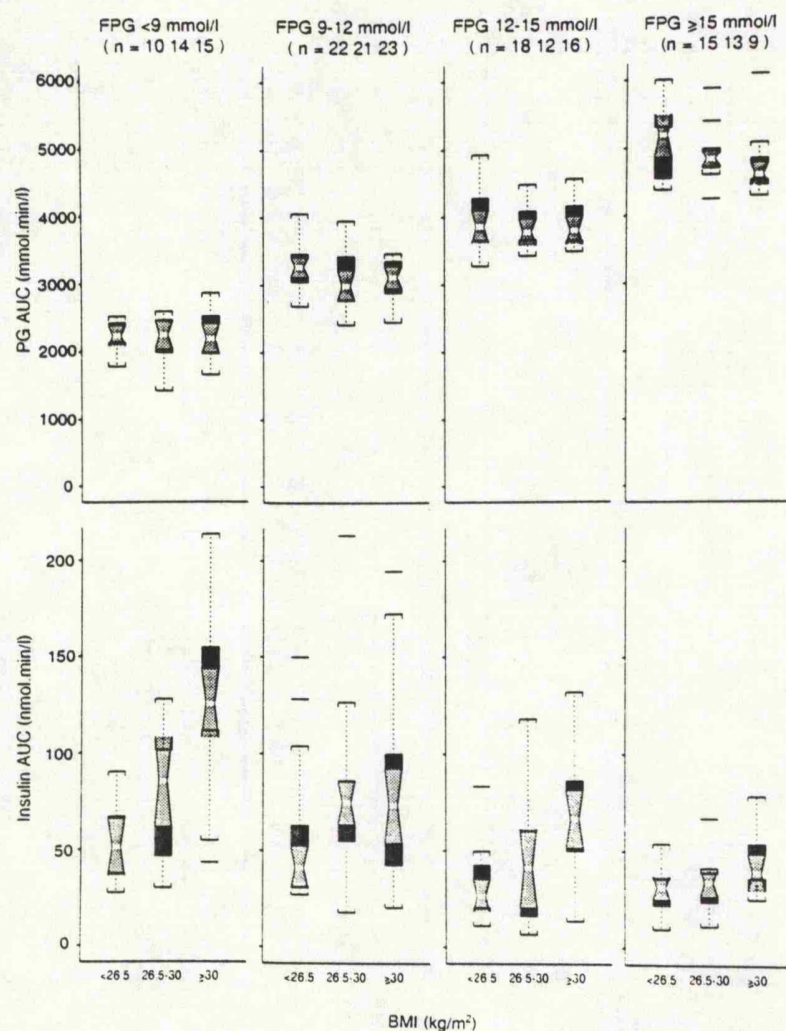
Table 6.2. Insulin AUC concentrations



Median fasting IRI concentrations were similar for normal and diabetic subjects (Table 6.3). Median peak IRI concentrations fell progressively from 590 pmol/l in normals to 197 pmol/l in subjects with FPG > 15 mmol/l. Median IRI AUC values in the first hour of the test were subnormal for all NIDDM groups (Figure 6.2, panel 1), whereas over the 2 (Figure 6.2, panel 2) and 4 hour post-prandial periods (Figure 6.2, panel 3) the median total IRI AUC was

supranormal in subjects with FPG < 9 mmol/l, similar to normal in subjects with FPG 9-12 mmol/l and becoming sub-normal in the remaining diabetic groups with FPG in excess of 12 mmol/l. Trends in the fall of median IRI AUC with increasing FPG over 1, 2 and 4 h post-prandially were statistically significant ($p < 0.0001$, whether adjusted for BMI or not). Figure 6.3 illustrates the AUC glucose and insulin data from the MTT stratified according to BMI.

Figure 6.3.



In comparing the three arbitrarily defined BMI groups, glucose exposure (AUC) was relatively constant within each FPG sub-groups (Figure 6.3, upper

panels, $p > 0.95$, $p > 0.1$, $p > 0.9$ and $p > 0.3$, respectively). Within each of the four FPG subgroups, increasing obesity was associated with increasing concentrations of IRI ($p < 0.001$, $p < 0.02$, $p < 0.001$ and $p < 0.01$, respectively for trends). However, as the FPG rose the difference between the median IRI AUC for each BMI group fell (Figure 6.3, lower panels). Figure 6.4 presents the post-prandial NEFA profiles.

Figure 6.4. Post-prandial NEFA profiles

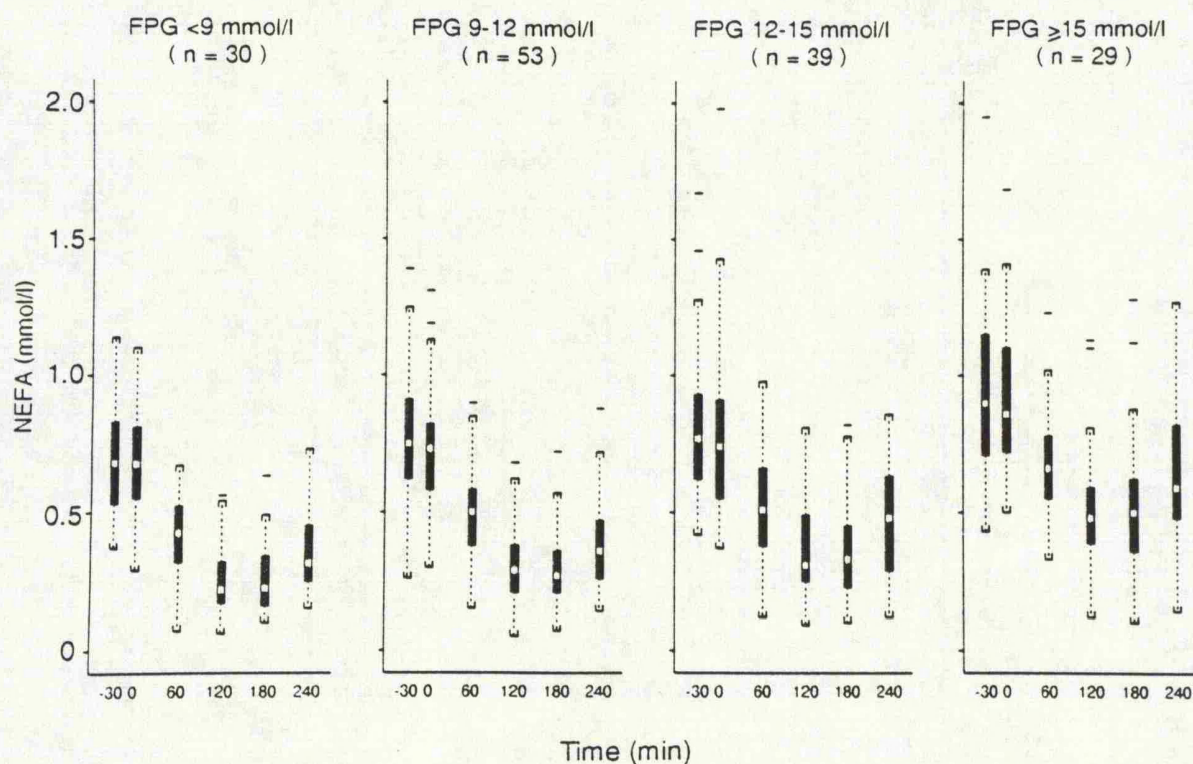


Figure 6.5 illustrates the fasting and cumulative AUC (0-240 minutes) NEFA concentrations stratified by FPG.

Figure 6.5. AUC NEFA concentrations

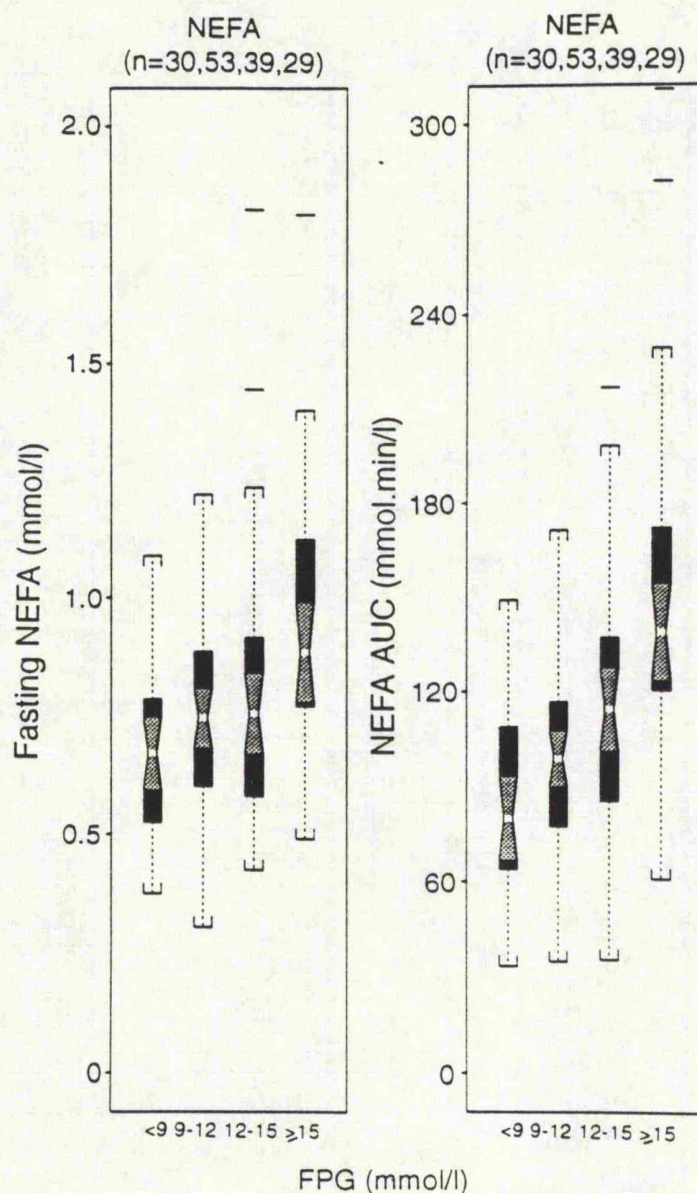


Figure 6.5 (panel 1) illustrates the presence of increasing fasting NEFA with increasing FPG ($p < 0.0001$ for the trend). The degree of NEFA suppression in each FPG sub-group during the mixed meal was similar (Figure 6.4) with post-prandial NEFA AUC concentrations (Figure 6.5, panel 2) significantly increasing with increasing FPG ($p < 0.001$ for the trend, whether adjusted for BMI or not). However, this should not be over-interpreted because of the wide spread of the data.

6.4 Discussion

The current study attempts to describe the spectrum of metabolic and hormonal responses to a standard test meal of a large group of normal subjects and subjects with NIDDM at time of presentation stratified according to the fasting plasma glucose and degree of obesity. The normal subjects were included for comparative purposes with respect to insulin secretion but were not specifically matched for BMI or sex.

Previous attempts to describe the metabolic and hormonal responses of ^{subjects} with NIDDM to glucose stimulation have produced varied results which may be related to the use of oral or intravenous glucose, differing assay procedures, variable duration of diabetes and treatment, and also the possibility of inclusion of subjects who would now be classified as having impaired glucose tolerance [Temple et al,1992]. Using data from oral glucose tolerance tests only, fasting IRI concentrations have been shown to increase with increasing FPG up to the level of 7.8 mmol/l and subsequently decline - the so-called 'Starling curve' of the pancreas [DeFronzo et al,1992]. Additionally, integrated insulin concentrations over a 2-hour glucose tolerance test are said to be augmented compared to normal subjects, in those subjects with only mildly elevated FPG (6.4 mmol/l) but then progressively decline with increasing fasting hyperglycaemia [DeFronzo et al,1992].

The data from these newly diagnosed subjects with NIDDM, using a mixed meal stimulus, broadly supports this view, although the fasting median IRI concentrations in the mildly hyperglycaemic group with FPG < 9 mmol/l (median 8.0 mmol/l) were similar to normals in whom the median FPG was lower at 5.5 mmol/l. For diabetic subjects, including those subjects with FPG < 9 mmol/l there was a gross reduction in IRI AUC during the first hour of the

test which deteriorated exponentially with increasing fasting plasma glucose concentrations. Augmentation of the insulin response compared to the normal subjects only occurred in the second hour of the test and only in subjects in the lowest FPG group. Total insulin response then fell progressively with increasing fasting hyperglycaemia in the remaining diabetic groups.

The adverse impact of obesity on insulin sensitivity is also suggested by these data. Despite equivalent glucose exposure within each FPG group, the most obese subjects had higher IRI AUC concentrations. This trend was greatest for the 'mildest' diabetic subjects (FPG < 9 mmol/l) and least in the most hyperglycaemic group (FPG > 15 mmol/l). Although the aetiology of severe hyperglycaemia in obese subjects with NIDDM is often believed to be mainly insulin resistance [Reaven et al,1982] similar subjects in this study also had marked relative insulinopenia, not too dissimilar to their less obese equally hyperglycaemic counterparts and the role of hyperglycaemia itself as a desensitiser of the β -cell [Unger et al,1985] cannot therefore be discounted.

Additionally, these findings and those of others aiming to describe the pathophysiology of NIDDM must be viewed with a degree of caution as they are based on the radioimmunoassay (RIA) of insulin which results in an overestimation of circulating plasma insulin concentrations [Temple et al,1990]. The availability of highly specific insulin and proinsulin assays using the ELISA technique [Andersen et al,1993] or immunoradiometric assay [Sobey et al,1989] will allow better characterisation of the β -cell secretory dysfunction in NIDDM.

This study has attempted to describe the spectrum of metabolic and hormonal responses observed in NIDDM as near to the time of diagnosis as possible

and prior to the institution of any treatment. These responses were observed utilising a physiological challenge in the form of a standard meal, without the complicating factor of any therapeutic intervention and with the patients arbitrarily divided into sub-groups according to the fasting glucose concentrations and degree of obesity. A marked and increasing loss of the early-phase β -cell secretory response as depicted by the 1 hour IRI secretion in response to the mixed meal in all sub-groups of diabetic subjects was clearly demonstrated. Compensatory hypersecretion over 2 and 4 hours after the mixed meal compared to healthy subjects occurred only in those subjects with the mildest fasting hyperglycaemia (< 9 mmol/l). Thereafter with FPG > 9 mmol/l the insulin response was subnormal despite increasing ambient plasma glucose concentrations. Increasing obesity was accompanied by increasing insulin secretion counterbalanced by insulin resistance which was most evident in the lowest FPG group (< 9 mmol/l).

In conclusion subjects with established NIDDM have an inadequate early-phase insulin response to a mixed meal, accompanied by an apparent and transient compensatory late-phase insulin response only in the mildest subjects with FPG < 9 mmol/l. Obesity at all levels of hyperglycaemia was associated with relative hypersecretion of insulin indicating a state of insulin resistance. Thus these data both confirm the presence of β -cell dysfunction and insulin resistance in established NIDDM and indicate the heterogeneity of the syndrome even when assessed by a standardised meal and attempting to control for the level of FPG and degree of obesity.

Chapter 7 - Specific assessment of β -cell function and insulin sensitivity at the time of presentation in a cohort of NIDDM subjects

7.1 Introduction

In the previous chapter the degree of fasting hyperglycaemia and obesity were used in an attempt to 'metabolically stage' patients with newly presenting NIDDM using the plasma glucose, immunoreactive insulin and non-esterified fatty acid responses to a mixed meal. The findings demonstrated the existence of β -cell dysfunction in these subjects and also suggested the co-existence of insulin resistance, increasingly evident as body mass index increased. More specific quantification of both β -cell function and insulin sensitivity at the time of presentation may further improve the hormonal and metabolic 'staging' both for prognostic purposes and for monitoring responses to established or novel therapeutic interventions.

For these purposes, accurate assessment of β -cell function and estimation of insulin sensitivity is increasingly important. As a result of the relatively recent availability of highly sensitive and specific assays for insulin [Andersen et al,1993] and proinsulin [Kjems et al,1993] it is now possible and very necessary to re-assess β -cell function in NIDDM subjects in response to a variety of different insulin secretagogues with the aid of these new technologies. In addition, the use of 'minimal' model (MINMOD) analysis of the insulin-modified FSIVGTT allows relatively simple quantification of insulin sensitivity. Previous studies [Pedrosa et al,1990; Coates et al,1995] have contributed to the validation of this dynamic technique in NIDDM subjects against the more frequently used adynamic isoglycaemic hyperinsulinaemic glucose 'clamp'. The impact of differing sampling schedules and insulin assay methods on the accuracy of estimates of insulin sensitivity from the technique

have also been examined elsewhere [Coates et al,1993; Lovejoy et al,1994] and in previous chapters.

The aim of the current study was, therefore, to assess both β -cell function during a standardised mixed meal test with highly specific insulin and proinsulin assays, and also estimate insulin sensitivity with the extended sampling schedule insulin-modified FSIVGTT in newly diagnosed NIDDM subjects. This cross sectional study provides baseline clinical and metabolic data on a cohort of newly diagnosed NIDDM subjects who were then followed and investigated over the subsequent 2 year period.

For comparative purposes, the NIDDM subjects were matched for age (within 5 years), sex and body mass index with normal control subjects who underwent the same investigations i.e. meal tolerance and insulin-modified FSIVGT tests.

7.2 Subjects and methods

Subjects with NIDDM and normal healthy volunteers were recruited as described in Chapter 2. All investigations in the NIDDM subjects took place within two weeks of presentation to the hospital out-patient clinic and prior to any therapeutic intervention. All subjects underwent both an MTT and an insulin-modified FSIVGTT in random order using the protocols previously described (Chapter 2).

Both procedures were generally well tolerated. No subject, developed symptomatic hypoglycaemia during the insulin-modified FSIVGTTs. Adverse events were entirely related to cannula placement and were typically

vasovagal in nature with rapid recovery. One subject (female, diabetic) developed mild thrombophlebitis of an antecubital vein following glucose administration which responded to anti-inflammatory measures (oral non-steroidal anti-inflammatory medication).

Analytical methods

Plasma glucose, specific insulin, total plasma proinsulin and plasma lipid estimations were assayed as previously described (Chapter 2)

7.3 Results

Table 7.1 gives the baseline clinical and metabolic data for all the NIDDM subjects (n=35) and their matched normal controls (n=35).

Table 7.1. Demographic data at presentation - all NIDDM subjects (n=35) (median (IQR))

	NIDDM subjects	Normals	p value
Age	54.0 (15.0)	49.0 (4.3)	n/a
BMI (kg/m ²)	29.6 (7.3)	28.4 (7.6)	0.82
Systolic bp (mmHg)	130 (20.0)	118 (10.0)	0.004
Diastolic bp (mmHg)	82 (8.0)	80 (16.0)	0.44
FPG (mmol/l)	11.6 (5.7)	4.8 (0.8)	0.0001
HbA _{1c} (%)	10.1 (4.3)	5.7 (0.7)	0.0001
Total cholesterol (mmol/l)	6.5 (1.4)	5.3 (0.5)	0.03
HDL cholesterol (mmol/l)	0.95 (0.4)	1.38 (0.5)	0.0001
LDL cholesterol (mmol/l)	3.9 (1.5)	3.3 (0.7)	0.044
Triglycerides (mmol/l)	2.3 (1.6)	1.2 (1.5)	0.018

Matching of normal and NIDDM subjects was achieved satisfactorily. The glycaemic indices were clearly different as expected between the two groups. The NIDDM subjects had higher systolic blood pressures (130 vs 118 mmHg), total cholesterol (6.5 vs 5.3 mmol/l), LDL-cholesterol (3.9 vs 3.3 mmol/l) and total triglyceride concentrations (2.3 vs 1.2 mmol/l) along with lower HDL-cholesterol concentrations (0.95 vs 1.38 mmol/l).

Figure 7.1 represents the plasma glucose, insulin and proinsulin profiles from the MTT for the normal and NIDDM subjects. Table 7.2 includes the metabolic data from the MTT also stratified according to fasting plasma glucose as in Chapter 6. Figure 7.2 shows the median AUC values for glucose, insulin and proinsulin for 0-60, 0-120, 0-180 and 0-240 minutes of the MTT.

Table 7.2. Meal test data at presentation (median (interquartile range))

	Normals (n=35)	FPG <9 (n=7)	FPG 9-12 (n=14)	FPG >12-15 (n=6)	FPG >15 (n=8)
Glucose					
Fasting (mmol/l)	4.9 (0.8)	7.2 (2.1)	11.2 (1.5)	12.9 (1.9)	15.9 (3.5)
AUC ₆₀ (mmol/l)	404 (114)	607 (132)	836 (88)	876 (309)	1098 (309)
AUC ₂₄₀ (mmol/l)	1281 (423)	2215 (946)	3292 (400)	3561 (1073)	4701 (1024)
Insulin					
Fasting (pmol/l)	59 (52.0)	69.5 (46.5)	49.8 (24)	49 (22)	27 (25.5)
AUC ₆₀ (nmol/l)	18.1 (10.1)	13.8 (9.9)	6.1 (6.2)	5.1 (2.6)	5.3 (4.7)
AUC ₂₄₀ (nmol/l)	42.2 (25.7)	58.9 (30.0)	30.1 (39.4)	25.1 (7.8)	27.8 (4.8)
Proinsulin					
Fasting (pmol/l)	8 (20.5)	22 (36.5)	30.3 (32.0)	28.8 (22.5)	22 (36.5)
AUC ₆₀ (nmol/l)	1.7 (1.4)	2.3 (3.8)	2.6 (1.6)	2.3 (1.8)	1.6 (1.8)
AUC ₂₄₀ (nmol/l)	8.4 (8.5)	15.5 (18.8)	16.7 (9.3)	12.2 (8.4)	10.3 (10.2)
Ratios					
FI:FPG	12.3 (7.2)	9.7 (10.6)	4.2 (3.0)	3.7 (1.6)	3.1 (4.7)
FI:FPI	5.5 (11.3)	2.8 (3.3)	1.6 (1.0)	2.2 (0.8)	1.4 (1.6)

FPG=fasting glucose; FI=fasting insulin; FPI=fasting proinsulin

Figure 7.1. Plasma glucose, insulin and proinsulin profiles during the MTT

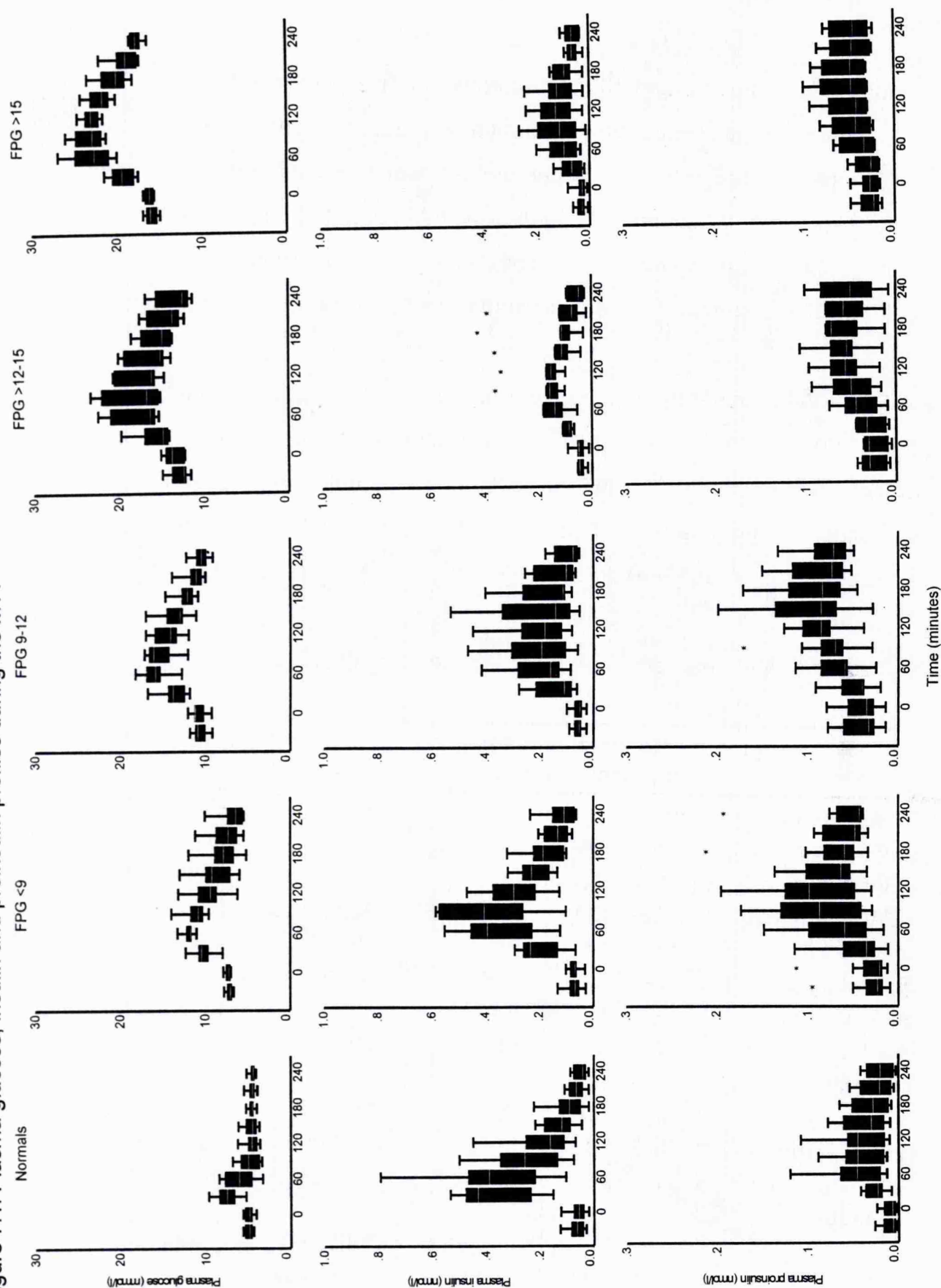
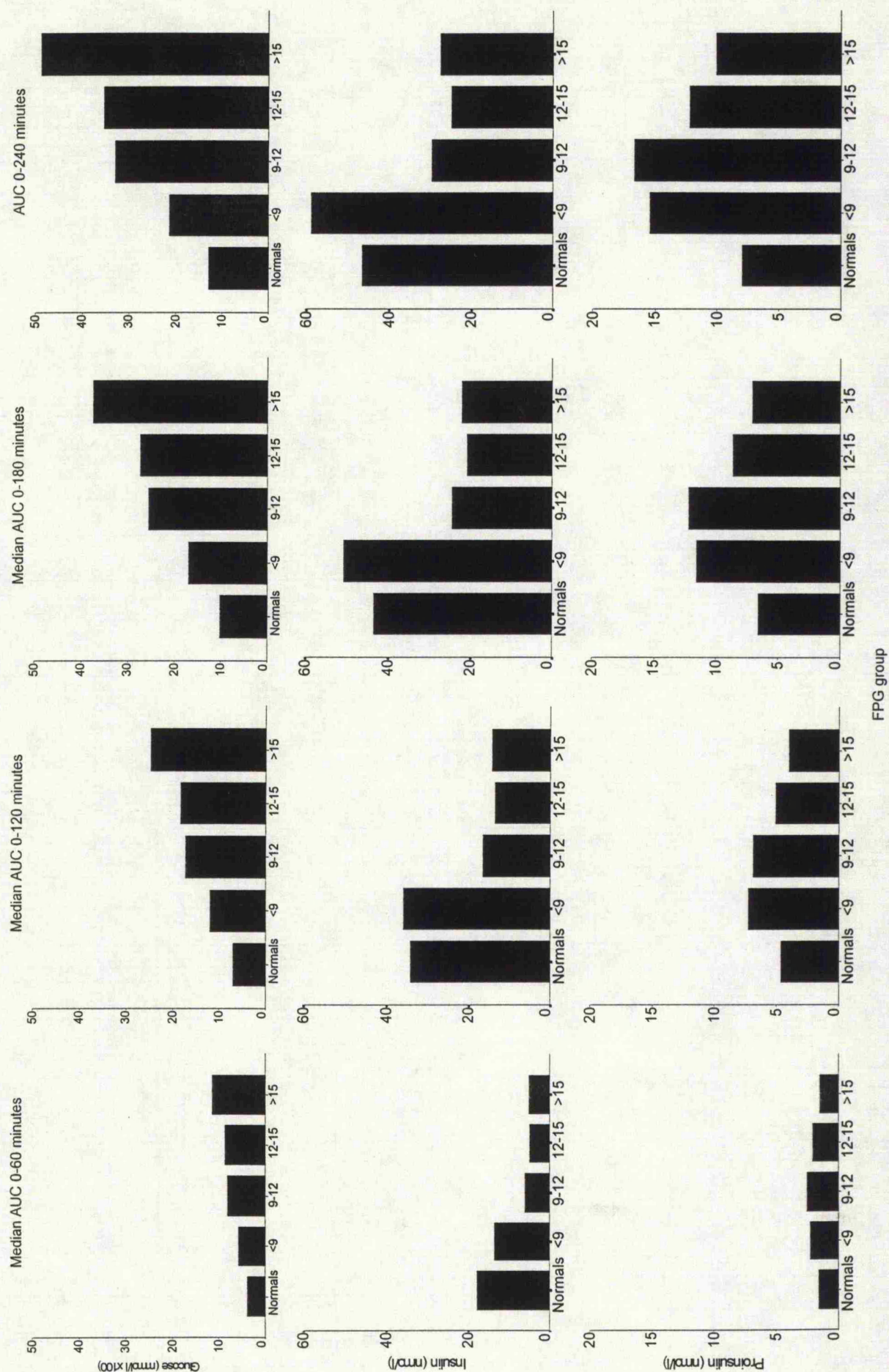


Figure 7.2. Plasma glucose, insulin and proinsulin AUC concentrations during the MTT



Plasma glucose was significantly higher throughout the test in all the NIDDM subjects and demonstrated delayed peaking of glucose concentrations and a progressive failure of return to basal values with increasing FPG. Fasting plasma insulin concentrations were similar to the normals for those subjects with a FPG <15 mmol/l but significantly lower ($p < 0.05$) in those subjects with a FPG >15 mmol/l. The trend for an inverse relationship between fasting insulin concentrations and fasting plasma glucose fell just below the level of statistical significance ($p = 0.068$). The insulin response to the mixed meal was uniformly delayed in the NIDDM subjects with a plateau type response and delay in the fall of the insulin concentrations in response to the ongoing hyperglycaemia. Fasting proinsulin was significantly higher ($p < 0.05$) than in the normals for all NIDDM subjects except for those with FPG >12 mmol/l and the profiles mirror those of the measured insulin concentrations with a sluggish initial response followed by a prolonged plateau phase. The ratio of fasting insulin to glucose also illustrates the reduction in basal insulin secretion relative to prevailing glycaemia. All NIDDM subject groups had a significantly ($p < 0.05$) lower insulin to glucose ratio than the normal subjects and the trend of lower insulin to glucose ratio with increasing FPG was also highly significant ($p < 0.0001$). Fasting insulin to proinsulin ratios were all significantly lower ($p < 0.05$) in the NIDDM subjects irrespective of FPG, and the trend of falling insulin to proinsulin ratio with increasing FPG was highly significant ($p < 0.0001$).

There was a significant decrease in insulin AUC during the first 60 minutes post-prandially in the all the NIDDM subjects compared to the normals which worsened as the FPG increased ($p < 0.0001$ for the trend). This was also evident over the subsequent second and third hours of the test with the exception of those NIDDM subjects with a FPG < 9 mmol/l (Figure 7.2, middle panels). Total insulin AUC concentrations (0-240 minutes) were not

statistically different between the normal and NIDDM subjects, however, the trend for falling total insulin AUC with increasing FPG was highly significant ($p = 0.008$).

Early phase proinsulin concentrations (AUC 0-60 minutes) were higher in the NIDDM subjects with a FPG <12 mmol/l compared to normals ($p < 0.05$). Proinsulin AUC concentrations for these subjects continued to rise during the test (Figure 7.2, lower panels) and total proinsulin AUC concentrations were significantly higher ($p < 0.05$) than in the normals. For NIDDM subjects with a FPG > 12 mmol/l proinsulin AUC concentrations generally fell with increasing FPG and there was no difference in total proinsulin secretion between these NIDDM subjects the normals. The trend for lower total AUC proinsulin with increasing FPG for these NIDDM subjects was highly significant ($p < 0.0001$).

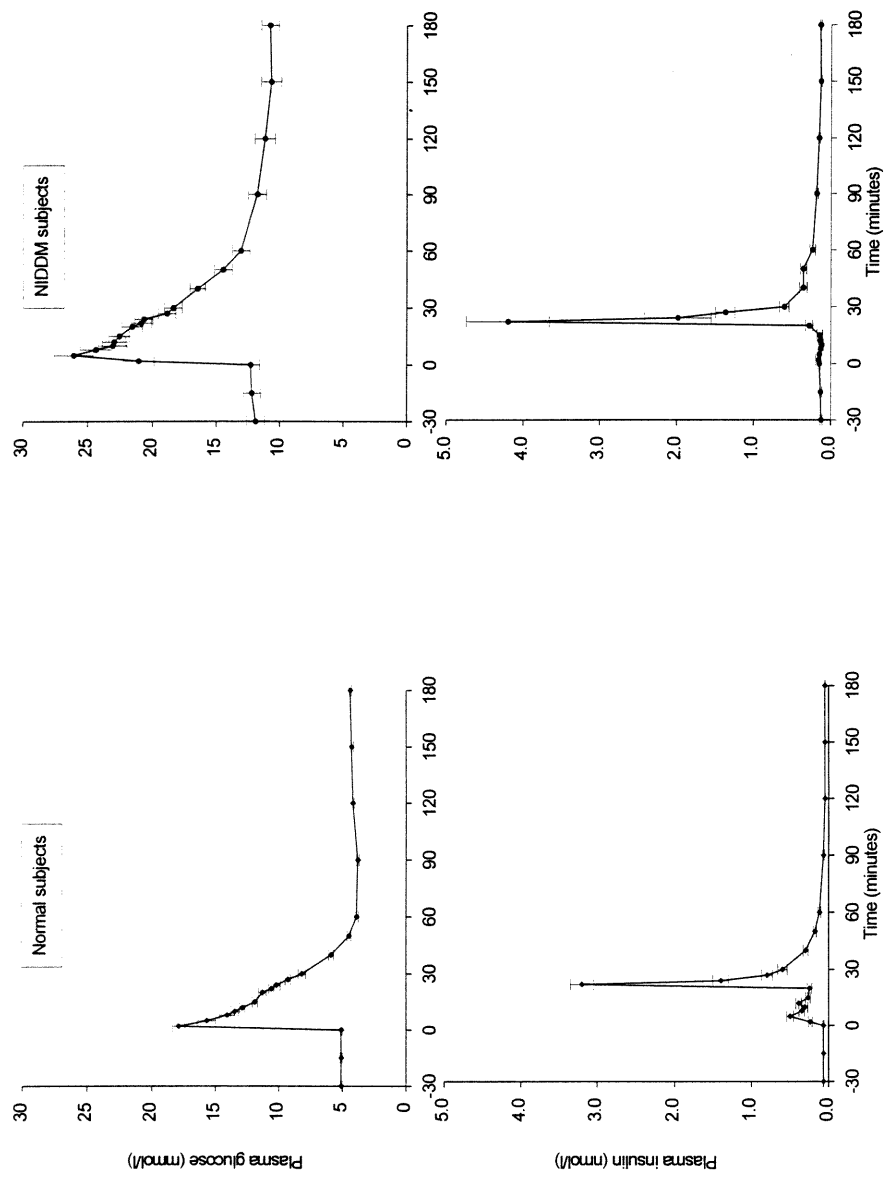
Table 7.3 summarises the calculated glucose clearance rate (K_{g4-20}), insulin sensitivity (S_i) and glucose effectiveness (S_g) and data. Figure 7.3 illustrates the mean plasma glucose and insulin concentrations from the insulin-modified FSIVGTT.

Table 7.3 Glucose disposal, glucose effectiveness and insulin sensitivity data at presentation (median (IQR))

	Normals	FPG <9	9-12	$>12-15$	>15
kg_{4-20}^*	2.1 (0.89)	1.8 (1.22)	1.1 (0.48)	0.99 (0.41)	1.57 (1.1)
S_i^\dagger	2.85 (2.72)	0.7 (0.45)	0.39 (0.76)	0.44 (0.5)	0.34 (0.47)
S_g^\ddagger	0.01951 (0.01)	0.01708 (0.01)	0.01723 (0.01)	0.01883 (0.01)	0.0184 (0.02)

*($10^{-2} \cdot \text{min}^{-1}$), $^\dagger(\text{min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4)$, $^\ddagger(\text{min}^{-1})$

Figure 7.3. Glucose and insulin profiles from the FSIVGTT



Kg as a measure of the rate of glucose clearance prior to exogenous insulin injection was significantly lower ($p < 0.05$) in the NIDDM subjects with FPG $> 9 \text{ mmol/l}$ when compared to normal subjects. The general trend of falling Kg with increasing FPG was highly significant ($p < 0.0001$). S_i as a direct index of insulin sensitivity was significantly lower in the NIDDM subjects and fell steeply as FPG increased ($p < 0.0001$ for the trend) with little or no further decline with FPG $> 9 \text{ mmol/l}$. S_G which reflects glucose uptake at basal insulin concentrations was lower in the NIDDM subjects but did not differ significantly between the normals and NIDDM subjects, or the individual NIDDM groups. The insulin profile from the insulin-modified FSIVGTT shows complete absence of a first-phase insulin response to the intravenous glucose bolus.

Figure 7.4 shows the relationship between insulin sensitivity and fasting plasma glucose for the normal subjects and all NIDDM subjects as a single group. Figure 7.5 illustrates the relationship between insulin sensitivity and fasting insulin concentrations for the normal subjects. Table 7.4 gives the univariate correlation coefficients of the insulin sensitivity index against baseline demographic and metabolic data for all subjects i.e. normal and NIDDM subjects together.

Figure 7.4. Relationship between S_i and FPG for all subjects

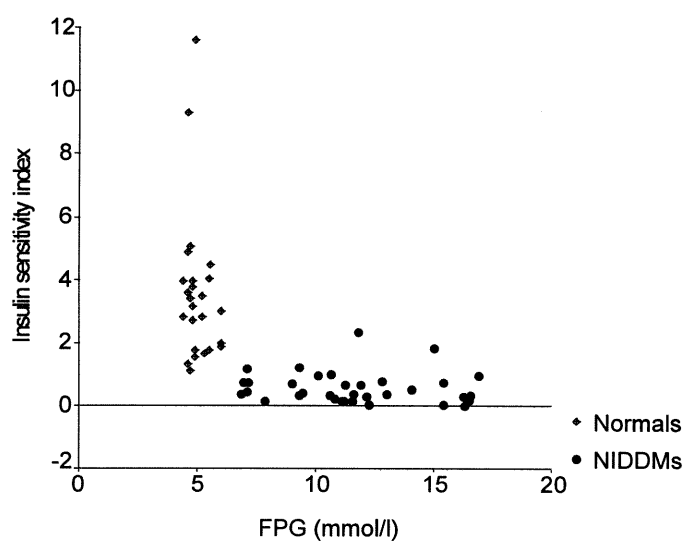


Figure 7.5. Relationship between S_i and fasting insulin concentration (normals)

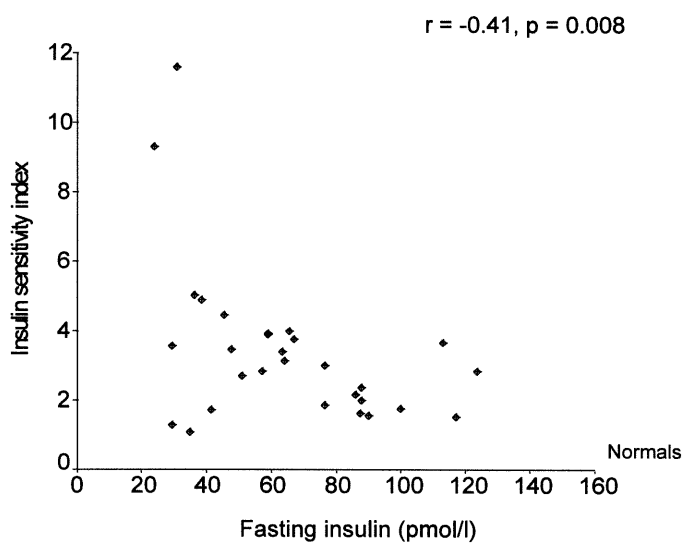


Table 7.4. Correlates of S_I at presentation (all subjects n=70)

Factor	r value	p value
BMI (kg/m ²)	-0.19	0.06
Systolic bp (mmHg)	-0.41	0.0001
Diastolic bp (mmHg)	-0.21	0.04
Fasting glucose (mmol/l)	-0.56	0.0001
Total cholesterol (mmol/l)	-0.24	0.026
HDL cholesterol (mmol/l)	+0.50	0.0001
LDL cholesterol (mmol/l)	-0.23	0.032
Triglycerides (mmol/l)	-0.36	0.002
Fasting insulin (pmol/l)	-0.15	0.11
Fasting proinsulin (pmol/l)	-0.46	0.0001
K _g (10 ⁻² .min ⁻¹)	+0.36	0.003
S _G (min ⁻¹)	-0.29	0.014

Table 7.5 (overleaf) gives the results of a stepwise logistic multiple regression analysis to determine from the variables measured those capable of acting as predictors of S_I for the normal subjects.

Table 7.5. Predictors of S_i (Normal subjects n=35)

Factor	beta	t	sig t
BMI (kg/m ²)	-0.16	0.90	0.37
Systolic bp (mmHg)	-0.31	1.99	0.05
Diastolic bp (mmHg)	-0.16	0.98	0.33
Fasting glucose (mmol/l)	-0.03	0.18	0.86
Total cholesterol (mmol/l)	+0.04	0.23	0.82
HDL cholesterol (mmol/l)	+0.26	1.26	0.22
LDL cholesterol (mmol/l)	-0.04	0.24	0.81
Triglycerides (mmol/l)	-0.03	0.13	0.89
Fasting insulin (pmol/l)	-2.48	2.95	0.006
Fasting proinsulin (pmol/l)	+0.27	1.38	0.18

7.4 Discussion

This study aimed to document in greater detail the degree of both β -cell dysfunction and insulin resistance in a group of newly diagnosed NIDDM subjects. Assessment of β -cell function was accomplished using the glucose, specific insulin and specific total proinsulin responses to a mixed meal and insulin sensitivity by MINMOD analysis of the data from insulin-modified FSIVGTTs. Similar studies involving age, sex and BMI matched healthy subjects without a family history of NIDDM were performed to provide comparative normal data.

Baseline clinical and metabolic variables were substantially different between the two groups. Apart from the expected differences in glycaemic indices (fasting plasma glucose and glycosylated haemoglobin), the NIDDM subjects

had significantly higher systolic blood pressures, total cholesterol, LDL-cholesterol and total triglyceride concentrations and significantly lower HDL-cholesterol concentrations. These differences serve to reinforce that NIDDM rather than being an isolated problem of hyperglycaemia, is, as has been suggested by others, part of a complex syndrome of clinical and metabolic abnormalities including obesity, dyslipidaemia, hypertension and possibly coronary artery disease [Reaven,1988; Reaven et al,1994; Hansen,1995].

β -cell dysfunction in the NIDDM subjects was apparent from a variety of indices obtained from the mixed meal test. The observed absolute fasting insulin concentrations were not different between the normals and the NIDDM groups. However, relative to the degree of hyperglycaemia there was clear insulin deficiency in the fasting state in all the NIDDM subjects as reflected in the reduced fasting insulin to glucose ratio, which deteriorated with increasing fasting plasma glucose. For the NIDDM subjects, there was also excessive secretion of proinsulin relative to insulin in the fasting state with both elevated fasting concentrations and a significantly reduced insulin to proinsulin ratio which fell as the FPG rose. In addition, all NIDDM subjects had a characteristically absent 'first phase' insulin response to intravenous glucose during the FSIVGTT [Cerasi et al,1967]. During the first hour of the meal test when plasma glucose exposure increased significantly with increasing FPG in the NIDDM subjects, insulin secretion was grossly deficient and fell significantly compared to normals. There was no evidence of early augmentation of insulin secretion in the NIDDM subjects with lowest FPG concentrations as described in Chapter 6 and elsewhere [DeFronzo et al,1992]. Late persistent secretion of insulin in response to continuing elevation of the plasma glucose concentrations occurred only in the NIDDM subjects with FPG <9, however the total insulin AUC secretion for this group was not different from the normal subjects. Absolute insulin secretion fell

sequentially with increasing FPG in the other NIDDM subjects, with all subjects with an FPG of > 9mmol/l secreting approximately 28% of the insulin secreted by normals in the first 60 minutes of the test and 60% of the insulin secreted by normals over the four hour post-prandial period. Absolute proinsulin concentrations were also higher in all NIDDM subjects with FPG < 12 mmol/l both at one hour and over the four hour post-prandial period. For those subjects with a FPG > 12 mmol/l however, early phase and total proinsulin concentrations were not different from those of the normal subjects.

The importance of the interpretation of the insulin secretion data in relation to the prevailing plasma glucose concentrations cannot be overstated. Previous studies reported as showing 'hyperinsulinaemia' in response to oral glucose [DeFronzo et al,1992] frequently failed to take account of both the inherent overestimation of insulin concentrations by the radioimmunoassay of insulin and the 'inappropriateness' of the absolute insulin concentrations when measured in relation to the prevailing level of hyperglycaemia [Temple et al,1992]. The late hyperinsulinaemia documented in early studies was initially considered simply to be compensatory for the failure of early insulin secretion leading to ongoing hyperglycaemia, but subsequent investigators regarded this as evidence of insulin resistance [Porte Jr,1991]. This study clearly demonstrates equivalent absolute fasting insulin concentrations, significantly reduced absolute early phase insulin secretion which continues to fall with increasing FPG, and statistically equivalent (although 30% lower in absolute terms for those with FPG >9mmol/l) total insulin concentrations in response to the MTT when compared to BMI matched normal subjects. The late 'hyperinsulinaemia' documented here occurred only in those subjects with FPG < 9mmol/l and in the face of persistently elevated glucose exposure. The disproportionate elevation of proinsulin concentrations both basally and following the MTT further supports significant dysfunction of the β -cell

population. Having started the test with an elevated fasting proinsulin concentration those subjects with a FPG < 12 mmol/l displayed augmentation of early phase and total proinsulin concentrations however this did not apply to the subjects with an FPG >12 mmol/l. These data therefore indicate increasing β -cell dysfunction in terms of both insulin and proinsulin secretion in all FPG groups but particularly as the FPG increases above 9 mmol/l. Subjects with an FPG >12 mmol/l in particular displayed severe and progressive failure to secrete both insulin and its precursor molecule in response to insulin secretagogues.

Insulin sensitivity as estimated by the insulin-modified FSIVGTT was grossly reduced in the NIDDM subjects at presentation, as was kg a measure of glucose clearance derived from the same test prior to insulin injection. The degree of insulin insensitivity appeared to increase with increasing FPG although this was not a significant trend. There also appeared to be a hyperbolic relationship between S_I and FPG and this serves to illustrate that measured S_I in some normal subjects (~15%) falls into the same range as that for subjects with NIDDM. This phenomenon was recognised early in the use of the minimal model approach and developed more recently by Kahn and co-workers [Bergman,1989; Kahn et al,1993]. The proposed explanation for this phenomenon is that β -cell function and insulin sensitivity are inversely related so that insulin sensitivity \times insulin secretion = constant, and that glucose tolerance is dependent on this relationship [Bergman,1989]. In normal individuals therefore, reductions in insulin sensitivity are compensated for by the capacity to increase insulin secretion and maintain normal glucose tolerance. This would appear to hold true for the normals studied here who demonstrated a significant inverse relationship between insulin sensitivity and fasting insulin concentrations. There is also some limited evidence to suggest that those normal individuals with the lower estimated insulin sensitivity may

go on to develop NIDDM [Martin et al,1992]. In the case of subjects with NIDDM however, the co-existence of β -cell dysfunction precludes adequate compensation for insulin resistance in terms of increased insulin secretion and glucose intolerance results.

The relationship between S_I and other clinical and metabolic variables for the group as a whole serves to reinforce the role of insulin resistance in other facets of the 'metabolic syndrome'. Significant negative correlations with total cholesterol, LDL-cholesterol and total triglycerides confirm its association with known atherogenic lipid fractions. Similarly the negative correlations with fasting insulin and proinsulin concentrations illustrate the increased demands placed on the β -cell secretory capacity as discussed above. Hypertension has been demonstrated to be associated with insulin resistance [Reaven,1988; Sowers et al,1991; Hansen,1995] and this is would also appear to be the case in these individuals for systolic blood pressure. The difficulty of predicting insulin sensitivity from a single metabolic variable is also highlighted by this data. For the normal subjects, fasting insulin concentrations and systolic blood pressure were strongly predictive of S_I but for the NIDDM subjects, none of the metabolic or clinical variables had sufficient predictive power to achieve significance - hence the requirement for specific methods to dynamically measure insulin action in these individuals.

Taken in context with the MTT data these findings illustrate the apparent heterogeneous nature of NIDDM. To develop the syndrome it would appear that defects both in β -cell function and insulin sensitivity must co-exist [Bergman,1989; Johnston et al,1990]. For subjects with only mildly elevated FPG (<9 mmol/l), insulin sensitivity is grossly reduced however insulin secretory capacity is augmented at least to the same level as that displayed by healthy subjects following a mixed meal stimulus. In this group however,

the failure of early insulin secretion results in persistent hyperglycaemia which subsequent 'compensatory' increases in insulin secretion are unable to correct. This persistent stimulation to the β -cell by ongoing hyperglycaemia also results in excessive secretion of proinsulin. For subjects with FPG $>9\text{mmol/l}$, insulin sensitivity is also grossly reduced ($\sim 50\%$ of those with FPG $<9\text{mmol/l}$) but is coupled with more severe and progressively deteriorating β -cell function. This is manifest by absolute early (0-60 minutes) and total (0-240 minutes) hypoinsulinaemia in all groups with FPG $>9\text{mmol/l}$ and supported by the failure of even proinsulin secretion in those subjects with FPG $> 12\text{ mmol/l}$.

In summary, this study suggests that when compared to age, sex and BMI matched normals subjects presenting with NIDDM have objective evidence of β -cell dysfunction which varies in severity depending on the fasting plasma glucose concentrations. All have an absolute reduction in insulin secretion during the first hour of a mixed meal test and only those with a FPG less than 9 mmol/l secrete equivalent amounts of insulin to normals over the course of the postprandial period although this is accompanied by excessive secretion of proinsulin and occurs in the face of significant hyperglycaemia. Those individuals with an FPG $>12\text{ mmol/l}$ show a 30% reduction in insulin secretion over the course of the postprandial period compared to normals with proinsulin secretion also generally reduced suggesting extreme β -cell dysfunction. These abnormalities occur in the context of grossly reduced insulin sensitivity as measured directly by the insulin-modified FSIVGTT when compared to the same normal subjects. The suggested equilibrium between β -cell function and insulin sensitivity in maintaining glucose tolerance is clearly disrupted in NIDDM subjects as a result of abnormalities of both these metabolic variables. Newly presenting NIDDM subjects also demonstrate a range of other metabolic abnormalities in comparison to normal individuals

which may also have reduced sensitivity to insulin as part of the pathophysiological basis for their development.

The ability to identify these abnormalities by objective testing provides the ability to monitor changes over time in respect of specific therapies and may also help to illustrate the natural history of the disease once established

Chapter 8 - The effects of dietary intervention alone on β -cell function and insulin sensitivity in NIDDM subjects - a one year study

8.1 Introduction

Dietary treatment remains the mainstay of therapy for NIDDM. As the majority of NIDDM subjects are obese, the aim of this mode of therapy is weight reduction resulting in improvements in glycaemic control with additional beneficial effects on other features of the syndrome such as plasma lipoprotein concentrations and blood pressure [Alberti et al,1994]. Weight loss is usually achieved by moderate calorific restriction and a shift in the emphasis of the diet towards complex carbohydrates in the form of soluble fibre with a reduction in the contribution of saturated fats to the total energy intake.

Several studies have documented the success of weight reduction on metabolic control in NIDDM subjects e.g. [Hadden et al,1975; Henry et al,1985; UKPDS Group,1990], however, much less data is available as to the mechanism by which these improvements occur.

β -cell function in NIDDM subjects either in terms of insulin and proinsulin secretion [Yoshioka et al,1989; Davies et al,1993a] or CIGMA estimates [Hosker et al,1993] has been shown to improve following treatment with diet alone in a number of short term studies lasting between 3 and 5 months. Insulin sensitivity has also been shown to improve in NIDDM subjects following dietary therapy in most [Hughes et al,1984; Henry et al,1986; Laakso et al,1988; Yamanouchi et al,1995] but not all studies [Bogardus et al,1984b; Hosker et al,1993] and the significance of this improvement in

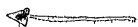
A number of techniques are available for dietary assessment including weighed records of all food consumed, food-frequency questionnaires, 24 hour recalls, estimated food records and diet diaries [Karvetti et al, 1992; Callmer et al, 1993; Bingham et al, 1995]. Methods relying on subject self-reporting - food-frequency questionnaires, 24 hour recalls, estimated food records and diet diaries - tend to result in under-reporting of consumed food [Mertz et al, 1991; de Vries et al, 1994; Schoeller, 1995]. Against this potential source of bias has to be considered the practical difficulties of either physically observing the diet consumed or weighing the individual components separately prior to consumption. In these studies, a 3-day diet diary was chosen as it was felt it would (i) provide an indication of the range of foods typically consumed by NIDDM subjects, (ii) be a practical method of assessing food consumption. In addition, several studies suggested that short (2-3 day) diet diaries provided reasonably accurate assessments of food intake when compared to more intrusive methods [Larkin et al, 1991; Karvetti et al, 1992].

relation to reductions in plasma glucose concentrations has been questioned [Hosker et al,1993].

This study represents the results of the effects of one years dietary therapy on β -cell function, measured during the standardised mixed meal test with specific insulin and proinsulin assays and insulin sensitivity measured by the insulin-modified FSIVGTT in a group of newly presenting diet responsive NIDDM subjects

8.2 Subjects and Methods

Subjects were recruited and monitored as documented in Chapter 2. Those subjects who had reduced their fasting plasma glucose to 9 mmol/l at the 3 months assessment were considered 'diet-responsive' and the results of their studies are reported here.



For the three days prior to their initial metabolic assessments each subject completed a 3 day diet diary detailing their food consumption following instruction from a dietician. Following their metabolic studies they were given specific instruction on a weight reducing, high fibre, low saturated fat diet. The dietary instruction was reinforced at 3, 6, 9 and 12 months after presentation. Immediately prior to their subsequent metabolic assessments i.e. at 6 and 12 months they again completed 3 day diet diaries which were returned to the dietician for analysis.

Each subject underwent a mixed meal test and an insulin-modified FSIVGTT according to the protocols in Chapter 2. The data from the diet diaries were

Table 8.1. Dietary, clinical and metabolic data - diet treated NIDDM subjects (n=24) (Median (interquartile range))

	0 Months	6 Months	12 Months	p value(0 vs 6)	p value (0 vs 12)	p value (6 vs 12)
Energy (kcal)	2186 (501)	1969 (672)	1763 (492)	*	**	ns
Carbohydrate (%)	40.5 (10)	43.2 (5.4)	42.7 (7.0)	ns	ns	ns
Protein (%)	17.6 (3.9)	19.43	18.6 (7.0)	**	*	**
Fat (%)	41.7(6.7)	36.8 (7.4)	33.96.4)	**	***	ns
Weight (kg)	89.5 (21.9)	84.3 (22.8)	85.6 (20.5)	***	**	ns
BMI (kg/m ²)	29.6 (5.5)	28.4 (4.6)	28.7 (4.7)	**	***	ns
Systolic bp (mmHg)	130 (26.5)	123 (17)	128 (23)	*	ns	ns
Diastolic bp (mmHg)	83 (16.5)	78 (15)	80 (12)	*	ns	ns
FPG (mmol/l)	10.9 (3.6)	8.2 (1.7)	8.2 (2.3)	***	**	ns
HbA _{1c} (%)	9.1 (2.4)	7.7 (0.9)	8.0 (1.9)	***	**	ns
Total cholesterol (mmol/l)	6.5 (1.5)	6.1 (1.2)	5.6 (1.1)	ns	**	**
HDL-cholesterol (mmol/l)	0.91 (0.35)	1.03 (0.29)	0.99 (0.42)	ns	ns	ns
LDL-cholesterol (mmol/l)	4.3 (1.5)	4.1 (0.8)	3.6 (0.7)	ns	ns	**
Triglycerides (mmol/l)	2.3 (1.5)	2.0 (1.4)	1.7 (2.1)	**	ns	ns

* p < 0.05, ** p < 0.01, *** p < 0.001

Wilcoxon signed rank test

coded by the dietician and then entered into the Microdiet software program which estimates dietary composition.

8.3 Results

Table 8.1 contains the dietary, clinical and metabolic data for the diet treated NIDDM subjects at presentation and after six and twelve months. Dietetic intervention resulted in a significant reduction in median total energy intake at 6 months which was sustained and further reduced at 12 months (2186 to 1969 to 1763 kilocalories respectively). This was associated with increases in the percentage of energy derived from carbohydrate and protein along with significant falls in energy derived from fat consumption (42 to 37 to 34%). Weight and BMI both fell significantly by 6 months and these improvements were also evident at the 12 months assessment. There were also gradual and consistent falls in the median total cholesterol (6.5 to 6.1 to 5.6 mmol/l) and LDL-cholesterol concentrations (4.3 to 4.1 to 3.6 mmol/l) over the 12 month period. The falls in LDL-cholesterol concentrations at 6 and 12 months were not statistically significant however the trend for falling LDL-cholesterol over time achieved significance ($p = 0.03$). Systolic (130 vs 123 mmHg) and diastolic (83 vs 78) blood pressure also fell significantly by the 6 months assessment.

A. MTT data

Figure 8.1 illustrates the glucose, insulin and proinsulin data during the meal tolerance tests at presentation, 6 months and 12 months. Table 8.2 includes the metabolic data from these meal tolerance tests in summary form.

Figure 8.1. Plasma glucose, insulin and proinsulin concentrations during the MTT

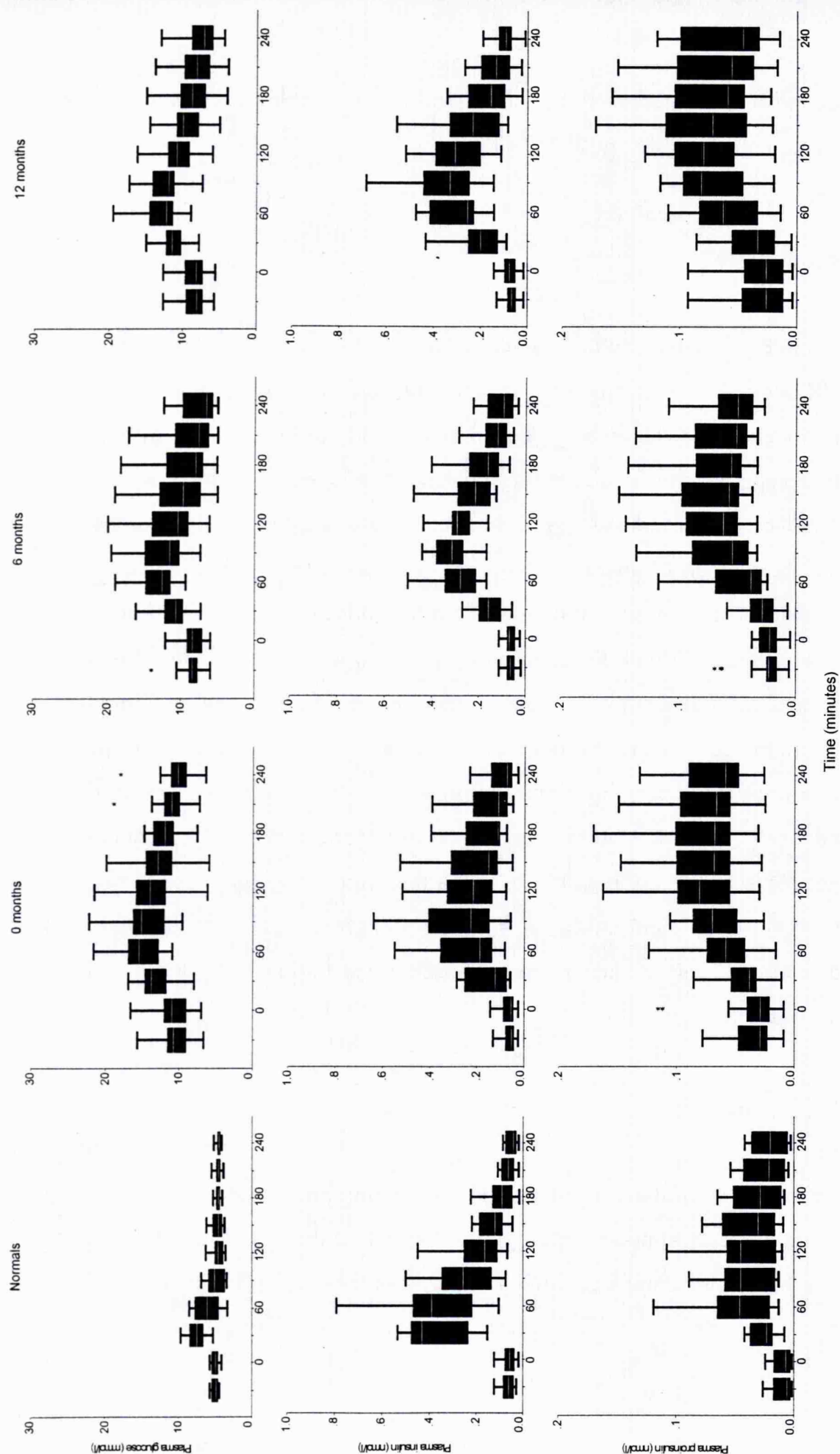


Figure 8.2. Plasma glucose, insulin and proinsulin AUC concentrations during the MTT

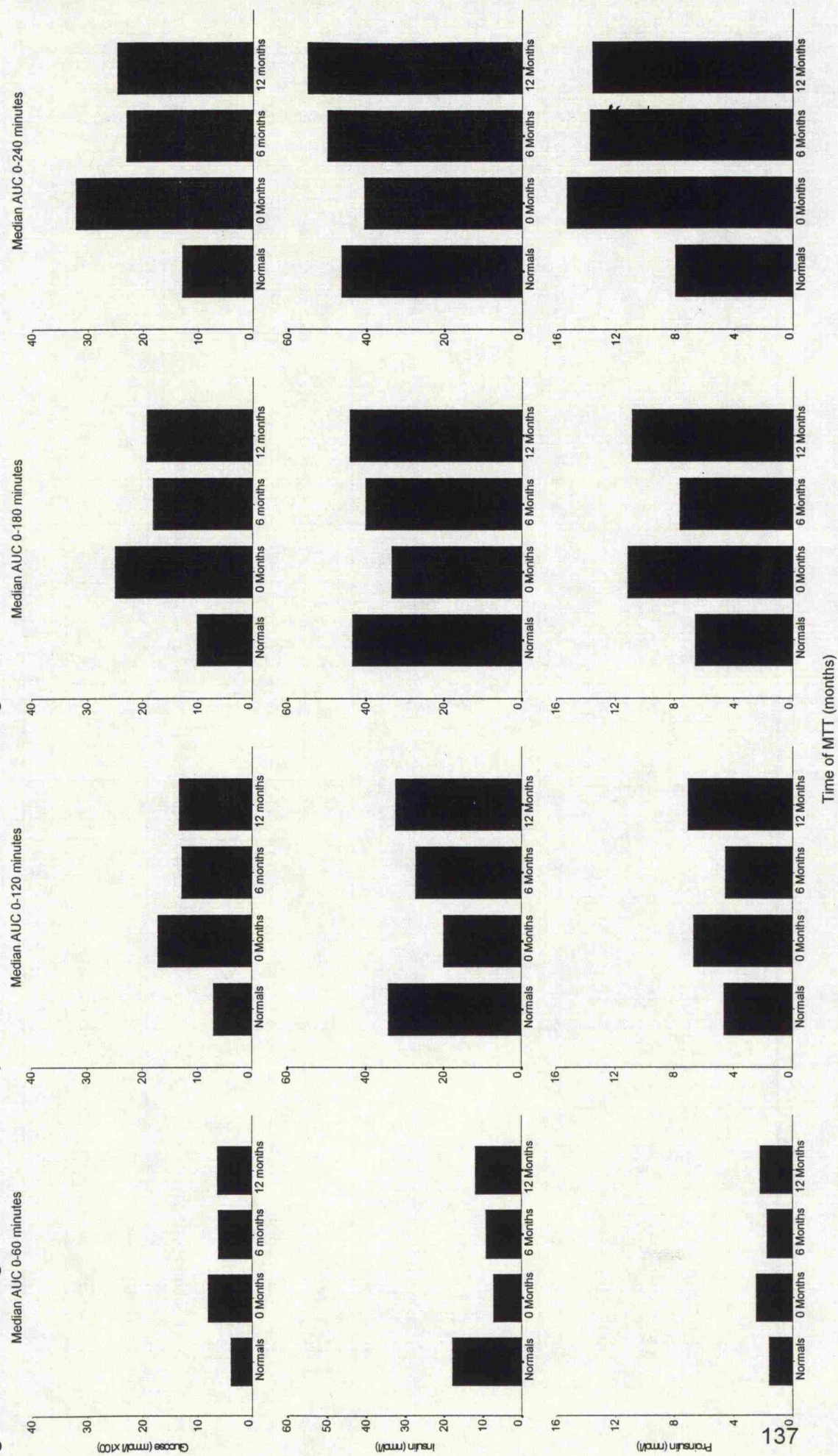


Table 8.2. Metabolic data from the MTT - diet treated NIDDM subjects (n=24) (median (interquartile range))

	0 Months	6 Months	12 Months	p value(0 vs 6)	p value (0 vs 12)	p value (6 vs 12)
Glucose						
Fasting (mmol/l)	10.9 (3.6)	8.2 (1.7)	8.2 (2.3)	***	**	ns
AUC ₀₋₆₀ (mmol/l)	810 (195)	614 (110)	640 (127)	***	***	ns
AUC ₀₋₂₄₀ (mmol/l)	3154 (755)	2166 (626)	2458 (549)	***	**	ns
Insulin						
Fasting (pmol/l)	52.8 (38.5)	56 (25.0)	71.3 (42.8)	ns	ns	ns
AUC ₀₋₆₀ (pmol/l)	7410 (7385)	9337 (3757)	11541 (6667)	ns	**	ns
AUC ₀₋₂₄₀ (pmol/l)	40818 (31849)	50227 (17152)	56216 (30453)	*	**	ns
Proinsulin						
Fasting (pmol/l)	30.3 (22.8)	23.0 (19.0)	22.9 (32.5)	**	**	ns
AUC ₀₋₆₀ (pmol/l)	2542 (1245)	1905 (1590)	2106 (365)	**	ns	ns
AUC ₀₋₂₄₀ (pmol/l)	15761 (9952)	13803 (9660)	13567 (12553)	*	ns	ns
Ratios						
FI:FPG	4.8 (5.2)	7.2 (4.2)	8.1 (4.9)	*	**	ns
FI:FPI	1.9 (1.8)	2.6 (1.9)	2.9 (3.3)	***	*	ns

* p < 0.05, ** p < 0.01, *** p < 0.001

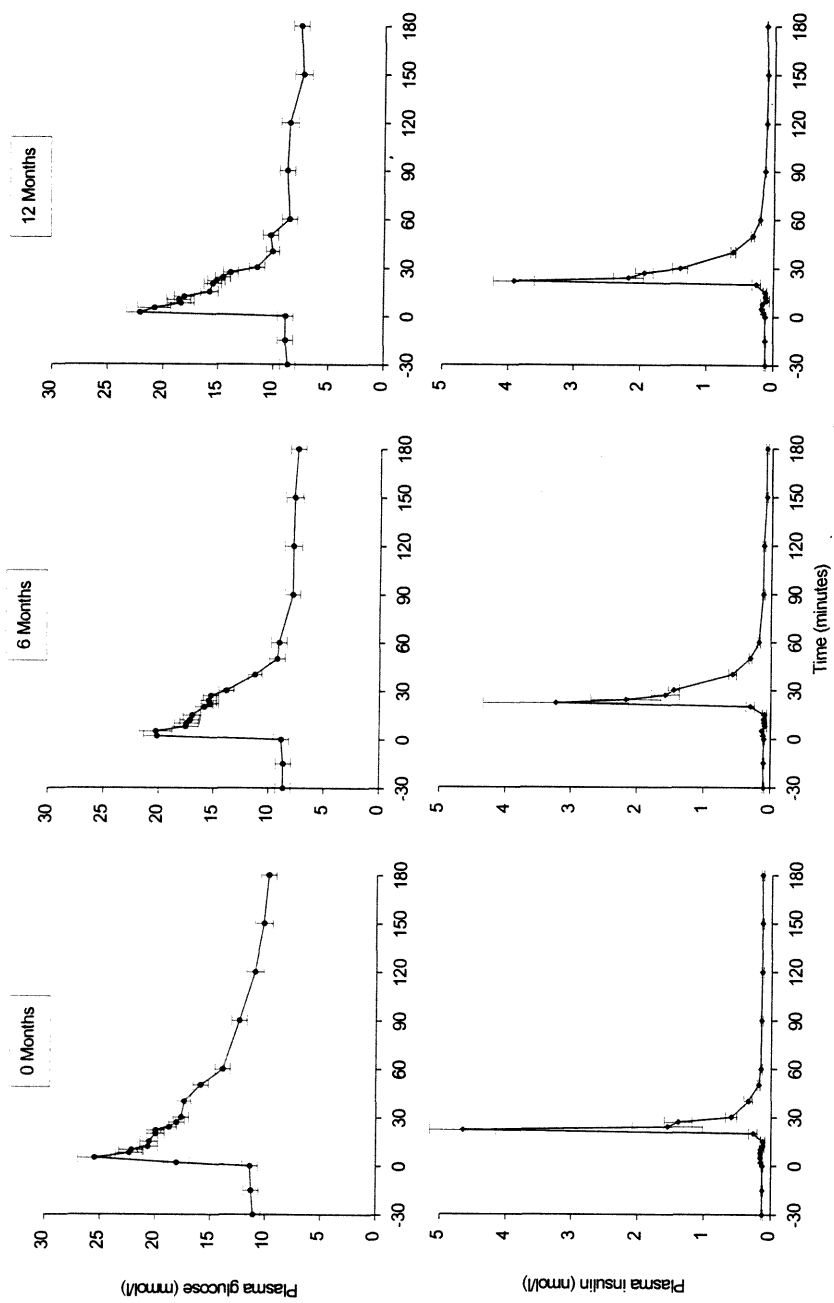
Wilcoxon signed rank test

Figure 8.2 illustrates the glucose, insulin and proinsulin AUC data from the meal tests in greater detail.

Median fasting plasma glucose concentrations fell significantly at 6 months and this was maintained at the 12 month assessment (10.9 to 8.2 to 8.2 mmol/l). Peak plasma glucose concentrations during the MTTs fell over the 12 month period with a suggestion of an earlier return to basal concentrations at 6 and 12 months compared to presentation. Median fasting insulin concentrations increased by 50% (52.8 to 71.3 pmol/l) over 12 months with post-prandial insulin secretion continuing to show a delayed peak and plateau pattern. As a result of these changes, the fasting insulin to glucose ratio was significantly increased at the 6 and 12 month assessments (4.8 to 7.2 to 8.1). Median fasting proinsulin concentrations fell significantly at 6 and 12 months (30.3 to 23.0 to 22.9 pmol/l) with a resultant highly significant increase in the fasting insulin to proinsulin ratio (1.9 to 2.6 to 2.9). Proinsulin profiles also continued to demonstrate delayed early concentrations with a prolonged later plateau phase at 6 and 12 months.

Post-prandial glucose early phase (0-60 minutes) AUC and total (0-240 minutes) AUC were both significantly reduced at 6 and 12 months. This pattern was seen throughout the MTTs but there was a non-significant trend towards an increase in early phase and total glucose AUC at 12 months compared to the 6 month data. This corresponds with the slight rise in HbA_{1c} at 12 months (9.1 to 7.7 to 8.0%) and the failure of the FPG to fall further (10.9 to 8.2 to 8.2 mmol/l). Post-prandial insulin early phase AUC (7410 to 9337 to 11541 pmol/l) and total AUC (40818 to 50227 to 56216 pmol/l) concentrations increased significantly at 6 and 12 compared to the presentation data. Both proinsulin early phase AUC (2542 to 1905 pmol/l) and

Figure 8.3. Mean glucose and insulin concentrations during the FSIVGTT at 0, 6 and 12 months



total AUC (15761 to 13803 pmol/l) concentrations fell significantly at 6 months and these reductions were maintained at 12 months.

B. Insulin-modified FSIVGTT data

Table 8.3 represents the glucose disposal, glucose effectiveness and insulin sensitivity data derived from the insulin-modified FSIVGTT at 0, 6 and 12 months. Figure 8.3 illustrates the mean glucose and insulin concentrations from the insulin-modified FSIVGTT at each assessment.

The median glucose disposal rate determined following the bolus of intravenous glucose (kg_{4-20}) improved significantly from presentation to 12 months (1.12 to 1.26 to $1.46 \times 10^{-2} \cdot \text{min}^{-1}$). Median estimated insulin sensitivity was very low compared to that from age, sex and BMI matched healthy subjects (median (IQR) 2.0 (2.2) $\text{min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$) but increased threefold over the first six months (0.44 to $1.23 \text{ min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$) and subsequently deteriorated at the 12 month assessment although remaining significantly higher than at presentation. Despite weight loss and improved FPG, there was no suggestion of a return of a first phase insulin response to the intravenous glucose bolus. Tables 8.4 - 8.6 give the results of multiple regression analyses to determine from the variables measured, those parameters capable of predicting fasting plasma glucose, β -cell function and insulin sensitivity at 6 and 12 months. As changing proinsulin concentrations have been regarded as a useful indicator of β -cell function (see Chapter 2) along with the fact that fasting insulin concentrations did not change significantly over the course of the study, the ratio of the two was used as a measure of the improvement in β -cell function.

Table 8.3. Glucose disposal, insulin sensitivity and glucose effectiveness data - diet treated NIDDM subjects (n=24)
(Median (interquartile range))

	0 Months	6 Months	12 Months	p value(0 vs 6)	p value (0 vs 12)	p value (6 vs 12)
Kg ^{4.20} (10 ⁻² .min ⁻¹)	1.12 (0.8)	1.26 (0.7)	1.46 (0.7)	ns	*	ns
SI (min ⁻¹ .uU ⁻¹ .ml x10 ⁴)	0.44 (0.53)	1.23 (0.71)	0.71 (0.50)	***	*	*
SG (min ⁻¹)	0.01558 (0.005)	0.01650 (0.005)	0.01491 (0.004)	ns	ns	ns

* p < 0.05, ** p < 0.01, *** p < 0.001

Wilcoxon signed rank test

Table 8.4. Predictors of FPG at 6 and 12 months

	0 vs 6 months			0 vs 12 months		
	Beta	t value	p value	Beta	t value	p value
Δ weight	-0.44	2.4	*	-0.37	2.0	*
Δ Si	+0.43	2.3	*	+0.32	1.6	ns
Δ FI:FPI	+0.25	1.4	ns	+0.16	0.7	ns
Δ insulin AUC ₀₋₂₄₀	+0.29	1.8	0.09*	+0.53	2.9	**
Δ Kg ₄₋₂₀	+0.06	0.3	ns	+0.37	1.9	*

Table 8.5. Predictors of FI:FPI at 6 and 12 months

	0 vs 6 months			0 vs 12 months		
	Beta	t value	p value	Beta	t value	p value
Δ weight	-0.40	2.0	*	-0.34	1.7	ns
Δ Si	+0.37	2.1	*	+0.17	0.7	ns
Δ FPG	-0.61	3.4	**	+0.11	0.2	ns
Δ glucose AUC ₀₋₂₄₀	-0.29	0.5	ns	-0.47	2.4	*

Table 8.6. Predictors of Insulin sensitivity at 6 and 12 months

	0 vs 6 months			0 vs 12 months		
	Beta	t value	p value	Beta	t value	p value
Δ weight	-0.60	3.3	*	-0.15	0.8	ns
Δ FPG	+0.73	1.7	ns	-0.61	3.5	*
Δ FI:FPI	+0.26	1.3	ns	+0.22	0.3	ns
Δ insulin AUC ₀₋₂₄₀	+0.99	1.7	ns	+0.21	1.0	ns

Δ = absolute change in value. * p < 0.05, ** p < 0.01, *** p < 0.0001

8.4 Discussion

This study aimed to document in detail the changes in β -cell function and insulin sensitivity following dietary intervention in a group of NIDDM subjects followed over one year from the time of diagnosis.

Dietetic advice reinforced every 3 months during the first year resulted in a significant fall in the total daily energy intake both at 6 and 12 months. The proportion of energy derived from carbohydrates and protein increased over this time but the major cause for the reduction in total energy intake was the significant fall in fat consumption over the study period. This change in calorific intake was associated with sustained weight loss at 6 and 12 months associated with falls in FPG and total cholesterol concentrations. Plasma triglyceride concentrations fell by 6 months and this reduction was maintained at 12 months as was the trend for falling LDL-cholesterol concentrations.

Post-prandial glucose AUC concentrations fell significantly by 6 months and remained lower than at presentation by 12 months despite small increases in both early phase and total AUC concentrations which were mirrored by a small increase in HbA_{1c}. Fasting insulin concentrations increased by 50% and post-prandial early phase AUC and total insulin AUC concentrations increased significantly at 6 and 12 months with a concomitant rise in the fasting insulin to glucose ratio. There were sustained falls in fasting and post-prandial early phase AUC and total AUC proinsulin concentrations at 6 and 12 months with associated increases in the fasting insulin to proinsulin ratio.

The rate of glucose disposal after intravenous injection as measured by Kg₄₋₂₀ increased at 6 and 12 months. Insulin sensitivity increased and remained

significantly higher than at presentation both at 6 and 12 months although there was a reduction in the SI estimate noted at the 12 month assessment.

Unfortunately, few similar long-term studies are available for comparative purposes. In terms of weight reduction, the median loss in this study over the 12 month study period was of the order of 5kg which compares favourably with studies in the USA, UK and Scandinavia [Wing et al,1987; UKPDS Group,1990; Uusitupa et al,1993]. Weight loss in the current study was associated not only with improvements in glycaemic indices (FPG and HbA_{1c}) but also other metabolic and clinical parameters associated with the syndrome of NIDDM. In particular, there were early and sustained falls in plasma triglyceride concentrations, systolic and diastolic blood pressure and later falls in total and LDL-cholesterol concentrations.

Treatment of NIDDM by any means (weight loss, sulphonylureas, insulin) results in improvements in β -cell function as measured by increases in immunoreactive insulin concentrations [Kosaka et al,1980]. More recently, using specific insulin and proinsulin assays to analyse oral glucose tolerance tests, a number of short term studies (2-5 months) have shown that weight loss is associated with an increase in the secretion of 'true' insulin and a decrease in the concentrations of total proinsulin [Yoshioka et al,1989] and other proinsulin-like molecules [Davies et al,1993a]. The current study fully supports these findings with increases in the secretion of true insulin and reductions in proinsulin concentrations following the mixed meal not only at 6 but also at 12 months following diagnosis.

As discussed in Chapter 1, the increased secretion of proinsulin in subjects with NIDDM is likely to be due to an increase in the activity of the regulated secretory pathway with mobilisation of immature secretory granules which are

rich in proinsulin. However, the mechanisms by which diet induced weight loss results in a reduction in the concentrations of proinsulin and an increase in the secretion of true insulin are not clear. The fasting insulin to proinsulin ratio at six months appeared to be determined by the absolute reductions in weight and fasting plasma glucose and the increase in insulin sensitivity. Of these, the reduction in fasting plasma glucose had the greatest predictive power and this would concur with the findings of Kosaka et al [Kosaka et al,1980] whose suggest that the reduction in 'glucose toxicity' may be the major determinant of improved β -cell function. The effects of improvements in insulin sensitivity on β -cell function do not necessarily require an alternative explanation. Improved insulin mediated glucose uptake would also result in falls in plasma glucose with resultant reduction of 'glucose toxicity' feeding back to the β -cell.

At 12 months, the best determinant of β -cell function was the absolute reduction in the total glucose AUC. This is of interest and explicable mainly as a result of the previous determinants failing to change (FPG) or falling (S_I). It also demonstrates the difficulty of using FPG as a measure of β -cell function as there were clear and continuing improvements in insulin secretion along with falls in proinsulin concentrations during the MTT at 12 months despite the lack of change in the FPG. The association with the reduction in total glucose AUC however again suggests an important influence of glucose per se on β -cell function.

In terms of insulin sensitivity, weight loss as a result of dietary manipulation has generally been shown to lead to improvements in insulin sensitivity in NIDDM subjects [Hughes et al,1984; Henry et al,1986; Laakso et al,1988; Wing et al,1994; Yamanouchi et al,1995]. In at least one of the studies in which insulin sensitivity failed to change, the weight loss associated with

dietary intervention was small (median 1.5kg, [Hosker et al,1993]) which may explain the lack of effect. The current study documented significant increases in insulin sensitivity (Median S_I = 0.44 at 0 months to median S_I = 1.26 at 6 months) over the first 6 months of weight loss, perhaps comparable to the results from the only other published study using the same technique for assessing insulin sensitivity (mean S_I = 0.5 at 0 months to mean S_I = 1.5 at 6 months) [Wing et al,1994]. However, despite the maintenance of weight loss, calorific restriction and reductions in fasting plasma glucose concentrations, the estimated S_I fell between 6 and 12 months although it remained significantly above the baseline measurement at presentation.

The mechanisms by which diet induced weight loss improves insulin sensitivity in NIDDM are not clear. Improvements in non-oxidative glucose disposal as a result of weight reduction have been previously documented [Laakso et al,1988]. Of additional interest however are the results from a further study in which the effects of weight loss on the different pathways of glucose metabolism were examined in obese non-diabetic subjects [Franssila-Kallunki et al,1992]. After very low calorie diets (VLCD) for 3 weeks, mean weight loss was 11kg and this was associated with significant increases in insulin sensitivity as measured by total glucose disposal, including increases in both oxidative and non-oxidative glucose disposal, and decreases in total plasma cholesterol, triglyceride concentrations and basal lipid oxidation. Hepatic glucose output remained unchanged. The improvement in oxidative glucose disposal was unexpected but the association with reductions in plasma lipoproteins and basal lipid oxidation suggested reversal of the activity the glucose-FFA cycle [Randle et al,1963] to be also associated with improved insulin sensitivity. Further supportive evidence for this concept came from the observation that the majority of the

weight loss came from a reduction in fat free mass which would result in a reduced flux of FFAs available for competition for oxidation with glucose.

Clearly the current study fails to directly address the metabolic mechanisms by which insulin sensitivity improves following diet-induced weight reduction, however fasting total cholesterol and triglyceride concentrations are correlated with fasting FFA concentrations [Lemieux et al,1994] and the reductions in these concentrations over the course of the current study may suggest that a similar mechanism i.e. reversal of the Randle cycle, may be contributing to the increase in insulin sensitivity brought about by diet alone.

Of the determinants of insulin sensitivity at 6 months, weight reduction as expected was the major factor (although the fall in FPG also appeared to have some predictive value). At 12 months however with weight reduction static or deteriorating marginally, the absolute fall in FPG over the 12 month period was the main determinant and hyperglycaemia has been demonstrated to independently increase insulin resistance [Yki-Järvinen,1990]. Despite the fall in insulin sensitivity at 12 months β -cell function continued to be improved compared to the time of presentation. Clearly this relates to the reduction of prevailing plasma glucose concentrations and the amelioration of 'glucose toxicity' however the hyperbolic relationship between β -cell function and insulin sensitivity may also help to explain the continued improvement. Kahn et al suggest that in subjects with very poor insulin sensitivity, small improvements will necessarily result in large improvements in β -cell function [Kahn et al,1993]. Although the absolute estimated insulin sensitivity index fell at 12 months it remained at twice the value estimated at presentation and perhaps resulted in continued improvements in β -cell function.

In summary therefore, the introduction of a weight reducing diet in newly presenting subjects with NIDDM has significant beneficial effects on glycaemic control as well as plasma lipoprotein concentrations and blood pressure. The improvements in glycaemic control appear to be sustained one year after the diagnosis and occur as a result of improvements both in β -cell function and insulin sensitivity. At 6 months after initiation of dietary therapy, there were significant increases in insulin secretion and falls in proinsulin concentrations and a threefold increase in insulin sensitivity. At 12 months, continuing increases in insulin secretion and reductions in proinsulin concentrations were associated with a 50% reduction in estimated S_i , although metabolic control in the NIDDM subjects remained stable. These results appear to suggest that β -cell function continues to improve in diet treated NIDDM subjects mainly as a result of the reduction in 'glucose toxicity'. Improvements in insulin sensitivity appear to have a permissive role in the improvements in metabolic control and β -cell function in both the early (6 month) and later (one year) stages of dietary treatment.

Chapter 9 - The effects of dietary intervention alone on β -cell function and insulin sensitivity in NIDDM subjects - a two year follow-up study

9.1 Introduction

The following chapter represents the results of the study of β -cell function and insulin sensitivity in a group of NIDDM subjects treated by diet alone for 24 months from the time of diagnosis.

9.2 Subjects and methods

Of the 24 subjects who completed the 12 month study, 16 were willing to be re-investigated at 24 months following diagnosis. Comparison of the baseline (0 month) clinical (age, weight, BMI, blood pressure) and metabolic (fasting plasma glucose, insulin and proinsulin, kg, S_e and S_i) data from the subjects willing to be re-investigated with those from the 8 subjects who were not revealed no evidence of systematic bias in the makeup of the former group. However, for comparative purposes the 16 subjects studied at 0 and 24 months were analysed as a separate data set. Each subject was reviewed clinically at 18 and 24 months and underwent an MTT and an insulin-modified FSIVGTT in the manner previously described at the 24 month assessment. Diet diaries were again kept by each NIDDM subject for three days prior to the metabolic assessment at 24 months and coded as before.

9.3 Results

Table 9.1 gives the comparative dietary, clinical and metabolic data for 0 and 24 months.

Table 9.1. Dietary, clinical and metabolic data 0 and 24 months - diet treated NIDDM subjects (n=16) (median (IQR))

	0 Months	24 Months	p value
Energy (kcal)	2209 (467)	2243 (515)	0.12
Carbohydrate (%)	40.0 (9.3)	40.5 (5.2)	0.58
Fat (%)	41.2 (8.0)	36.2 (4.7)	0.03
Protein (%)	17.3 (4.0)	18.5 (3.4)	0.23
Weight (kg)	92.9 (22.9)	89.9 (25.1)	0.02
BMI (kg/m ²)	29.3 (5.6)	28.2 (5.2)	0.008
Systolic bp (mmHg)	129 (25)	123 (20)	0.33
Diastolic bp (mmHg)	82 (14)	84 (13)	0.51
FPG (mmol/l)	9.7 (4.9)	8.4 (2.5)	0.28
HbA _{1c} (%)	8.9 (2.4)	8.1 (1.7)	0.04
Total cholesterol (mmol/l)	6.5 (1.5)	5.7 (0.95)	0.03
HDL-cholesterol (mmol/l)	0.91 (0.41)	0.99 (0.28)	0.35
LDL-cholesterol (mmol/l)	4.44 (1.5)	3.60 (1.1)	0.002
Triglycerides (mmol/l)	1.6 (1.2)	1.7 (1.5)	0.87

There was little overall change in energy intake as assessed by the diet diaries over the two year period, however there was a significant reduction in the percentage of energy derived from fat (41% to 36%). Weight fell significantly over the two year period (92.9 to 89.9 kg) and this was mirrored by fall in BMI (29.3 to 28.2 kg/m²). Overall glycaemic control as measured by

HbA_{1c} also improved significantly (8.9 to 8.1%). There were also significant reductions in total (6.5 to 5.7 mmol/l) and LDL-cholesterol (4.44 to 3.60 mmol/l) concentrations over the two years

A. MTT data

Table 9.2 gives the metabolic data from the MTT in detail. Figure 9.1 illustrates the MTT data with comparative data from age, sex and BMI matched healthy subjects. Figure 9.2 shows the AUC data throughout the MTT.

Table 9.2. Meal test data 0 and 24 months - diet treated NIDDM subjects (n=16) (median (IQR))

	0 Months	24 Months	p value
Glucose			
FPG (mmol/l)	9.7 (4.9)	8.4 (2.5)	0.28
AUC ₀₋₆₀ (mmol/l)	802 (239)	642 (180)	0.04
AUC ₀₋₂₄₀ (mmol/l)	3055 (973)	2657 (946)	0.08*
Insulin			
FI (pmol/l)	52.8 (29.5)	55.8 (37)	0.88
AUC ₀₋₆₀ (pmol/l)	6934 (7417)	7222 (9864)	0.35
AUC ₀₋₂₄₀ (pmol/l)	35857 (34560)	41948 (21562)	0.41
Proinsulin			
FPI (pmol/l)	28.8 (17.3)	15.3 (19.8)	0.03
AUC ₀₋₆₀ (pmol/l)	2475 (915)	1805 (971)	0.02
AUC ₀₋₂₄₀ (pmol/l)	15307 (7241)	11122 (4852)	0.02
Ratios			
FI:FPG	4.8 (6.3)	5.6 (5.2)	0.38
FI:FPI	1.9 (0.9)	3.3 (3.6)	0.02

The plateau-type profile of post-prandial glucose concentrations and insulin secretion improved over the 24 months with more rapid return to baseline values than at 0 months. There also appeared to be a lowering of post-

Figure 9.1. Plasma glucose, insulin and proinsulin profiles during the MTT

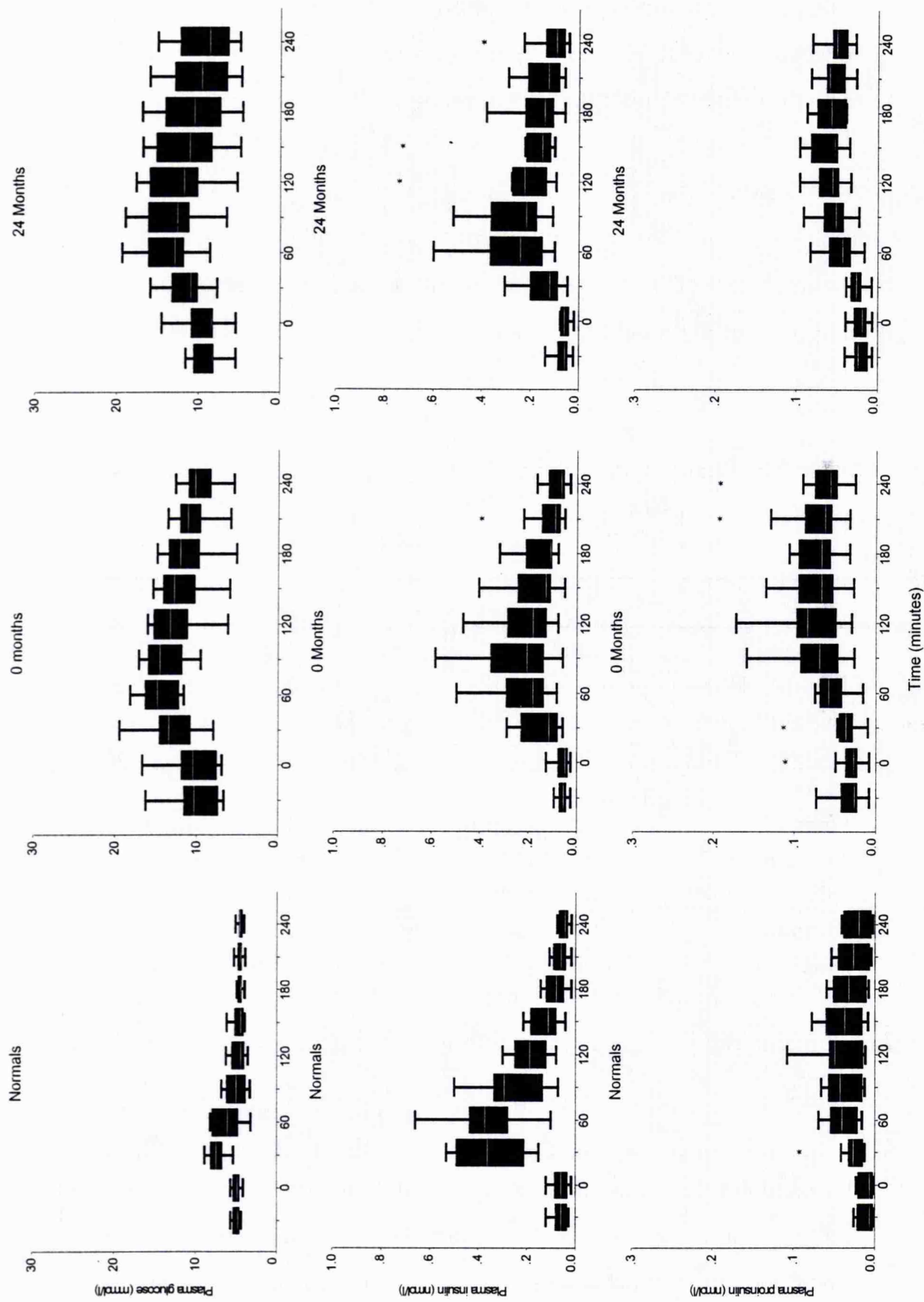
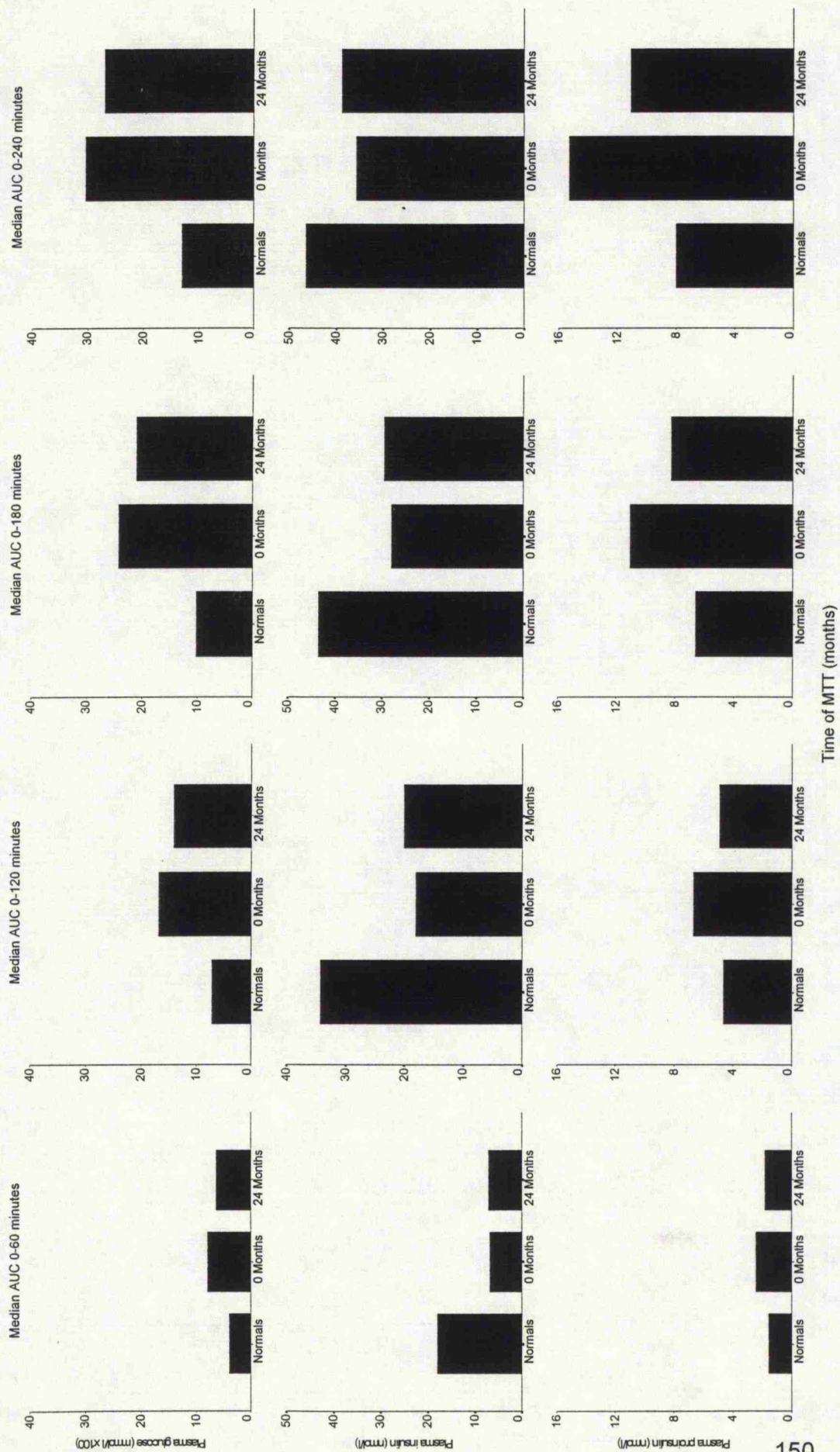


Figure 9.2. Plasma glucose, insulin and proinsulin AUC concentrations during the MTT



prandial proinsulin concentrations throughout the test at 24 months compared to the baseline study.

Improvements in post-prandial hyperglycaemia were reflected in the falls in early phase AUC (802 to 642 mmol/l) and total AUC (3055 to 2657 mmol/l) concentrations although the latter fell just below statistical significance ($p = 0.08$). Total AUC insulin concentrations increased by 17% over the two year period although this did not achieve statistical significance (see Figure 9.2, middle panels) however there were significant falls in fasting (28.8 to 15.3 pmol/l), early phase AUC (2475 to 1805 pmol/l) and total AUC (15307 to 11122 pmol/l) proinsulin concentrations at 24 months (see Figure 9.2, lower panels). Consequently, there was a twofold increase (1.9 to 3.3) in the fasting insulin to proinsulin ratio.

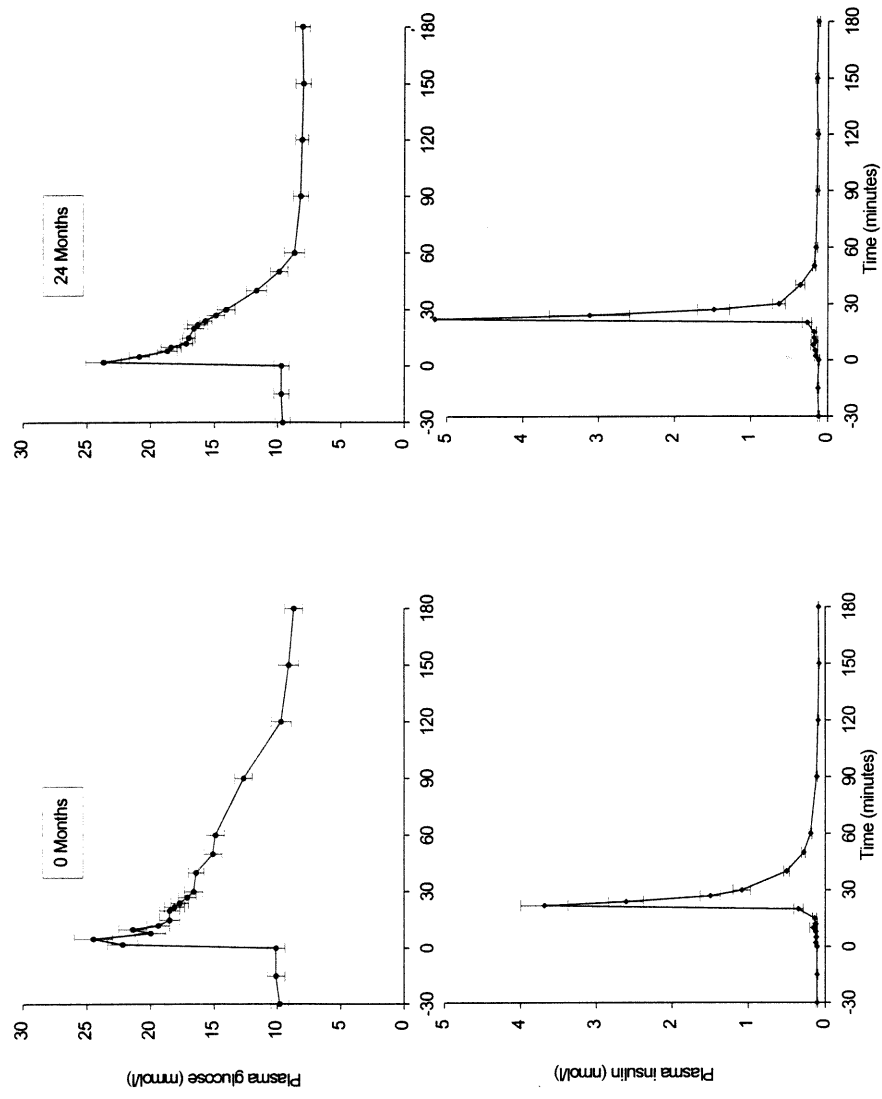
B. Insulin-modified FSIVGTT data

Table 9.3 gives the glucose clearance, insulin sensitivity and glucose effectiveness data for 0 and 24 months. Figure 9.3 illustrates the mean plasma glucose and insulin concentrations from the insulin-modified FSIVGTTs at these assessments.

Table 9.3. Glucose clearance, insulin sensitivity and glucose effectiveness data 0 and 24 months - diet treated NIDDM subjects (n=16) (median (IQR))

	0 Months	24 Months	p value
kg $_{4-20}$ ($10^{-2} \cdot \text{min}^{-1}$)	1.12 (0.8)	1.67 (0.5)	0.08*
S_i ($\text{min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$)	0.42 (0.6)	0.95 (0.8)	0.03
S_G (min^{-1})	0.01673 (0.006)	0.01397 (0.009)	0.08*

Figure 9.3. Glucose and insulin profiles from the FSVGTTs



At 24 months from presentation, the calculated glucose clearance following the intravenous glucose bolus had increased by 50% along with a significant rise in estimated insulin sensitivity (0.42 to $0.95 \text{ min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$). Glucose effectiveness fell by a smaller margin (17%) over the same time period. Some of these changes are reflected in the glucose profiles shown in Figure 9.3 with a steeper slope of falling glucose concentrations prior to the insulin bolus (kg) and a more rapid return to baseline glucose following the insulin bolus (Si) at 24 months.

Table 9.4 gives the results of a stepwise multivariate analysis to determine from the variables measures the factors predictive of improved β -cell function at 24 months as represented by the fasting insulin to proinsulin ratio, and Table 9.5 gives a similar analysis for insulin sensitivity.

Table 9.4. Predictors of FI:FPI at 24 months

	beta	t value	pvalue
Δ BMI	0.43	2.18	0.04
Δ Si	-0.45	2.29	0.04

Δ = absolute change

Table 9.5. Predictors of insulin sensitivity at 24 months

	beta	t value	pvalue
Δ BMI	0.60	2.87	0.01
Δ fasting proinsulin	0.26	1.17	0.26
Δ FI:FPI	-0.27	1.20	0.25

Δ = absolute change

Both the increase in insulin sensitivity and the fall in BMI were highly predictive for β -cell function, whereas for insulin sensitivity, the fall in BMI alone was the major determinant.

9.4 Discussion

This study documents the overall changes in β -cell function measured by the MTT and insulin sensitivity as estimated by the insulin-modified FSIVGTT which occurred in a group of NIDDM subjects treated by diet alone over 24 months from the time of diagnosis.

Dietary treatment resulted in falls in weight and BMI and improvements in the atherogenic lipid profile and glycaemic control as judged by glycosylated

haemoglobin and post-prandial glucose AUC concentrations. Post-prandial area under the curve insulin concentrations increased marginally over 24 months however fasting and post-prandial proinsulin concentrations fell significantly. Glucose disposal at basal insulin concentrations fell whilst the glucose clearance rate in response to intravenous glucose (Kg) and subsequently in response to intravenous insulin (Si) increased by the order of 50%.

These results require comparison to those achieved at 12 months in the slightly larger (although not systematically differing) group of 24 NIDDM subjects who commenced the study. In this group at 12 months, glycaemic control had improved dramatically compared to at presentation (median FPG 8.2 vs 10.9 mmol/l, median total glucose AUC 2458 vs 3154 mmol/l) associated with a 40% increase in median total insulin secretion over the test and a 15% fall in median proinsulin concentrations. Median insulin sensitivity also increased by 60%. In comparison by 24 months, the improvement in basal and post-prandial hyperglycaemia were maintained but were associated with a return to levels of fasting and post-prandial insulin secretion similar to those at the time of diagnosis along with a continuing decrease in fasting and post-prandial proinsulin concentrations. Insulin sensitivity continued to be maintained at over 100% of the original value. In assessing those factors most predictive of β -cell function (falling proinsulin, stable insulin) at 12 months the reduction in total glucose AUC concentrations appeared most important whereas at 24 months the improvement in insulin sensitivity and the fall in BMI were the major predictors. For insulin sensitivity, the major predictor at 12 months was the fall in FPG whilst at 24 months this had been replaced by the reduction in BMI.

This perhaps provides an insight into the potential mechanisms for the continuing improvement in glycaemic control in this group. At 12 months, it was suggested that the fall in glucose itself was the major factor in improving β -cell function i.e. reduction in 'glucose toxicity' and that improvements in insulin sensitivity played a permissive rather than a central role. At 24 months however, the continued improvement in β -cell function in the face of stable glycaemic control as judged by the reduction in proinsulin concentrations would appear to be secondary to the maintenance of improved insulin sensitivity although the positive effects of the reduction of 'glucose toxicity' on the β -cell must also be contributory.

In summary, at 24 months following diagnosis, subjects with NIDDM treated by diet alone exhibit sustained improvements in basal and post-prandial hyperglycaemia. This appears to occur as a result of the maintenance of weight-loss induced improvements in insulin sensitivity, reduced 'glucose toxicity' and a subsequent continuing improvement in β -cell function.

Chapter 10 - The effects of sulphonylurea treatment on β -cell function and insulin sensitivity in NIDDM subjects - a one year study

10.1 Introduction

Subjects with NIDDM who fail to show improvements in glycaemic control after an appropriate period of dietary intervention are normally considered for therapy with an oral hypoglycaemic agent. For NIDDM subjects who are not severely obese, sulphonylureas (SFU) remain the drugs of choice [Alberti et al,1994] and estimates suggest that up to 30% of subjects with NIDDM are currently treated with a sulphonylurea agent [Kennedy et al,1988].

The hypoglycaemic properties of the sulphonamide antibiotics from which all sulphonylureas are derived were discovered accidentally in 1942 [Jambon et al,1942] and subsequently a variety of sulphonylureas have been developed for therapeutic use in NIDDM subjects. Numerous studies have since documented improved glycaemic control after treatment with sulphonylureas [Lavaux et al,1972; Owens et al,1980; Hernandez et al,1986] and the mechanisms of their pancreatic and extra-pancreatic effects have been closely studied.

The major mode of action of sulphonylureas on the pancreas is to enhance glucose-stimulated insulin secretion from the β -cell. This is brought about by the specific blockade of an ATP-sensitive potassium channel in the β -cell membrane [Sturgess et al,1985] resulting in the opening of voltage-dependent transmembrane calcium channels, influx of calcium, cellular depolarisation and insulin secretion [Malaisse et al,1990].

The evidence for major extrapancreatic effects of sulphonylureas is less clear. A number of studies employing the euglycaemic hyperinsulinaemic clamp have documented increased insulin-mediated glucose disposal in NIDDM subjects after treatment with a variety of sulphonylureas [Greenfield et al,1982; Kolterman et al,1984; Ward et al,1985; Firth et al,1986b; Wajchenberg et al,1993]. Although muscle glucose uptake was documented to increase in these studies sulphonylureas appeared to have a greater effect on reducing hepatic glucose output. The mechanism by which sulphonylureas bring about improvements in insulin sensitivity appears unclear as in some studies improvements have been related entirely to increased insulin secretion [Firth et al,1986c] whilst in others the effect has been attributed to some intrinsic property of the drugs with no documented changes in plasma insulin concentrations [Beck-Nielsen et al,1988]. In this latter respect, some in-vitro studies have demonstrated sulphonylurea induced increases in post-binding insulin action [Ward et al,1985] or the number of insulin receptors expressed on hepatocytes [Feinglos et al,1978]. In contrast to these studies however, a number of other investigators have failed to demonstrate any changes in insulin sensitivity following sulphonylurea therapy in NIDDM subjects [Hosker et al,1985; Marchand et al,1985; Hosker et al,1989]

The aim of the current study was to document in detail the changes in β -cell function and insulin sensitivity estimated by the insulin-modified FSIVGTT brought about by treatment with the sulphonylurea gliclazide in NIDDM subjects who did not adequately respond to 3 months intensive dietary management.

10.2 Subjects and methods

Subjects were recruited as documented in Chapter 2. Treatment with gliclazide was commenced in subjects whose fasting plasma glucose remained above 9 mmol/l after 3 months of dietary treatment. Ten subjects satisfied this inclusion criterion and were started on gliclazide. The initial dose was 80mg daily, increasing to a maximum of 160mg twice daily on clinical review at 6, 9 and 12 months in an attempt to achieve fasting plasma glucose concentrations of 9 mmol/l or less.

After the initial metabolic assessments at the time of presentation, those subjects assigned to sulphonylurea therapy had repeat assessments of β -cell function and insulin sensitivity at 12 and 24 months. Diet diaries were kept by each subject for the three days prior to each metabolic assessment and the data processed as previously described. The comparative data over the first 12 months is reported here.

The test protocols along with the analytical and statistical methods are as described in Chapter 2.

10.3 Results

Baseline data

Table 10.1 represents the demographic, clinical and metabolic data at the time of presentation for those NIDDM subjects who were subsequently treated with sulphonylureas at the 3 month clinical review in comparison to those subjects who remained on diet alone.

Table 10.1. Baseline demographic, metabolic and clinical data - pre-SFU (n=10) and pre-diet treated (n=24) NIDDM subjects (median (IQR))

	pre-SFU treated	pre-Diet treated	p value
Weight (kg)	73.9 (12.8)	89.5 (21.9)	0.001
BMI (kg/m ²)	26.3 (6.2)	29.6 (5.5)	0.01
Systolic bp (mmHg)	130 (10.0)	130 (26.5)	0.82
Diastolic bp (mmHg)	81 (2.0)	83 (16.5)	0.57
FPG (mmol/l)	15.8 (1.5)	10.9 (3.6)	0.0002
HbA _{1c} (%)	12.6 (1.4)	9.1 (2.4)	0.001
Total cholesterol (mmol/l)	6.6 (1.0)	6.5 (1.5)	0.26
HDL-cholesterol (mmol/l)	1.02 (0.5)	0.91 (0.35)	0.96
LDL-cholesterol (mmol/l)	4.15 (1.3)	4.3 (1.5)	0.67
Triglycerides (mmol/l)	2.3 (4.5)	2.3 (1.5)	0.64

In comparison to the subjects who were to remain on diet alone, the NIDDM subjects who required SFU treatment 3 months after presentation were of lower weight (73.9 vs 89.5 kg) and BMI (26.3 vs 29.6 kg/m²) and had significantly worse glycaemic control as judged by FPG (15.8 vs 10.9 mmol/l), HbA_{1c}, (12.6 vs 9.1%)

A. MTT data

Table 10.2 shows similar comparative metabolic data from the MTT in greater detail.

Table 10.2. Meal test data - pre-SFU (n=10) and pre-diet treated (n=24) NIDDM subjects (median (IQR))

	pre-SFU treated	pre-Diet treated	p value
Glucose			
FPG (mmol/l)	15.8 (1.5)	10.9 (3.6)	0.0002
AUC ₀₋₆₀ (mmol/l)	1138 (153)	810 (195)	0.0003
AUC ₀₋₂₄₀ (mmol/l)	4790 (382)	3154 (755)	0.0001
Insulin			
FI (pmol/l)	39 (38)	52.8 (38.5)	0.04
AUC ₀₋₆₀ (pmol/l)	3990 (4729)	7410 (7385)	0.02
AUC ₀₋₂₄₀ (pmol/l)	22170 (22887)	40818 (31849)	0.02
Proinsulin			
FPI (pmol/l)	24.8 (22.5)	30.3 (22.8)	0.25
AUC ₀₋₆₀ (pmol/l)	2040 (1770)	2542 (1245)	0.09
AUC ₀₋₂₄₀ (pmol/l)	12090 (9225)	15761 (9952)	0.02
Ratios			
FI:FPG	3.1 (2.3)	4.8 (5.2)	0.002
FI:FPI	1.4 (1.1)	1.9 (1.8)	0.47

For those subjects destined to be treated with SFUs the post-prandial early phase AUC (1138 vs 810 mmol/l) and total AUC (4790 vs 3154 mmol/l) glucose concentrations during the MTT were significantly higher than those of subjects who remained on diet alone. In addition, the subjects requiring SFU therapy displayed significantly worse β -cell function in the face of this higher glucose exposure with lower fasting insulin concentrations (39 vs 58.2 pmol/l) and a 50% reduction in post-prandial early phase AUC and total AUC insulin

secretion. Total AUC proinsulin concentrations were also reduced by the order of 20%.

B. Insulin-modified FSIVGTT data

Table 10.3 compares the pre-treatment glucose disposal, glucose effectiveness and insulin sensitivity data between the two groups.

Table 10.3. Glucose clearance, insulin sensitivity and glucose effectiveness data - pre-SFU (n=10) and pre-diet treated (n=24) NIDDM subjects (median (IQR))

	pre-SFU treated	pre-Diet treated	p value
Kg ₄₋₂₀ (10 ⁻² .min ⁻¹)	1.4 (0.9)	1.12 (0.8)	0.72
S _I (min ⁻¹ .uU ⁻¹ .ml x10 ⁴)	0.31 (0.85)	0.44 (0.53)	0.35
S _G (min ⁻¹)	0.01825 (0.014)	0.01558 (0.005)	0.04

The glucose effectiveness i.e. glucose disposal at basal insulin concentrations was significantly higher in the NIDDM subjects destined to be treated with sulphonylureas (0.01825 vs 0.01558 min⁻¹) but the glucose disposal rate following the intravenous glucose bolus (kg₄₋₂₀) and insulin sensitivity were not different from the diet treated NIDDM subjects.

Sulphonylurea treated subjects - comparative data 0 and 12 months

Table 10.4 gives the dietary, clinical and metabolic data for the SFU treated group at 0 months (presentation) and after 12 months therapy.

Table 10.4. Dietary, demographic, metabolic and clinical data for SFU treated NIDDM subjects at 0 and 12 months (median (IQR))

	0 Months	12 Months	p value
Energy (kcal)	2327 (1691)	2110 (699)	0.50
Carbohydrate (%)	45.3 (11.5)	45.9 (9.5)	0.50
Protein (%)	17.0 (4.3)	17.3 (6.8)	0.22
Fat (%)	37.7 (8.1)	36.8 (10.3)	0.22
Weight (kg)	73.9 (12.8)	74.9 (14.4)	0.05
BMI (kg/m ²)	26.6 (6.2)	26.8 (6.1)	0.60
Systolic bp (mmHg)	130 (10.0)	130 (6.0)	0.88
Diastolic bp (mmHg)	81 (2.0)	79 (20.0)	0.72
FPG (mmol/l)	15.8 (1.5)	9.8 (3.2)	0.005
HbA _{1c} (%)	12.6 (1.4)	9.5 (1.9)	0.005
Total cholesterol (mmol/l)	6.6 (1.0)	6.3 (1.5)	0.10
HDL-cholesterol (mmol/l)	1.02 (0.5)	1.08 (0.3)	0.92
LDL-cholesterol (mmol/l)	4.15 (1.3)	4.01 (1.6)	0.72
Triglycerides (mmol/l)	2.3 (4.5)	2.4 (1.7)	0.50

Despite specific dietary advice there were no significant changes in the energy content or the composition of the diets at 0 and 12 months. Weight increased marginally and body mass index remained essentially unchanged but glycaemic control as judged by FPG (15.8 vs 9.8 mmol/l) and HbA_{1c} (12.6

vs 9.5%) improved significantly. There were small but non-significant falls in total cholesterol and LDL-cholesterol concentrations.

A. Meal Tolerance test data

Table 10.5 gives data from the MTTs in detail. Figure 10.1 displays the data from the MTTs at 0 and 12 months and Figure 10.2 shows the glucose, insulin and proinsulin AUC concentrations throughout the tests compared to the responses from age, sex and BMI matched healthy subjects.

Table 10.5. Meal test data - SFU treated NIDDM subjects at 0 and 12 months (median (IQR))

	0 Months	12 Months	p value
Glucose			
FPG (mmol/l)	15.8 (1.5)	9.8 (3.2)	0.005
AUC ₀₋₆₀ (mmol/l)	1138 (153)	825 (307)	0.005
AUC ₀₋₂₄₀ (mmol/l)	4790 (382)	3285 (1237)	0.005
Insulin			
FI (pmol/l)	39 (38)	53 (22)	0.009
AUC ₀₋₆₀ (pmol/l)	3990 (4729)	7830(4838)	0.005
AUC ₀₋₂₄₀ (pmol/l)	22170 (22887)	42052 (32588)	0.005
Proinsulin			
FPI (pmol/l)	24.8 (22.5)	23.1 (17.5)	0.33
AUC ₀₋₆₀ (pmol/l)	2040 (1770)	2186 (1204)	0.11
AUC ₀₋₂₄₀ (pmol/l)	12090 (9225)	16383 (11786)	0.01
Ratios			
FI:FPG	3.1 (2.3)	5.6 (3.7)	0.005
FI:FPI	1.4 (1.1)	2.3 (1.8)	0.20

Figure 10.1. Plasma glucose, insulin and proinsulin profiles during the MTT

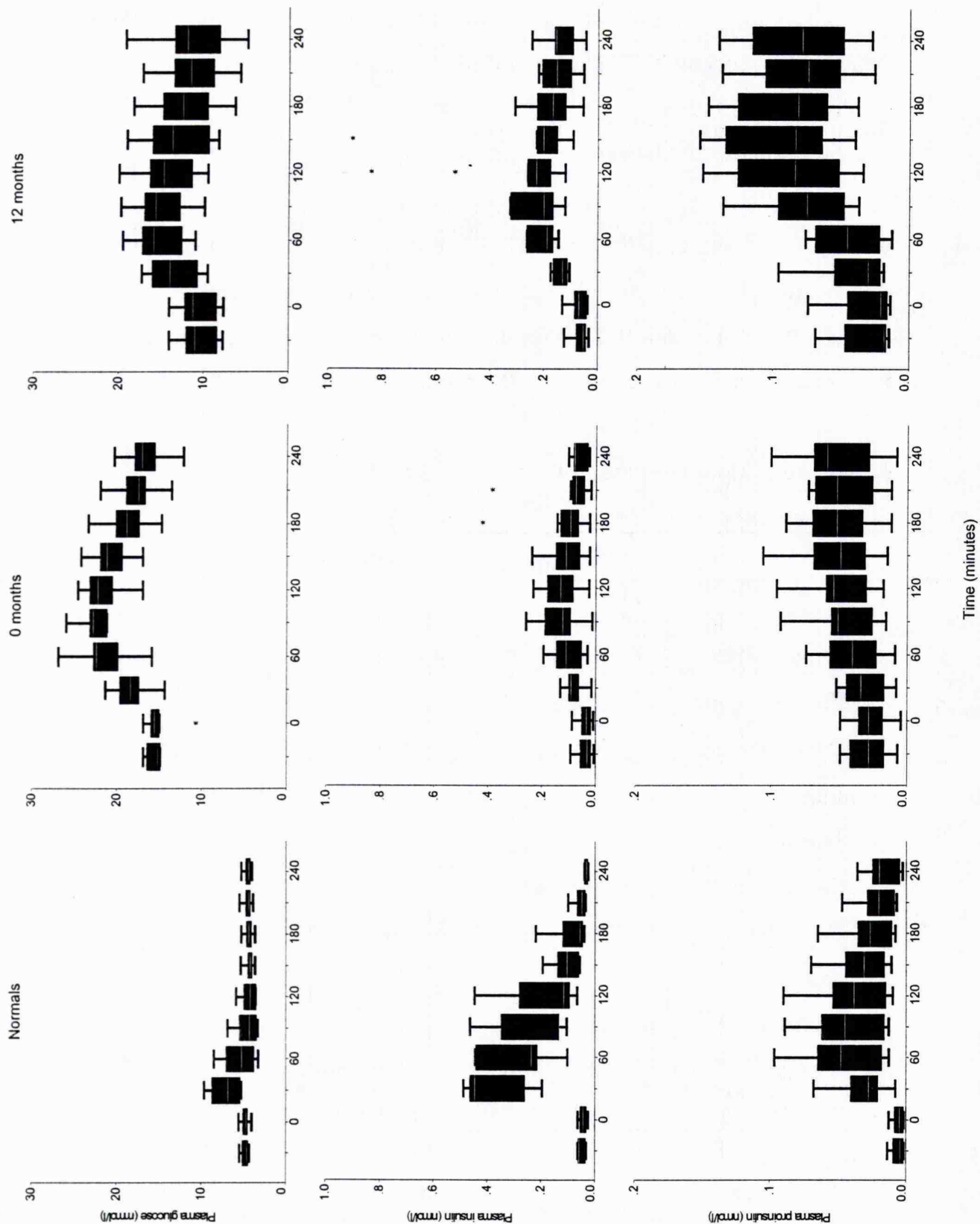
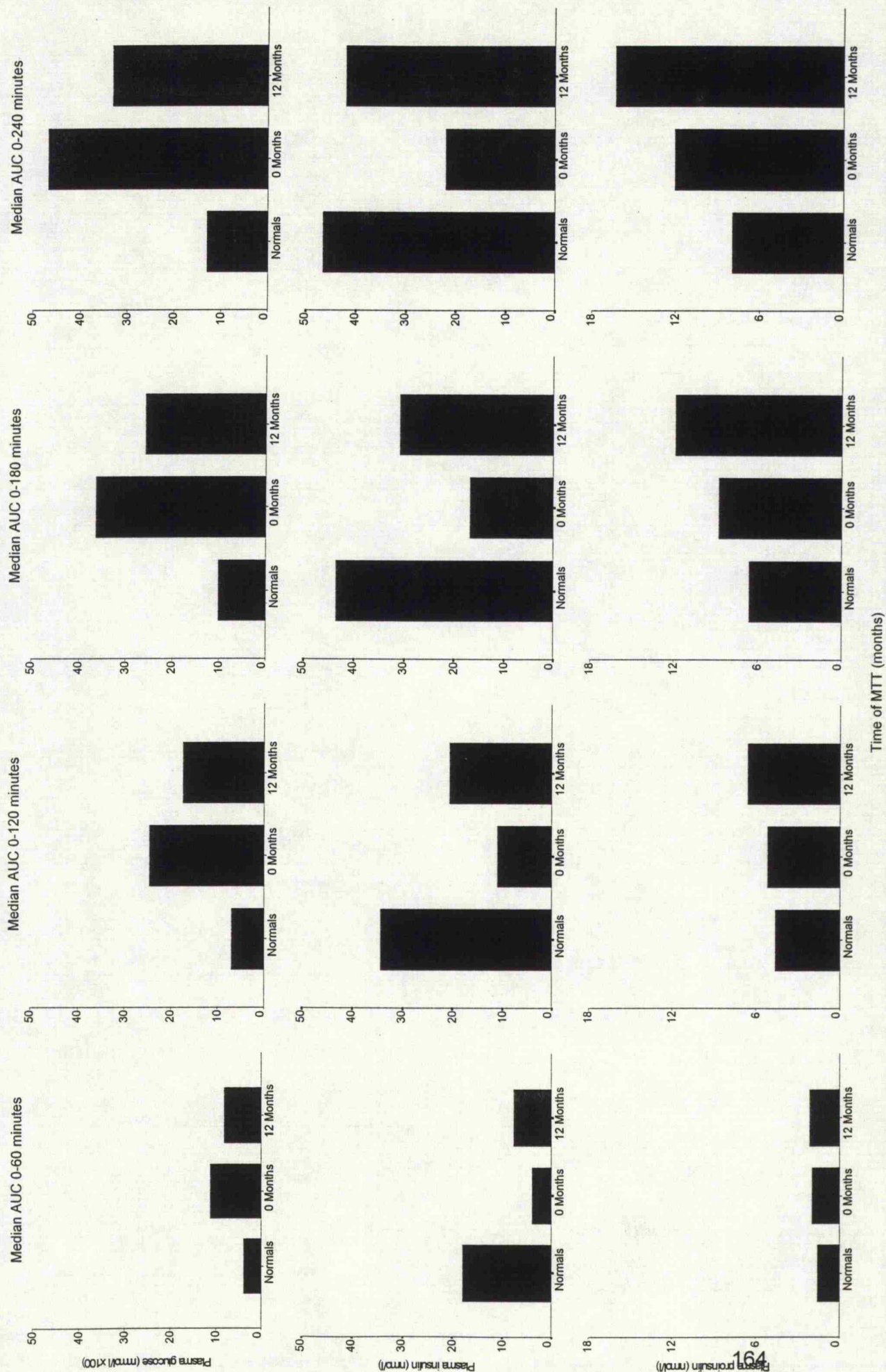


Figure 10.2. Plasma glucose, insulin and proinsulin AUC concentrations during the MTT



The post-prandial glucose profile from the MTT reflected the improvement in glycaemic control but continued to display a prolonged hyperglycaemic plateau after the meal. The insulin secretory profile still remained abnormal at 12 months with a delayed onset of secretion after the meal followed by a prolonged plateau phase with failure to return to basal concentrations by 240 minutes. For proinsulin concentrations the virtually flat profile at 0 months changed to one of reduced early concentrations followed by prolonged elevation of plasma concentrations with failure to return to basal by the end of the meal.

Fasting plasma glucose concentrations fell by 30% over the first 12 months and post-prandial early phase AUC (1138 vs 825 mmol/l) and total AUC (4790 vs 3285 mmol/l) glucose concentrations also fell significantly, the improvement being evident at all stages of the MTT (Figure 10.2, top panels). Fasting plasma insulin concentrations also increased significantly (39 to 53 pmol/l) with a consequent increase in the fasting insulin to glucose ratio (3.1 to 5.6). Post-prandial insulin secretion also improved significantly at 12 months, again evident at each stage of the MTT (Figure 10.2, middle panels) but specifically there was a 50% increase in both early phase AUC and total AUC insulin secretion. Fasting and early phase AUC proinsulin concentrations in response to the MTT did not change over the 12 month period however total AUC proinsulin increased significantly by 25% (12090 to 16383 pmol/l).

B. Insulin-modified FSIVGTT data

Table 10.6 represents the glucose disposal, insulin sensitivity and glucose effectiveness data from the insulin-modified FSIVGTTs at 0 and 12 months.

Figure 10.3. Glucose and insulin concentrations during the FSIVGTTs

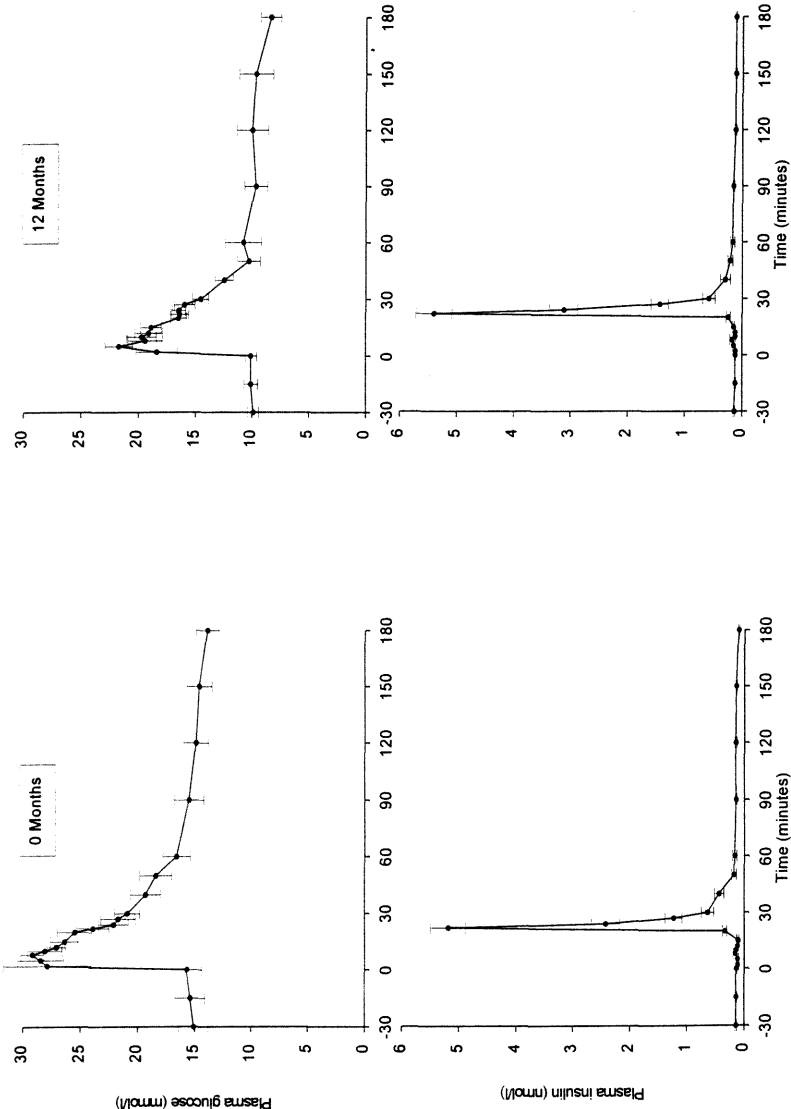


Figure 10.3 illustrates the mean glucose and insulin concentrations from these latter assessments.

Table 10.6. Glucose clearance, glucose effectiveness and insulin sensitivity data - SFU treated NIDDM subjects at 0 and 12 months (median (IQR))

	0 Months	12 Months	p value
Kg_{4-20} ($10^{-2} \cdot \text{min}^{-1}$)	1.4 (0.9)	1.1 (0.3)	0.39
S_I ($\text{min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$)	0.31 (0.85)	1.02 (0.9)	0.01
S_G (min^{-1})	0.01825 (0.014)	0.01271 (0.001)	0.01

The calculated glucose clearance rate remained unchanged at 12 months however glucose effectiveness fell significantly (0.01825 to 0.01271 min^{-1}). Median insulin sensitivity increased threefold over the course of the assessments and the improved glycaemic control resulted in a leftward shift of the plasma glucose concentrations during the insulin-modified FSIVGTT. Despite the fall in FPG, there was no suggestion of a return of first-phase insulin response to the injected glucose.

Tables 10.7-10.9 show the results of stepwise multiple regression analysis to determine from those parameters measured the factors capable of predicting fasting plasma glucose, fasting plasma insulin and insulin sensitivity at 12 months in these SFU treated NIDDM subjects.

Table 10.7. Predictors of fasting plasma glucose concentrations at 12 months

	beta	t value	pvalue
Δ fasting insulin	-0.78	3.6	0.007
Δ insulin AUC 0-60	-0.34	1.02	0.34
Δ insulin AUC 0-240	-0.23	0.80	0.44
Δ SI	-0.32	1.11	0.30
Δ Kg	+0.16	0.66	0.53

Δ = absolute change

Table 10.8. Predictors of fasting plasma insulin concentrations at 12 months

	beta	t value	pvalue
Δ weight	+0.29	0.78	0.47
Δ fasting plasma glucose	+0.81	3.48	0.01
Δ glucose AUC 0-240	+0.31	0.12	0.91
Δ SI	-0.47	2.26	0.07

Δ = absolute change

Table 10.9. Predictors of insulin sensitivity at 12 months

	beta	t value	pvalue
Δ fasting plasma glucose	-0.07	0.25	0.81
Δ fasting insulin	-0.52	3.30	0.01
Δ insulin AUC 0-240	-0.01	0.06	0.95
Δ proinsulin AUC 0-240	-0.47	2.68	0.03
Δ FI:FPG	+0.04	0.12	0.91

Δ = absolute change

Both the fall in fasting plasma glucose and the increase in estimated insulin sensitivity at 12 months appeared to be best predicted by the increase in fasting plasma insulin concentrations over the one year treatment period.

Fasting plasma insulin concentrations at 12 months were best predicted by the fall in fasting plasma glucose concentrations. The rise in insulin sensitivity over the 12 month period also appeared to have predictive power for fasting insulin concentrations although this fell just below the level of statistical significance.

10.4 Discussion

Treatment of NIDDM relies initially on dietary manipulation and in up to 70% of subjects metabolic control can be achieved and maintained on diet alone providing patients remain motivated [Hadden et al,1986]. A significant minority of subjects will, therefore, fail to reach the required therapeutic targets or may remain with symptoms relating to hyperglycaemia and thus require treatment. Sulphonylurea therapy is recommended for the treatment of the non-obese (BMI generally $<27\text{kg/m}^2$ [Lemieux et al,1994]) NIDDM subject who fails to improve after diet therapy alone [Alberti et al,1994].

In this study, the NIDDM subjects who failed to achieve a fasting plasma glucose of 9 mmol/l after 3 months intensive dietary therapy were significantly less obese than the subjects who continued on diet alone. In addition their post-prandial glucose and insulin responses to the MTT were also significantly worse. In particular, in the face of higher early phase and total AUC glucose concentrations, insulin early phase AUC and insulin and proinsulin total AUC concentrations were significantly lower than in the diet treated subjects. The equivalence of the insulin sensitivity estimates between the two groups also suggested that glycaemic control was poorer in subjects ultimately requiring SFU treatment mainly as a result of significantly poorer β -cell function. It is also of interest to note that Sg i.e. glucose uptake at basal

insulin concentrations, was significantly higher in the SFU treated group. This may have represent a compensatory mechanism in view of the poor basal insulin secretion to limit the rise in fasting plasma glucose and impose a new basal glycaemic steady-state by increased 'mass effect' of glucose.

Dietary intervention over the 12 month investigation period resulted in little change in total energy consumption or the constituents of the diet. As the primary aim of dietary intervention in this group of non-obese NIDDM subjects would be weight maintenance however, this is not unexpected although it is disappointing that the contribution of fat to the energy content of the diet did not fall significantly.

The effects of the sulphonylurea therapy on β -cell function are clearly evident from the MTT data. Highly significant improvements in all indices of glycaemic control were associated with a doubling of insulin secretion over the course of the meal test although the profile of insulin secretion remained abnormal. These results are in accord with a number of other studies which have documented improved insulin secretion following improvements in glycaemic control brought about by a variety of treatment modalities [Kosaka et al,1980; Hosker et al,1985; Matsumoto et al,1994].

As the pancreatic effects of sulphonylureas are to chronically stimulate β -cell insulin secretion it may be expected that the proportions of both insulin and proinsulin released following the consumption of a meal would be altered. Proinsulin concentrations during the MTT were significantly elevated after 9 months SFU therapy which contrasts sharply with the falls in post-prandial proinsulin concentrations documented in the diet controlled subjects at 12 months for the same degree of improvement in glycaemic control. A number of studies have recently suggested that elevations of total and split-proinsulin

concentrations are more potent risk factors for coronary artery disease (the major cause of death in NIDDM subjects) than insulin concentrations [Nagi et al,1990; Davies et al,1993a]. Consequently the improvement in glycaemic indices brought about by sulphonylurea therapy after one year may occur at the expense of increased risks from coronary artery disease as a result of hyperproinsulinaemia in the short term.

In terms of the mechanism of improved β -cell function, multiple regression analysis suggested that the reduction in fasting plasma glucose concentrations by 12 months had the major beneficial effect although the improved insulin sensitivity appeared to contribute albeit to a lesser extent. This is of some interest as clearly in the acute phase of administration, SFUs result in increased insulin secretion from the β -cell and therefore the improvements in β -cell function would be expected to be entirely secondary to the therapy. In Duckworth's long-term (one year) study [Duckworth et al,1972] plasma IRI concentrations following oral and intravenous glucose returned to those measured at the start of the study, the implication being that sulphonylureas had only a temporary effect on insulin secretion. This conclusion was however erroneous as the plasma IRI responses obtained at the start and end of the study were obtained at different plasma glucose concentrations. Kosaka et al demonstrated using three groups of subjects with identical levels of hyperglycaemia that the improvement in β -cell function occurs independently of the mode of therapy (diet, sulphonylurea or insulin) and is therefore related to the reduction in plasma glucose concentrations [Kosaka et al,1980]. In this study, as fasting and post-prandial insulin concentrations and the total post-prandial AUC proinsulin concentration were significantly higher at 12 months on gliclazide therapy effects of both the drug itself and the reduction in glucose toxicity on β -cell function are apparent.

A third potential cause for the increased concentrations of insulin after SFU therapy is the possibility of reduced clearance of insulin precipitated by the drugs themselves. An early report that SFUs resulted in a reduction in the activity of the enzyme insulinase and thus an increase in circulating insulin concentrations was regarded as improbable [Mirsky et al,1956] however several more recent studies have documented increased displacement of insulin from its receptors brought about by SFUs [Almer et al,1982; Scheen et al,1984; Groop et al,1988].

As well as having a significant effect on β -cell function, sulphonylurea therapy in this study was associated with a threefold increase in estimated insulin sensitivity, confirming an earlier report using the insulin-modified FSIVGTT to investigate the effects of short-term (3 months) gliclazide therapy in NIDDM subjects [Wajchenberg et al,1993]. The mechanism behind this improvement in insulin sensitivity after SFU administration as discussed earlier is likely to be mainly as a result of increased hepatic sensitivity to insulin resulting in reduced hepatic glucose output. From the multiple regression analysis it would appear that the increase in fasting insulin concentration over the 12 month period had the major influence on insulin sensitivity and this may be linked directly to reductions in HGO. It is equally plausible however that SFU induced increases in insulin secretion sufficient to overcome initial insulin resistance lead to a reduction in hyperglycaemia, itself an independent cause of insulin resistance [Yki-Järvinen,1990], and contributed to the improvement in estimated insulin sensitivity.

It is of interest to also note that Sg fell following SFU therapy presumably reflecting the reduction in FPG brought about by the improved β -cell function and therefore the possible re-imposition of control of basal glucose

concentrations by the basal insulin concentrations as opposed to a global increase in disposal of glucose by mass action.

In conclusion, SFU therapy in subjects with NIDDM results in significant improvements in glycaemic control as a result of improved β -cell function and insulin sensitivity. The post-prandial improvements in insulin secretion at one year are mirrored by increases in proinsulin concentrations which have previously been suggested to have detrimental effects on the morbidity profile in NIDDM. The mechanism of the improved β -cell function would appear initially to relate primarily to the specific pharmacological action of the sulphonylureas with secondary beneficial effects as a result of the reduction in 'glucose toxicity' acting on the β -cell and the reduction in peripheral insulin resistance. Improved insulin sensitivity associated with sulphonylurea treatment has been confirmed in this study and the mechanism appears to be secondary to increases in insulin secretion presumably reducing hepatic glucose output. The ameliorating effect of a reduction in glucose concentrations on insulin action cannot however be discounted.

Chapter 11 - The effects of sulphonylurea treatment on β -cell function and insulin sensitivity in NIDDM subjects - a two year follow-up study

11.1 Introduction

The following chapter gives the results of the follow-up study of β -cell function and insulin sensitivity at 24 months in the group of NIDDM subjects treated with gliclazide as a result of the failure to improve glycaemic control on diet alone some 3 months following diagnosis.

11.2 Subjects and Methods

All ten of the subjects commenced on SFUs at 3 months after diagnosis completed the two year study. Each underwent a final MTT and an insulin-modified FSIVGTT as detailed previously. Again, each subject completed a three day diet diary prior to the final metabolic assessment which was coded as before.

11.3 Results

Table 11.1 gives the dietary, clinical and metabolic data for 0 and 24 months.

Table 11.1. Dietary, demographic, metabolic and clinical data for SFU treated NIDDM subjects at 0 and 24 months (median (IQR))

	0 Months	24 Months	p value
Energy (kcal)	2327 (1691)	1852 (464)	0.14
Carbohydrate (%)	45.3 (11.5)	44.0 (15.1)	0.14
Protein (%)	17.0 (4.3)	19.7 (18.0)	0.06*
Fat (%)	37.7 (8.1)	29.0 (8.8)	0.06*
Weight (kg)	73.9 (12.8)	78.1 (20.2)	0.03
BMI (kg/m ²)	26.6 (6.2)	29.5 (4.2)	0.02
Systolic bp (mmHg)	130 (10.0)	129 (4.0)	0.40
Diastolic bp (mmHg)	81 (2.0)	80 (18.0)	0.44
FPG (mmol/l)	15.8 (1.5)	9.5 (2.7)	0.005
HbA1 (%)	12.6 (1.4)	7.9 (1.0)	0.005
Total cholesterol (mmol/l)	6.6 (1.0)	5.4 (1.4)	0.05
HDL-cholesterol (mmol/l)	1.02 (0.5)	1.0 (0.4)	0.15
LDL-cholesterol (mmol/l)	4.15 (1.3)	3.51 (0.89)	0.14
Triglycerides (mmol/l)	2.3 (4.5)	2.4 (2.2)	0.11

There was a 20% fall in median daily energy intake brought about mainly by the fall in the percentage of energy derived from fat (37.7 to 29.0%). Weight rose significantly over the 21 months of SFU therapy (73.9 to 78.1kg) with a concomitant increase in BMI (26.6 to 29.5 kg/m²). There were however significant falls in fasting plasma glucose (15.8 to 9.5 mmol/l) matched by glycosylated haemoglobin (12.6 to 7.9%) and a reduction in total plasma cholesterol concentrations (6.6 to 5.4 mmol/l).

A. MTT data

Table 11.2 gives the comparative MTT data in detail. Figure 11.1 illustrates the MTT profiles and Figure 11.2 shows the AUC data from the MTT at 0 and 24 months with comparative data from age, sex and BMI matched healthy subjects.

Table 11.2. Meal test data - SFU treated NIDDM subjects at 0 and 24 months (median (IQR))

	0 Months	24 Months	p value
Glucose			
FPG (mmol/l)	15.8 (1.5)	9.5 (2.7)	0.005
AUC ₀₋₆₀ (mmol/l)	1138 (153)	697 (185)	0.005
AUC ₀₋₂₄₀ (mmol/l)	4790 (382)	3010 (922)	0.005
Insulin			
FI (pmol/l)	39.0 (38)	79.0 (70.5)	0.005
AUC ₀₋₆₀ (pmol/l)	3990 (4729)	11212 (10732)	0.005
AUC ₀₋₂₄₀ (pmol/l)	22170 (22887)	54382 (47602)	0.005
Proinsulin			
FPI (pmol/l)	24.8 (22.5)	31.0 (10.5)	0.76
AUC ₀₋₆₀ (pmol/l)	2040 (1770)	2471 (1102)	0.72
AUC ₀₋₂₄₀ (pmol/l)	12090 (9225)	14696 (10042)	0.14
Ratios			
FI:FPG	3.1 (2.3)	9.1 (4.9)	0.005
FI:FPI	1.4 (1.1)	2.2 (1.8)	0.13

The plasma glucose profile improved over the 24 months as did the profile for insulin secretion, however early insulin secretion following the meal remained

Figure 11.1. Plasma glucose, insulin and proinsulin profiles during the MTT

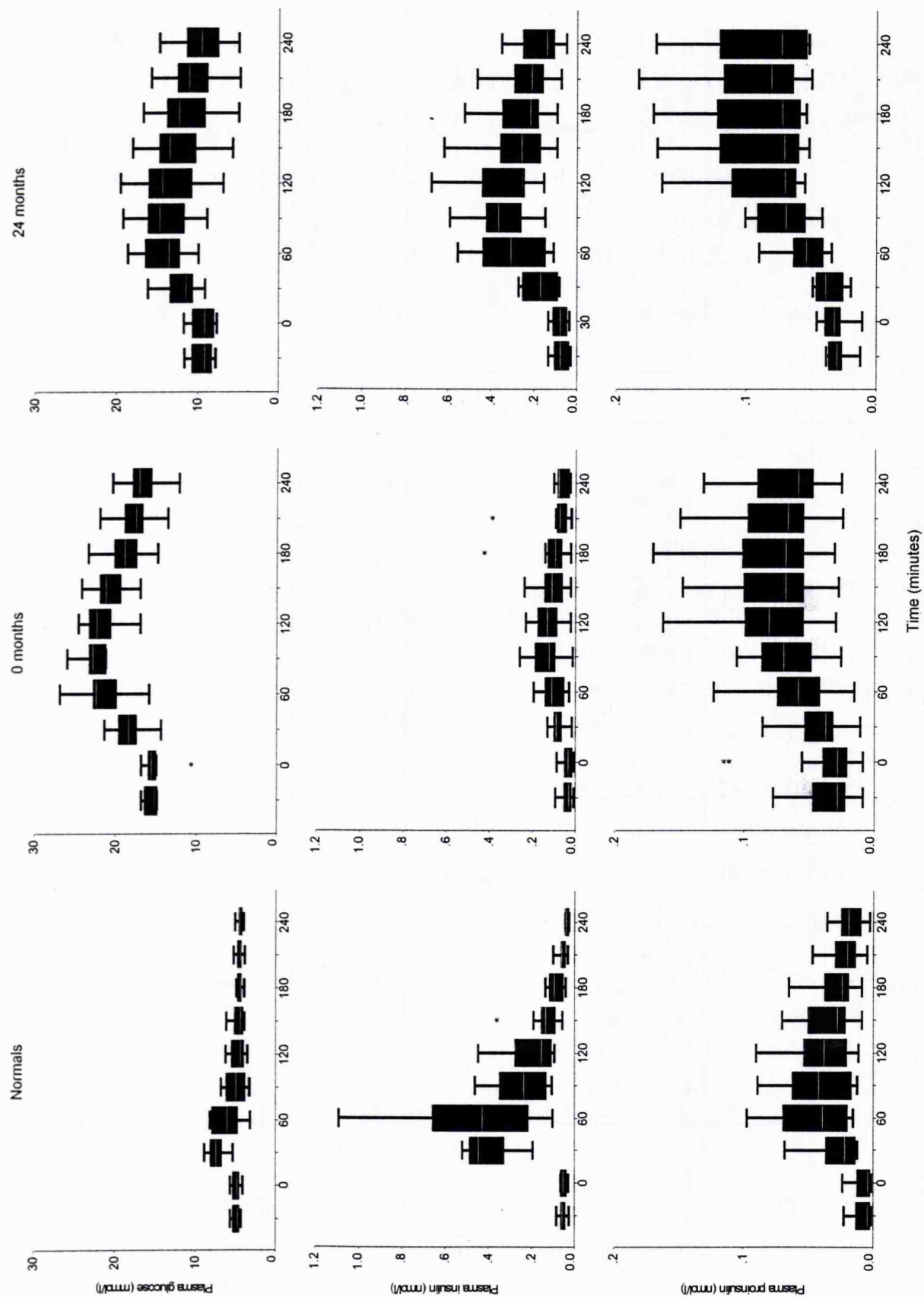
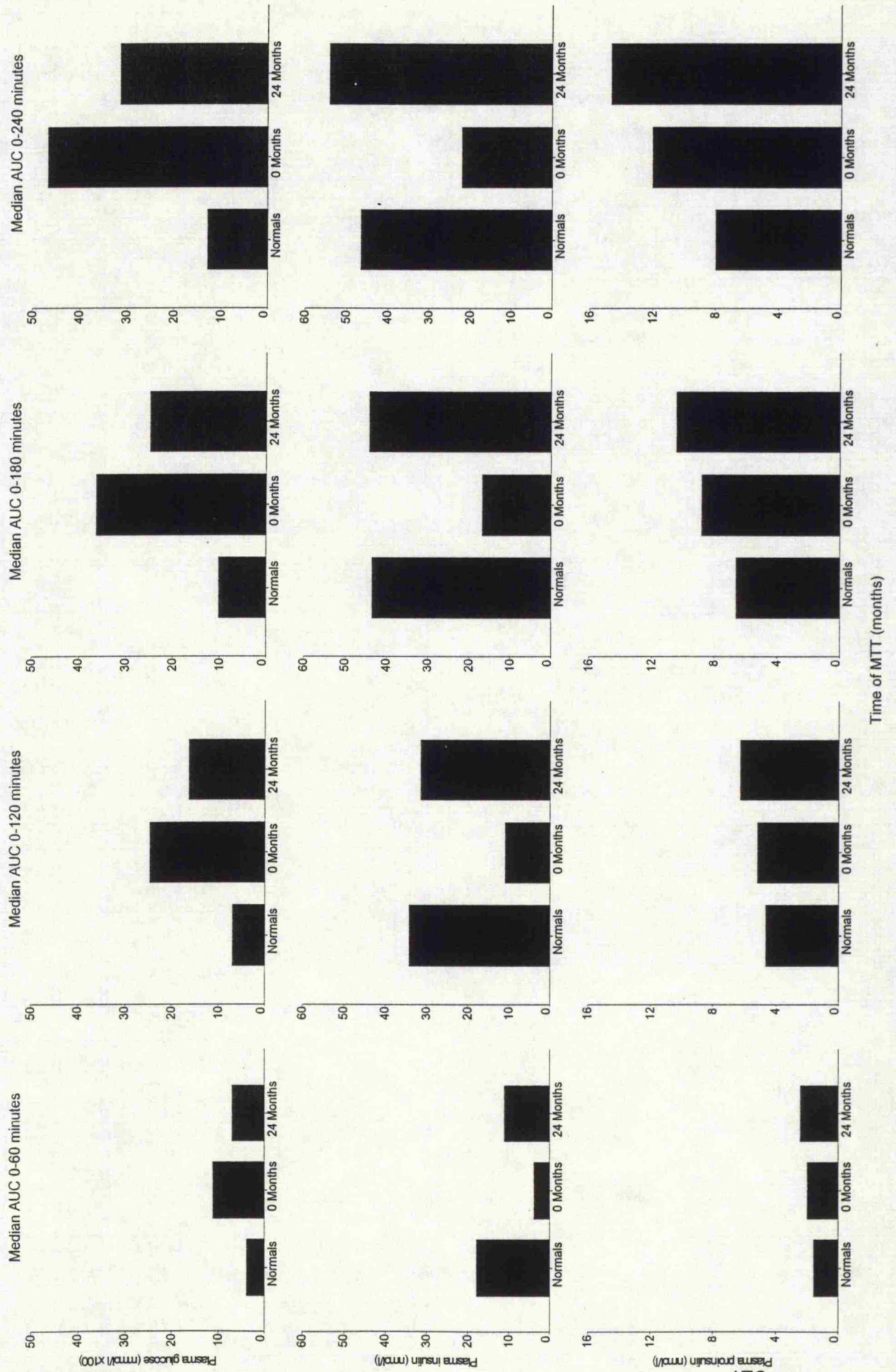


Figure 11.2. Plasma glucose, insulin and proinsulin AUC concentrations during the MTT



delayed and both plasma glucose and insulin concentrations showed delayed returns to basal concentrations.

Glycaemic control improved significantly over the 24 months with falls in fasting, post-prandial early phase AUC (1138 to 697 mmol/l) and total (4790 to 3010 mmol/l) glucose AUC concentrations (Figure 11.2, upper panels). There were significant increases in insulin secretion over the 24 months with a 100% increase in fasting concentrations (39 to 79 pmol/l), a threefold increase in post-prandial early phase AUC concentrations (3990 to 11212 pmol/l) and a twofold increase in total insulin AUC concentrations (22170 to 54382 pmol/l). These concentrations were also 30-50% higher than the equivalent values at 12 months. As a consequence of the reduction in fasting plasma glucose and the increase in fasting plasma insulin concentrations there was a threefold increase in the fasting insulin to glucose ratio. Fasting, post-prandial early phase AUC and total AUC plasma proinsulin concentrations showed no significant changes over the two year follow-up (Figure 11.2, lower panels) with total AUC concentrations falling compared to those at 12 months.

B. Insulin-modified FSIVGTT data

Table 11.3 shows the glucose clearance, insulin sensitivity and glucose effectiveness data from the insulin-modified FSIVGTTs at 0 and 24 months. Figure 11.3 illustrates the mean plasma glucose and insulin concentrations from these assessments.

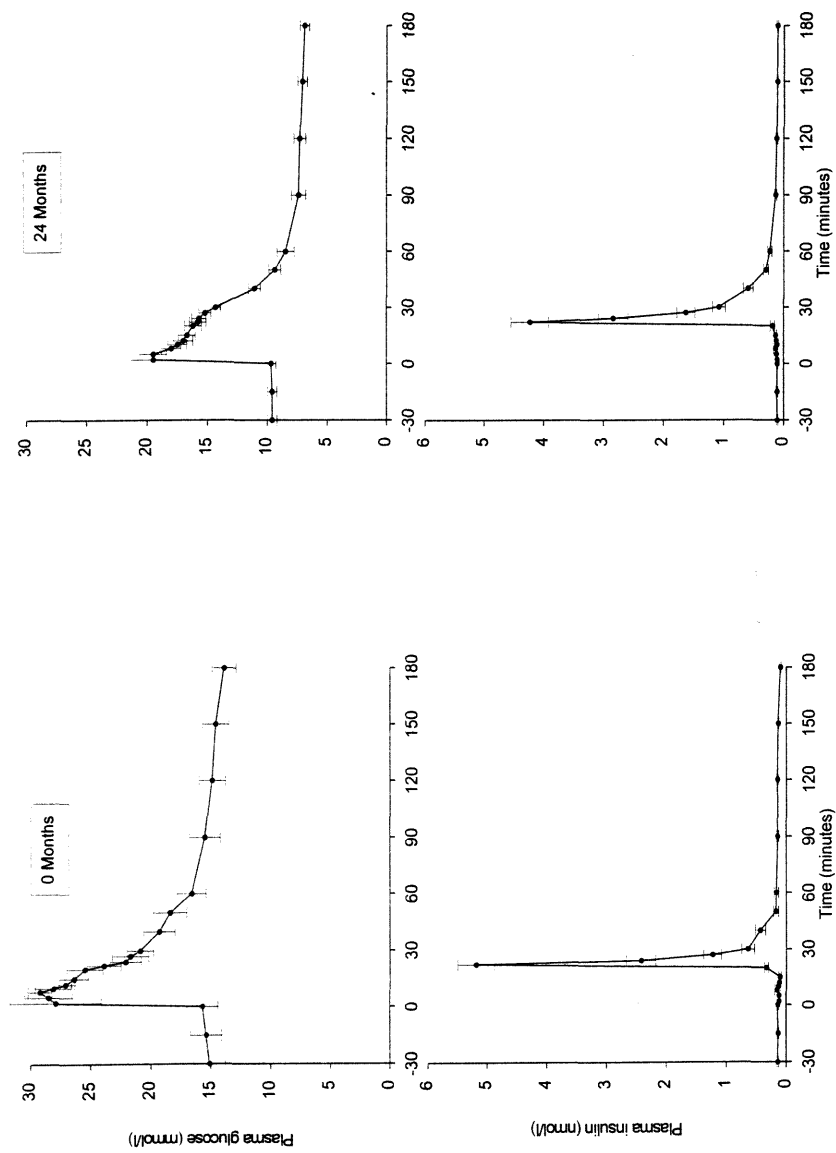


Figure 11.1. Glucose and insulin profiles from the FSVGTTs

Table 11.3. Glucose clearance, insulin sensitivity and glucose effectiveness data - SFU treated NIDDM subjects at 0 and 24 months (median (IQR))

	0 Months	12 Months	p value
Kg_{4-20} ($10^{-2} \cdot \text{min}^{-1}$)	1.4 (0.9)	1.2 (0.3)	0.76
S_I ($\text{min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$)	0.31 (0.85)	0.95 (0.5)	0.009
S_G (min^{-1})	0.01825 (0.014)	0.01322 (0.006)	0.65

There were no significant changes in the calculated glucose clearance rate over the 24 months however there was a threefold increase in insulin sensitivity (0.31 to 0.95 $\text{min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$). These changes are partially reflected in the plasma glucose profiles from the insulin-modified FSIVGTT illustrating equivalent rates of decline in plasma glucose concentrations following the glucose bolus but an increased rate of decline following the insulin bolus at 24 months.

Tables 11.4 - 11.6 give the results of stepwise multivariate analyses to determine predictors of fasting plasma glucose and fasting insulin concentrations along with insulin sensitivity at 24 months.

Table 11.4. Predictors of fasting plasma glucose concentrations at 24 months

	beta	t value	pvalue
Δ BMI	-0.26	3.14	0.02
Δ fasting insulin	0.71	8.52	0.0001
Δ Kg	0.03	0.42	0.69
Δ SI	-0.24	3.19	0.02

Δ = absolute change

Table 11.5. Predictors of fasting plasma insulin concentrations at 24 months

	beta	t value	pvalue
Δ BMI	0.20	0.68	0.51
Δ fasting plasma glucose	0.18	0.27	0.79
Δ glucose AUC 0-240	0.82	4.37	0.002
Δ SI	-0.02	0.14	0.90

Δ = absolute change

Table 11.6. Predictors of insulin sensitivity at 24 months

	beta	t value	pvalue
Δ BMI	-0.12	0.33	0.75
Δ fasting plasma glucose	0.63	2.43	0.04
Δ fasting insulin	-0.08	0.17	0.87
Δ FI:FPG	0.14	0.29	0.78

Δ = absolute change

Fasting plasma glucose at 24 months was best predicted by the increase in plasma insulin concentrations but also determined by the increases in both SI and BMI. Fasting insulin concentrations were predicted by the fall in total glucose AUC concentrations and SI by the fall in fasting plasma glucose concentrations.

11.4 Discussion

This study documented the long term effects of SFU therapy on β -cell function measured by the specific insulin and proinsulin responses to the MTT and insulin sensitivity estimated by the insulin-modified FSIVGTT in NIDDM subjects unresponsive to dietary therapy 3 months after presentation.

Gliclazide therapy over 21 months was associated with a significant degree of weight gain but resulted in a highly significant improvement in glycaemic control as judged by the reduction in glycosylated haemoglobin. Total plasma cholesterol concentrations also fell over the same period. Fasting plasma glucose, post-prandial early phase and total glucose AUC concentrations all fell significantly associated with increases in fasting and post-prandial insulin concentrations. Insulin sensitivity increased threefold over the course of the study but remained well below that of age, sex and BMI matched healthy subjects (median (IQR) $3.63 (3.4) \text{ min}^{-1} \cdot \text{uU}^{-1} \cdot \text{ml} \times 10^4$).

It is again of some importance to compare the results of the current study to those involving the same NIDDM subjects at 12 months from diagnosis. At 12 months similar improvements in glycaemic control were evident, as were the increases in insulin secretion and insulin sensitivity. However, the improved insulin secretion was associated with significant increases in proinsulin concentrations following the MTT which as has been suggested by others could have a deleterious effect on cardiovascular risk in NIDDM [Nagi et al,1990; Davies et al,1993a]. At 12 months the major determinant of the improved basal glycaemia appeared to be the increase in fasting insulin concentrations and this would also appear to be the case at 24 months. The maintenance of the improvement in insulin sensitivity at the latter assessment would also however appear to be significant.

The nature of the β -cell response to SFUs was clearly different at 24 months compared to 12 months with the improvements in basal and post-prandial insulin secretion not being matched by similar increases in proinsulin concentrations during the MTT. The earlier i.e from 3-12 months β -cell stimulation by gliclazide appeared to lead to increases in both insulin secretion and proinsulin concentrations postprandially but also falls in basal and post-prandial glucose concentrations. This reduction in the level of 'glucose toxicity' acting upon the β -cell along with the maintenance of improved insulin sensitivity could have resulted in reduced 'stress' to the β -cell and subsequently improved insulin secretory granule processing with the resultant fall in proinsulin concentrations documented during the MTT at 24 months. Certainly, the best predictor of fasting insulin concentrations at 24 months was the fall in total glucose AUC concentrations following the MTT suggesting that amelioration of 'glucose toxicity' was critical. The continuing improvement in estimated insulin sensitivity in addition to the reduced pressure on the β -cell also appeared to be related to the reduction in overall hyperglycaemia, presumably via the mechanism previously described.

True insulin secretion in this study was maintained at almost twice that measured at the baseline assessment both at one and two years although postprandial proinsulin concentrations did return to their pre-treatment levels. This tends to call into question the assertion that β -cell function returns to the level seen at the time of presentation after long-term SFU therapy, albeit in association with reduced levels of hyperglycaemia [Duckworth et al,1972; Beck-Nielsen et al,1988]. If SFUs do have a chronic effect on reducing the clearance of insulin from the circulation then the data from these studies would tend to support the concept.

In summary, SFU therapy over 24 months in NIDDM subjects results in improved glycaemic control as a result of specific augmentation of β -cell function (increased insulin secretion) after an initial period of apparently non-specific stimulation (increased insulin and proinsulin secretion) with subsequent falls in ambient glucose concentrations. The maintenance of the improvements in insulin sensitivity may be a specific effect of the drugs themselves but would appear more likely to be secondary to the reduction in 'glucose toxicity' although the impact of the higher insulin sensitivity on the more specific improvements in β -cell function may also be of significance.

Chapter 12 - General Summary and Discussion

This Chapter provides a general summary and discussion of the pathophysiology of NIDDM in relation to the studies contained in this thesis concerning both the specific methodologies for assessing β -cell function and insulin resistance and their application to the study of treatment effects in NIDDM subjects. Suggestions are made at the conclusion for further potentially fruitful areas of research.

Non-insulin dependent diabetes mellitus represents a growing problem for health care services as a result of its increasing prevalence and high rates of morbidity and mortality. As it typically affects middle aged individuals in developed countries there exists the potential for even greater problems in the future as economic prosperity spreads.

The increase in research interest in the condition over the last 30 years stems primarily from the development of the radioimmunoassay for insulin [Yalow et al,1960a] which allowed investigators to link glucose metabolism to insulin secretion. Unfortunately much of the early work in the field has been undervalued on the basis of poor study design, poor subject definition or disagreements over the interpretation of results [Porte Jr,1991]. It is ironic however that as a direct consequence of such disagreements much of the work elaborating the major pathophysiological abnormalities resulting in the development of NIDDM has been carried out. Despite the exponential increase in research activity brought about by these conflicting interpretations the relative contributions of impaired insulin sensitivity and reduced β -cell function to the development of NIDDM remains in dispute at the present time

[Reaven,1988; Porte Jr,1991; DeFronzo et al,1992; Kahn,1994; Reaven et al,1994; Taylor et al,1994].

The disease undoubtedly has a major genetic element [DeMeyts,1993; Kahn,1994] however, single gene defects e.g. MODY [Tattersall,1974] account for only a small percentage of individuals with the disease and as such NIDDM must be regarded as being a polygenic condition. Of these genes, some may be primary i.e. required to initiate the development of the disease and others secondary, either allowing progression of the condition or simply activated by its development. Environmental factors such as adverse intrauterine conditions, diet, exercise and migration appear to either initiate the development of the disease in the predisposed or to allow the disease to progress in those in whom the disease has already developed.

Abnormalities of β -cell function are present in all NIDDM subjects at the time of diagnosis [Porte Jr,1991; Coates et al,1994]. Characteristic abnormalities include the loss of basal oscillatory insulin secretion [Lang et al,1979], loss of first-phase insulin secretion following intravenous glucose [Cerasi et al,1967], reductions in post-prandial insulin concentrations [Temple et al,1992] and hyperproinsulinaemia [Yoshioka et al,1988; Porte Jr et al,1989; Davies et al,1993b]. A number of studies have documented these abnormalities in the glucose tolerant offspring of NIDDM subjects, subjects who go on to develop NIDDM and subjects with impaired glucose tolerance [Cerasi et al,1972; Kosaka et al,1977; O'Rahilly et al,1986; Johnston et al,1990; Davies et al,1993c]. These studies suggest that β -cell dysfunction may be a primary abnormality in NIDDM with insulin resistance a secondary phenomenon developing as a result of hyperglycaemia [Yki-Järvinen,1990].

Insulin resistance is also found almost universally in subjects with NIDDM [DeFronzo et al,1992; Coates et al,1994]. Typically NIDDM subjects demonstrate reduction hepatic and peripheral skeletal muscle resistance to insulin-mediated glucose uptake and disposal. Similar abnormalities have been demonstrated in individuals who go on to develop NIDDM, individuals at high risk for the development of NIDDM and those with impaired glucose tolerance [Eriksson et al,1989; Martin et al,1992; Lillioja et al,1993]. Many investigators regard insulin resistance as the primary genetic abnormality in NIDDM [DeFronzo et al,1992; Kahn,1994] with β -cell dysfunction developing as a consequence following a period of 'compensatory hyperinsulinaemia' [Reaven et al,1994]. Insulin resistance is however a feature of a number of other common disorders e.g. obesity, ischaemic heart disease, hypertension and dyslipidaemias and as such, NIDDM may represent only one facet of the so-called 'Insulin resistance syndrome' [Reaven,1988; Hansen,1995].

Other more recent suggestions concerning the pathophysiology of NIDDM point out the potential bias inherent in our current diagnostic criteria for IGT and NIDDM, suggesting that these criteria inevitably lead to the over-representation of insulin-resistant subjects in studies [O'Rahilly et al,1994]. Other concerns lie with the techniques available for the detection of β -cell dysfunction and insulin resistance which appear to have different sensitivities at different period in the evolution of the disease and therefore may again bias the relative contributions of these pathophysiological processes [Groop,1995].

In spite of this variety of views, many investigators prefer to consider the pathogenesis of NIDDM as a continuum with both β -cell dysfunction and insulin resistance contributing to different degrees to produce the disease phenotype [Taylor et al,1994]. The acceptance of this synthesis of the

available research data with all its potential methodological ambiguities presupposes a relationship between β -cell function and insulin sensitivity which is crucial to glucose tolerance. Evidence for this relationship has been presented in the last 10 years but is frequently ignored [Bergman,1989; Johnston et al,1990; Kahn et al,1993].

The relationship between β -cell function and insulin sensitivity in non-diabetic subjects appears to be hyperbolic in nature. Thus reductions in insulin sensitivity in very insulin sensitive individuals places little or no increased demand on β -cell function to maintain glucose tolerance. Similarly, reductions in insulin secretion in individuals with good β -cell function requires little or no change in insulin sensitivity to retain glucose tolerance. At the opposite end of the scale however, individuals with poor β -cell function or with severe insulin resistance require large compensatory changes to avoid glucose intolerance. It is this area of the relationship which best defines most individuals with NIDDM i.e lacking the compensatory capacity either from improved β -cell function or insulin sensitivity to prevent glucose intolerance.

The study of the pathophysiology of NIDDM remains fruitful but controversial as by the time the disease develops 'the horse has bolted'. As there has yet to be any effective, easily applicable and widely acceptable method of prevention of NIDDM, attention must also be paid to the study of those individuals in whom the disease has developed and the mechanisms by which our currently available treatment modalities influence the major pathophysiological processes present. To enable these studies of established NIDDM in terms of β -cell function and insulin sensitivity to take place, standardised techniques must be available and repeatedly applied to a fixed cohort of affected subjects.

The study of β -cell function in the studies reported here used a mixed meal tolerance test as a challenge to the pancreas. This was used in preference to an oral glucose tolerance test as it represents both a more physiological and 'day-to-day' stimulus to islet function in individuals living with the disease [Lefebvre et al,1976]. In all studies except that reported in Chapter 6, specific assays for true insulin and intact proinsulin were used to attempt to describe in detail the β -cell responses to insulin secretagogues contained within the test meal.

The study of insulin resistance presents a number of challenges, not least the plethora of techniques available. The glucose clamp technique has long been the benchmark technique for these assessments [DeFronzo et al,1979] however it is unsuitable for routine use because of its complexity. Bergmans 'minimal' model approach to the assessment of insulin sensitivity offers advantages in terms of the technique and the subsequent derivation of an estimate of insulin sensitivity [Bergman et al,1979]. Unfortunately this technique requires modification for application to subjects with NIDDM. In particular the poor insulin response to intravenous glucose, characteristic of subjects with NIDDM precludes modelling in many subjects.

In this thesis a primary aim was to standardise and validate 'minimal' model analysis of the insulin-modified FSIVGTT for use in NIDDM subjects. Chapter 3 presented the first successful validation of 'minimal' model analysis of the insulin-modified FSIVGTT against the isoglycaemic hyperinsulinaemic clamp. The study was able to demonstrate good correlations between the two techniques using both an extended (240 minute) and the more traditional (180 minute) sampling time frames [Coates et al,1995]. This study contrasted with that of the IRAS study group who demonstrated a weaker correlation compared to the isoglycaemic hyperinsulinaemic clamp in their NIDDM

subjects using the full sampling schedule, no correlation using the reduced sampling schedule and significant difficulty modelling all the data [Saad et al,1994].

One of the reasons for these difficulties in the IRAS study was the lower dose of insulin used during the FSIVGTT and a further element was explained in Chapter 5 in which the sampling schedules themselves were investigated following the suggestion of Steil that reduced sampling schedules during the FSIVGTT could produce equally accurate estimates of insulin sensitivity [Steil et al,1993]. In Chapter 5, I was able to demonstrate that the suggested 12 sample protocol lead to the introduction of significant bias and inaccuracy into the estimation of insulin sensitivity and that this could be marginally improved by the use of an additional time point at 25 minutes [Coates et al,1993].

A further investigation of the potential effects of insulin assay method on the accuracy of estimates of insulin sensitivity from the insulin-modified FSIVGTT in Chapter 5 allowed a final definition of a standardised and validated technique for the assessment of insulin sensitivity in NIDDM subjects.

As the results of studies of β -cell dysfunction and insulin resistance in NIDDM subjects has been subject to a degree of controversy involving selection of subjects, methods employed and interpretation of results it is prudent to examine a large number of individuals from the population to be investigated in a cross-sectional manner to ascertain the general characteristics which are likely to apply to a smaller study group.

Using the immunoreactive insulin, glucose and non-esterified fatty-acid responses to a mixed meal tolerance test of over 180 Caucasian subjects at the time of presentation with NIDDM there was evidence of both β -cell

dysfunction and insulin resistance. β -cell function was impaired in all NIDDM subjects regardless of their fasting plasma glucose and deteriorated exponentially with increasing hyperglycaemia. Post-prandial 'hyperinsulinaemia' was documented only in the sub-group of NIDDM subjects with the lowest FPG and this was relative to the insulin responses of normal subjects studied, not to the level of glycaemia. Insulin resistance was evident in all NIDDM subjects but appeared to increase with increasing BMI and was also evident in the impaired suppression of NEFA concentrations post-prandially [Coates et al,1994].

Following the description of the spectrum of abnormalities present in a large number of subjects with NIDDM at the time of presentation, β -cell function and insulin resistance were measured specifically in a smaller cohort of NIDDM subjects who were then followed over the course of treatment with diet alone or a combination of diet and sulphonylurea agents.

When compared to age, sex and BMI matched healthy subjects, NIDDM subjects demonstrate other features of the 'Insulin resistance syndrome' [Reaven,1988; Hansen,1995]. In particular they display marked dyslipidaemia with elevations of total cholesterol, LDL-cholesterol and triglyceride concentrations with reduced HDL-cholesterol concentrations in addition to hypertension. When assessing β -cell dysfunction and insulin sensitivity more specifically at the time of presentation again compared to age, sex and BMI matched healthy subjects it is clear that all NIDDM subjects have absolute insulin deficiency in the first hour post-prandially. Some augmentation of insulin secretion occurs subsequently in those with the lowest FPG, but not to a significantly greater extent than that found in the normals. All other NIDDM subjects remain absolutely hypoinsulinaemic compared to the healthy subjects and the level of hypoinsulinaemia deteriorates with increasing FPG.

This hypoinsulinaemia is associated with significantly increased fasting and postprandial proinsulin concentrations which also fall with increasing FPG. All NIDDM subjects are grossly insulin resistant at presentation with estimated values some six times lower than age, sex and BMI matched subjects and estimated insulin sensitivity appears to continue to fall with increasing FPG.

For healthy subjects, the fasting insulin concentration provides a good predictor of insulin sensitivity however for NIDDM subjects no single variable acts as a suitable predictor and this remains the experience of many [Bergman,1989; DeFronzo et al,1992; Kahn et al,1993] if not all [Turner et al,1995] investigators.

Dietary treatment of NIDDM remains the mainstay of therapy [Alberti et al,1994] whether other modalities are subsequently introduced or not. A hypocaloric diet maintained over one year results in weight loss, improved glycaemic control and improved lipid profiles in NIDDM subjects. β -cell function appears to improve with increases in post-prandial true insulin concentrations and falls in proinsulin levels. These changes also occur in the face of a doubling of insulin sensitivity over the same period. In these diet treated individuals β -cell function improved predominantly as a result of a reduction in the ambient glucose concentrations and although insulin sensitivity initially improved as a result of weight loss, its continuing maintenance at over 100% of the baseline estimate was also related to continuing reduction in hyperglycaemia.

Two years after the initiation of dietary therapy, the improved glycaemic control was static, post-prandial insulin secretion had returned to that seen at presentation however proinsulin concentrations in response to the meal continued to fall. Insulin sensitivity was maintained at the level achieved at

one year. With glycaemic control static, the continued improvement in β -cell function was a function both of the maintenance of weight loss over the two years and the improvement in insulin sensitivity, itself maintained by the reduction in BMI.

These observations in diet treated NIDDM subjects may help to further illustrate some of the pathophysiological processes in NIDDM. The reduction in glucose toxicity following one years dietary therapy appears to have a finite effect on β -cell function and insulin sensitivity both in quantitative and temporal terms. This model of the effect of diet may help to explain the gradual deterioration in glycaemic control which appears to affect most diet-controlled NIDDM subjects as time passes. If diet rapidly maximises β -cell function and insulin sensitivity to some predefined level (? as a result of diabetes-induced expression of secondary 'diabetogenes') at which glycaemic control is also maximised then only other external factors e.g. weight, exercise, diet, the passage of time, are able to influence glycaemic control beyond this point. As these are traditionally the most difficult (if not impossible) to maintain then despite improved β -cell function and insulin sensitivity, glycaemic control will deteriorate and further therapeutic intervention be required e.g. insulin, sulphonylureas.

Treatment with SFUs is required in approximately 30% of NIDDM subjects [Kennedy et al,1988]. Individuals in this study who required SFU therapy at 3 months after presentation tended to be less obese than those who responded to diet alone. In addition, they were significantly more hyperglycaemic and had poorer indices of β -cell function. Treatment with SFUs resulted in dramatic improvements in post-prandial insulin secretion at the cost of increased proinsulin concentrations. Insulin sensitivity also improved significantly and whilst the improvements in these two abnormalities were

interdependent, the reduction in glucose toxicity appeared to be the major determinant of improved β -cell function.

After 2 years of SFU treatment, despite significant weight gain, glycaemic control remained significantly improved whilst β -cell function continued to improve in terms of maintenance of increased post-prandial insulin secretion. The previously documented rise in proinsulin concentrations was reversed and the increased insulin sensitivity documented at one year, was maintained. At 2 years the major determinant of both the improvement in β -cell function and insulin sensitivity appeared to be the reduction in 'glucose toxicity'.

Again, these findings perhaps cast light on the interaction between β -cell dysfunction and insulin resistance in NIDDM subjects. Subjects who require SFU treatment tend to have failed to respond to an adequate trial of dietary therapy and as such must represent subjects who have progressed further down the pathophysiological ladder in NIDDM. For these individuals therefore, the severity of their metabolic defects must be greater than those individuals who respond readily to simple dietary manipulation. These studies generally found that this was the case with the SFU treated subjects displaying significantly worse β -cell function than their diet treated counterparts. Insulin sensitivity was also 25% lower in this group although not significantly so. Although reduced 'glucose toxicity' acting both on the β -cell and on insulin resistance was demonstrable in this group, the effects of the drugs themselves were significant. The early increase in proinsulin concentrations was suggestive of a SFU effect and the continuing increase in insulin concentrations post-prandially at 2 years also raises suspicions of a major drug effect as opposed to a beneficial effect of reduced ambient glucose concentrations on β -cell function. In this case therefore, it is tempting

to suggest that many of the so-called 'failures' on SFU therapy are as a result ultimately of this form of treatment. If one accepts that during the genesis of NIDDM a period of increased pressure on the β -cell, either as a primary process or secondary to insulin resistance, will result in eventual β -cell failure and the onset of hyperglycaemia, then it is easy to speculate that SFUs may precipitate further β -cell failure. Having improved β -cell function as a result primarily of the drug specific action, along with the improvement in insulin sensitivity and the reduction in ambient glycaemia, the continuing stimulation of the β -cell to secrete insulin by the SFU may be detrimental. If this is the case then studies such as these would be helpful in deciding whether SFU treatment should be stopped after assessment of the changes brought about in β -cell function and insulin sensitivity. In the SFU treated subjects here, glycaemic control and insulin sensitivity were equivalent to the diet treated subjects and insulin secretion significantly higher - these would seem adequate reasons for a period of drug withdrawal to observe the effects on diabetic control as well as metabolic parameters.

Future directions

Work of this nature appears useful in determining the extent and nature of the metabolic defects present at the time of diagnosis in NIDDM and the changes brought about by therapeutic interventions. The combination of accurate assessment of β -cell function and insulin sensitivity using the techniques described is fairly simple but the insulin-modified FSIVGTT is still not easily applicable to large population groups. This latter technique needs to be further refined to be of widespread value and the area of most interest lies in the reduction of the sampling frame. Although Chapters 3-6 suggest the full-sampling time frame is essential, it is possible by the application of advanced mathematical modelling and other computing concepts such as 'fuzzy logic'

that estimates of insulin sensitivity may be derived from time frames as short as 50 minutes and some preliminary work in this area has already begun.

Methodological issues apart it would seem vital to extend these studies over 3 and 5 years to attempt to fully delineate the changes in β -cell function and insulin sensitivity in both diet and SFU treated NIDDM subjects. It would also be of value to study subjects treated with SFUs both during and after treatment to assess the impact of the drugs themselves on these pathophysiological processes.

In conclusion, these studies examine the development of a specific technique of assessing insulin sensitivity in NIDDM subjects and its application along with modern methods of assessing β -cell function to the study of NIDDM subjects during treatment. NIDDM is characterised by severe insulin resistance and progressive β -cell failure. Treatment either by diet or sulphonylurea agents improves the clinical and metabolic state, however the impact of these strategies on the basic pathophysiological processes producing the disease appears differ in both temporal, quantitative and qualitative terms. Neither treatment modality normalises these abnormalities and both appear destined to be unsuccessful in the long-term, either as a result of the underlying disease process or because of the re imposition of stress on β -cell insulin secretion brought about by chronic therapy. The longer term follow up of NIDDM subjects in the manner described here may allow better identification of these problems, a better definition of the relationship between the pathophysiological processes and subsequently lead to improved treatment strategies.

Appendix 1

Calculation of insulin sensitivity from the clamp can be complex. If endogenous glucose production has been measured, then the following equation applies [Bergman et al,1985]

$$SI_{(clamp)} = \Delta R_d / (\Delta I \times G)$$

Where ΔR_d is the difference in glucose utilisation between the basal and clamped states (R_d = Glucose infusion rate - calculated endogenous glucose production). ΔI is the increment in insulin concentrations from basal and G is the glucose concentration at steady state.

A variety of simpler indices of insulin sensitivity can also be calculated [Bergman et al,1985]:-

$$(1) SI_{(clamp)} = M$$

Where M = the glucose infusion rate at steady state

$$(2) SI_{(clamp)} = M / (\Delta I \times G)$$

$$(3) SI_{(clamp)} = R_d$$

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