

ASPECTS OF INSULIN SECRETION AND
ACTION IN NON-INSULIN-DEPENDENT
DIABETES MELLITUS

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To Louise,
who will probably never read this, but
without whom it might never have been
completed

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Declaration

I hereby declare that this thesis has been composed entirely by me, under the supervision of Dr Malcolm Natrass.

The design and protocol for the work described in chapter 3 was devised by me; the protocols for the studies described in chapters 4 and 5 were prepared by Dr Malcolm Natrass with subsequent modifications by me, and the study design for the work described in chapter 6 was by me in conjunction with Dr Jonathan Webber.

Execution of all the clinical work described in this thesis was by me with the exception of some of the individual procedures described in chapter 6, which were carried out by Dr Jonathan Webber as part of a collaborative study. Laboratory analyses were carried out separately and were not performed by me.

Data analysis and interpretation were carried out by me; responsibility for any errors is therefore mine.

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Abstract

Following a review of the published literature regarding insulin secretion and action, with particular reference to non-insulin-dependent diabetes (NIDDM) and insulin resistance, four major studies have been undertaken and are described here.

In a cross-sectional survey of coronary risk factors in subjects with recently diagnosed NIDDM I confirm the high prevalence of individual risk factors described by others, and show a broadly normal distribution pattern for the clustering of risk factors forming Reaven's Syndrome X, in contrast to that reported in other populations. I suggest that the risk factor clustering present may be better described by a three-component model than by a single underlying factor as proposed by Reaven.

In a study to examine the effects of the new oral hypoglycaemic drug A4166 in NIDDM I confirm its major effect as an insulin secretagogue but find limited effects on intermediary metabolism when assessed during an intravenous glucose tolerance test. Any enhancement of glucose clearance appears to be secondary to the increase in insulin secretion.

If hypertriglyceridaemia exacerbates insulin resistance in NIDDM, then its treatment may reverse this process. In a randomised study comparing the fibrate drug gemfibrozil with placebo I show that reduction in serum triglyceride concentrations with gemfibrozil is associated with lower concentrations of non-esterified fatty acid (NEFA) and ketones but not glucose, when assessed using a low-dose incremental insulin infusion technique; this suggests a direct effect of the drug on lipid metabolism rather than any effect on insulin sensitivity.

In established diabetes (NIDDM) the contribution of body fat to insulin resistance is less clear than among non-diabetic subjects. In a group of NIDDM subjects I show no consistent effects of body fat on insulin resistance using the low-dose incremental insulin infusion, and no relation between body fat indices and euglycaemic clamp measures of insulin sensitivity. From the insulin infusion data I show that fasting glucose concentration has the greatest impact on insulin resistance, suggesting that hyperglycaemia or perhaps glucose toxicity has an effect overwhelming that of other factors.

Using the data from the body fat studies described, I compare measures of insulin resistance derived from insulin infusions, euglycaemic clamps and homeostatic modelling (HOMA-R) in NIDDM, and discuss uses and limitations of these techniques.

In a concluding chapter I attempt to summarise the findings from these studies and draw together a discussion of the results obtained in the context of the existing published literature.

CHAPTER 1

Introduction

Historical Background

The discovery of insulin and its introduction into clinical use in 1922 permitted a revolution not only in treatment of diabetes but also in concepts of the disease. That diabetes was a heterogeneous condition had been recognised by Arab and Indian physicians during the latter part of the first millennium with observations recorded of two types of diabetes, one predominantly affecting young thin individuals and proceeding to rapid death, and the other seen in older more obese people and running a less acute course.

Little more than a decade after the discovery of insulin Himsworth had proposed (Himsworth, 1934) and then described (Himsworth, 1936) a distinction between insulin-sensitive and insulin-resistant diabetic subjects. Lawrence (1951), from clinical observations, proposed a division into an insulin-deficient group and one which he supposed not to be insulin-deficient, subdividing this latter into a rare 'lipoatrophic' category and the much more common 'lipoplethoric' - representing a predominantly older, more obese and usually female population with typical symptoms at presentation but characteristically absent ketosis and a relative insensitivity to insulin therapy.

Hugh-Jones (1955), in an epidemiological study of his Jamaican patients, introduced the terms type I and type II diabetes, noting the similarities of his type II subjects to Lawrence's lipoplethoric group. He observed that such patients rarely required insulin treatment except during intercurrent infections as they were not ketosis-prone under normal conditions, and that they were also relatively insulin resistant.

These observations and much subsequent debate paved the way for the detailed consensus classifications of diabetes defined first by the U.S. National Diabetes Data

Group (1979) and then the World Health Organisation (1980). These give detailed definitions of the categories we recognise today. The NDDG allows the use of the terms type I and type II diabetes interchangeably with insulin-dependent (IDDM) and non-insulin dependent diabetes (NIDDM) respectively as well as dividing type II diabetes into obese and non-obese groups. Within the category of type II diabetes are included individuals who may be hyperinsulinaemic and insulin resistant, to be considered further below.

Synthesis and secretion of insulin

The insulin gene has been sequenced and located to the short arm of chromosome 11 (Owerbach et al, 1981). It encodes an mRNA precursor from which insulin mRNA is derived. This in turn controls synthesis of pre-proinsulin in the β -cell.

After cleavage to proinsulin on the rough endoplasmic reticulum the molecule is transferred to the Golgi apparatus for conversion to insulin and C-(connecting) peptide in clathrin-coated granules with subsequent storage prior to release from uncoated mature secretory granules (Orci, 1985). Glucose is a potent stimulus to proinsulin synthesis showing a sigmoidal dose-response curve with (in rats) maximal effect at 10mmol/l and greatest change at 2-5mmol/l, thus distinct from its effect on insulin secretion (Schuit et al, 1988).

Within the secretory granule proinsulin may be converted to insulin via either of two routes, as cleavage at both the 32-33 and 65-66 positions is required. Two endopeptidases, currently known as PC3 and PC2 respectively, perform these functions, with subsequent removal of the exposed C-terminal amino acid pair by a carboxypeptidase. Thus the intermediate molecules generated may be either split 32-33 and des 31-32 proinsulin or split 65-66 and des 64-65 proinsulin (Hutton, 1989;

Rhodes and Alarcón, 1994). The work of Hales and his colleagues in developing specific assays for these molecules has clarified the processes involved and suggested that the favoured pathway is normally via split 32-33 and des 31-32 proinsulin. Only very low concentrations of split 65-66 and des 64-65 proinsulin are detected, as PC2 is thought to work more efficiently on split than on whole proinsulin (Sobey et al, 1989; Rhodes and Alarcón, 1994).

The insulin molecule thus produced is composed of two polypeptide chains - an A chain of 21 amino acids and a B chain of 30 amino acids, linked by two disulphide bonds. Insulin and C-peptide are secreted in equimolar quantities from the islet β cell along with a small amount of unmodified proinsulin (Howell and Bird, 1989). Interest has been aroused by the possibility that defective proinsulin processing and abnormal secretion of proinsulin and intermediates may contribute to both development and complications of type II diabetes.

Although glucose is the principal regulator of proinsulin synthesis it has no influence on the rate of conversion to insulin. Some other sugars (eg. mannose) and metabolites (eg. dihydroxyacetone) (Ashcroft et al, 1978) can also stimulate proinsulin synthesis directly although most stimulants are believed to work via glucose. Similarly, the major stimulus for insulin secretion is also glucose, becoming effective only at extracellular concentrations above 5 mmol/L and with maximal effects seen at concentrations between 5.5 and 17 mmol/L (Howell and Bird, 1989), measurably higher than those required for proinsulin synthesis. A limited number of other agents are capable of independently stimulating insulin release (i.e. without requiring the presence of glucose); these include amino acids - most notably lysine and arginine, ketones, non-esterified fatty acids (NEFA), and the sulphonylurea group of oral hypoglycaemic drugs.

Insulin secretion enhancers or potentiators also have an important physiological role and these include cyclic AMP, cholinergic neurones (predominantly of muscarinic type), β_2 adrenergic stimulation, and some of the gut peptides, notably glucagon, glucagon-like peptide-1 (GLP-1) and possibly gastric inhibitory peptide (GIP) (Kreymann et al, 1987; Ahren et al, 1986). Important inhibitors of insulin secretion include somatostatin, α -adrenergic neuronal activity (mainly α_2), circulating adrenaline and noradrenaline, and other neurotransmitters such as galanin and neuropeptide Y (Kreymann et al, 1987).

Stimulus-secretion coupling (the mechanism linking extracellular glucose concentration with the release from the β cell of insulin) requires the presence of Ca^{2+} whose uptake into the cell is stimulated by glucose and appears to be mediated by protein kinases within the islet β cell cytoplasm (Howell and Bird, 1989). In common with that of many other hormones secretion of insulin can be shown to be pulsatile, with oscillations observed in studies of minute-to-minute variations (Lang et al, 1979) and of diurnal patterns (Polonsky et al, 1988a). Although most modifiers of insulin secretion seem to influence amplitude rather than frequency of secretory oscillations (Matthews et al, 1983) diabetes disrupts normal frequency patterns (Lang et al, 1981; Polonsky et al, 1988b). However the significance of these observations remains unknown and it has not been possible to restore normal secretory activity in type II diabetic subjects.

The insulin secretory response to intravenous injection of glucose has been demonstrated to be biphasic (Porte and Pupo, 1969), with an initial rapid release of insulin within about one minute of the onset of the stimulus and lasting up to 10 minutes, followed by a second phase beginning 10 minutes after the stimulus and lasting for the duration of hyperglycaemia (i.e as long as the stimulus persists). It has been suggested that this reflects release of stored insulin from secretory granules (1st

phase) followed by de novo synthesis (Pfeifer et al, 1981), although this hypothesis is currently undergoing critical reappraisal. This phenomenon is not observed following oral glucose ingestion where intestinal absorption, incretins and hepatic factors have important influences on the secretory response, and the resulting insulin secretory response is in fact greater.

Insulin-receptor interactions

The insulin receptor gene is located on the short arm of chromosome 19; genetic defects in the receptor are associated with some of the rare syndromes of severe insulin resistance (Krook and O'Rahilly, 1996). Insulin receptors are found, in varying concentrations, in almost all mammalian cell types; the number and affinity of insulin receptors varies under different conditions. The receptor is a heterodimer comprising two α subunits on the extracellular surface of the cell to which the insulin molecule binds, and two β subunits which are transmembrane protein structures and are responsible for insulin signalling (Czech, 1985). Maximal effects of insulin are often demonstrable at very low concentrations (eg. in adipose tissue) and hyperinsulinaemia appears to reduce receptor affinity, suggesting a critical role in some insulin-resistant states (Kolterman et al, 1981).

The initial biochemical changes leading to intracellular effects occur in the β subunit with activation of a tyrosine kinase resulting in a phosphorylation cascade within the molecule (Shia and Pilch, 1983; Kahn and White, 1988). Further phosphorylation takes place on the intracellular signalling protein IRS-1 (insulin receptor substrate-1) (Sun et al, 1991). In man the gene coding for IRS-1 is on chromosome 2. Although IRS-1 is probably important for most actions of insulin, signalling pathways independent of IRS-1 have been demonstrated and an alternative insulin receptor

substrate has been identified and designated IRS-2 (insulin receptor substrate-2) (Araki et al, 1994).

Phosphorylated IRS-1 appears to act primarily as a docking site for a number of intracellular proteins having SH2 (src homology 2) domains. Of major importance among these is the enzyme phosphatidylinositol 3-kinase (PI3-kinase), part of whose function may involve modulation of insulin action by phosphorylation at serine residues on IRS-1 (Tanti et al, 1994). Phosphorylation of serine and threonine sites on the insulin receptor itself may have an inhibitory role (Lavan and Lienhard, 1994). Association of IRS-1 with other SH2-domain proteins also takes place; among them Grb2 which stimulates a kinase cascade activating Ras and MAP kinase (mitogen-activated protein kinase). These proteins may be responsible for controlling the phosphorylation-dephosphorylation reactions which characterise insulin's regulatory action on several metabolic processes (White and Kahn, 1994).

Detailed characterisation and understanding of the effects of these signalling cascades is as yet incomplete, and individual pathways cannot yet be related to individual actions of insulin. The integrity of several signalling pathways may be required for some effects and these may vary under different conditions.

These findings have been largely established in rodents, and although similar phenomena may reasonably be expected in man direct extrapolation to human tissues must be cautious at this stage. Nonetheless, the emergence of details of the molecular signalling required for insulin action has offered insights into potential mechanisms for the development of insulin resistance. Recent findings in mice deficient in IRS-1 suggest that muscle glucose transport and protein synthesis may be significantly impaired (i.e. insulin resistant) in this situation but hepatic effects of insulin can be preserved by a compensatory effect of IRS-2 (Yamauchi et al, 1996). Future

examination both of molecular signalling defects and underlying genetic abnormalities is likely to prove fruitful.

Probably the most important endpoint of the insulin signalling cascade is the facilitated entry of glucose into the cell and this role is fulfilled by the glucose transporter proteins, designated in order of discovery GLUT1 to GLUT5 (GLUT7 also exists but its role is thought to be at intracellular organelle level, mainly in the liver). GLUT1 appears to be ubiquitous, and GLUT2 is found in liver and importantly in the pancreatic islet, where it has a role in glucose-stimulated insulin secretion. GLUT3 predominates in neural tissue, while GLUT4 is found principally in brown and white adipose tissue, and in cardiac and skeletal muscle. GLUT5 is primarily a fructose transporter (Kahn, 1995; Thorens, 1996). Although hepatic glucose metabolism is regulated by insulin this is not true of hepatic glucose transport, and the search for a glucose transport defect underlying insulin resistance has focused on the insulin-sensitive tissues and GLUT4 (Mueckler, 1990; Pessin and Bell, 1992; Kahn, 1995).

In both muscle and adipose tissue insulin acutely stimulates the translocation of GLUT4 from intracellular vesicles to the plasma membrane, possibly via a pathway involving IRS-1 and PI3-kinase (Yang et al, 1996). Tissue-specific regulation can be demonstrated: with increasing insulin resistance in rats, adipose tissue GLUT4 downregulates while muscle GLUT4 does not. Present and future studies are expected to address more closely the site of this defect (which may be in the insulin signalling pathway, at the level of GLUT4 translocation or GLUT4 activation); to date genetic defects in the GLUT4 molecule have not been identified.

An exciting recent development in this area has been the finding in vitro that in conditions of high ambient glucose concentration adipose cells become refractory to insulin-stimulated glucose transport (Marshall et al, 1991). The mechanism for this

appears to be the routing of glucose metabolism via the hexosamine synthesis pathway, whose rate-limiting step is the enzyme glutamine: fructose-6-phosphate amidotransferase. The presence of L-glutamine is essential to activate this pathway. A similar process has been implicated in muscle (Karam, 1996). While clearly of direct relevance primarily in diabetes, this represents the first description of a specific molecular mechanism inducing insulin resistance.

Physiological effects of insulin

As insulin receptors are present on almost all cell types, insulin can exert effects in most tissues. However its most important regulatory roles concern metabolism in liver, muscle and adipose tissue. Insulin can modify tissue uptake and intracellular transport of glucose and has a regulatory effect on several metabolic pathways, particularly those of glucose and lipid metabolism, but also to a lesser extent on protein metabolism. Most of the effects of insulin are anabolic or at least anti-catabolic and in normal metabolism a tight balance is maintained between these effects and those of other, antagonistic, hormones. In recent years evidence has emerged to show that insulin can modify gene expression of certain proteins directly, as well as affecting enzyme activity. Thus it can be shown that insulin enhances transcription of genes coding for the enzymes glucokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, 6-phosphofructose-2-kinase/fructose-2,6-bisphosphatase, glycerol-3-phosphate dehydrogenase and others as well as the glucose transporters GLUT1, GLUT2 and GLUT4. Insulin reduces transcription of fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase among others (O'Brien and Granner, 1991). The importance of some of these actions remains unclear, and measurement of the impact and extent of these effects may be confounded by regulatory effects at the level of the

enzyme involved and the fact that insulin may not be the major modulator of gene expression for individual enzymes.

Glucose metabolism

Utilisation of glucose as a metabolic fuel requires its uptake into the cell and this is achieved by a process of facilitated diffusion. The membrane glycoprotein glucose transporters (GLUT1-4; see above) perform this function; each has different tissue specificity.

Insulin promotes uptake and utilisation of glucose by cells for oxidation, storage as glycogen and, via glycolysis, for storage as fat. The action of insulin on glucose uptake varies between tissues, and to some extent this is dependent on prevailing blood glucose concentrations. DeFronzo et al (1983) demonstrated differing dose-response characteristics for stimulation of peripheral glucose uptake and inhibition of hepatic glucose output, the latter requiring much lower concentrations of insulin. Insulin alone had little influence on splanchnic glucose uptake, whereas glucose, in the presence of insulin, enhanced its own uptake by splanchnic tissues. Others have argued that hepatic glucose uptake, mainly for storage as glycogen, is critically dependent on portal insulin concentration (Pagliassotti and Cherrington, 1992).

Only in muscle and adipose tissue does insulin acutely regulate glucose uptake and in these tissues this is a rate-limiting step for glucose metabolism. It has been estimated using glucose clamp techniques in healthy volunteers that approximately 75% of whole body glucose uptake from plasma, largely by the brain, is independent of insulin and is regulated only by blood glucose levels until saturation of glucose transport channels takes place. The process follows Michaelis-Menten (i.e. saturable) kinetics and uptake can be enhanced by insulin in a linear fashion (Gottesman et al,

1983). These findings have been confirmed in post-absorptive diabetic subjects whose absolute glucose uptake was greater than that of controls because of their higher basal plasma glucose levels (Baron et al, 1985).

Following entry into the cell cytoplasm glucose is converted to glucose-6-phosphate by the enzyme hexokinase prior to any further metabolism. In the liver, and also in the pancreatic islet cell, this reaction is carried out by glucokinase (hexokinase IV) which as its name suggests is more specific for glucose. This process is essentially irreversible although in the liver free glucose can be regenerated for release into the circulation by the action of glucose-6-phosphatase. From glucose-6-phosphate several metabolic pathways are accessible.

In the liver glycogen synthesis is stimulated by insulin and glycogenolysis is simultaneously inhibited. Insulin stimulates glycogen synthase by dephosphorylation of the enzyme to its active form. This effect appears to be independent of any lowering of cytoplasmic cyclic AMP levels. (The stimulatory effect of glucagon and adrenaline on glycogenolysis is mediated by cyclic AMP.) Under basal conditions (i.e. in the post-absorptive state following an overnight fast) the suppression of hepatic glucose output by insulin is almost entirely mediated by inhibition of glycogenolysis with a much smaller contribution from inhibition of gluconeogenesis (McGuinness et al, 1987).

Gluconeogenesis can only occur in liver and kidney as these are the only organs possessing all the enzyme systems required. The liver has normally been considered to account for the vast majority of gluconeogenic activity, at least in the postabsorptive state, although this view has recently been challenged (Stumvoll et al, 1997). The process can be regulated by substrate supply, enzyme regulation and product

inhibition (it is inhibited by glucose). It is promoted principally by glucagon and inhibited by insulin (Krauss-Friedmann, 1984).

Glycolysis and gluconeogenesis can be seen as almost exact opposites, with enzyme reactions differing only at three steps - glucose/glucose-6-phosphate (mediated by glucokinase and glucose-6-phosphatase respectively), fructose-6-phosphate/fructose-1,6-bisphosphate (phosphofructokinase-1/fructose-1,6-bisphosphatase) and phosphoenolpyruvate/pyruvate (pyruvate kinase/pyruvate carboxylase and phosphoenolpyruvate carboxykinase) interconversion. These are thus the key regulatory steps governing the overall direction of reaction. In the fasted state, where the effects of glucagon, glucocorticoids and catecholamines predominate, gluconeogenesis is favoured, while in the fed state with insulin dominant, the process is reversed in favour of glycolysis (Pilkis and Granner, 1992; Van Schaftingen, 1993). Using an isotopic method Consoli et al (1987) found that in healthy volunteers studied in the post-absorptive state acetyl CoA entered Krebs' cycle at twice the rate of pyruvate. Under these conditions gluconeogenesis accounted for approximately 28% of total (hepatic) glucose output.

Gluconeogenesis is enhanced in type II diabetes (Consoli et al, 1989; Magnusson et al, 1992) as might be expected by the predominant action of pro-gluconeogenic hormones in conditions of insulin deficiency or resistance. Glycogenolysis appears to be relatively little affected; thus gluconeogenesis is the greater contributor to increased hepatic glucose output. Although greater absolute concentrations of both lactate and alanine substrates are available for this process in NIDDM, only the proportion of lactate used is increased and the major source of this appears not to be muscle (Consoli et al, 1990).

Studies of muscle glucose disposal suggest that pyruvate dehydrogenase may be a critical regulatory step in oxidative glucose metabolism, while glycogen synthase is a regulatory step for nonoxidative metabolism (Mandarino et al, 1987). Reduced muscle glycogen synthase activity has been found in obese nondiabetic and diabetic subjects (Damsbo et al, 1991) as well as nonobese type II diabetics (Johnson et al, 1991) and their first degree relatives (Vaag et al, 1992a) suggesting an important role in the development of insulin resistance and type II diabetes. Further study in diabetic subjects has suggested that hyperglycaemia itself may have a compensatory effect, allowing glycogen synthase activity to be preserved by increased availability of substrate (Vaag et al, 1992b).

Thiebaud et al (1982) reported that with increasing insulin levels whole body glucose oxidation was saturable and that nonoxidative metabolism then predominated. In a study of muscle metabolism, which was found to account for only a quarter of an oral glucose load, 50% was found to be oxidised, leaving only 35% for storage, with the remainder undergoing glycolysis to release lactate, pyruvate and alanine (Kelley et al, 1988). This finding is somewhat at variance with previously reported data suggesting that some 50-70% of an oral glucose load is accounted for by peripheral tissues (Ferrannini et al, 1985). The same group reported in a separate study that, although splanchnic glucose uptake was relatively little altered by diabetes, peripheral (lower limb muscle) glucose uptake was reduced by up to 50% compared with nondiabetics (DeFronzo et al, 1985). More recent studies using magnetic resonance imaging have shown that skeletal muscle glucose uptake, glycogen synthesis and total nonoxidative glucose disposal are all markedly reduced in type II diabetes, suggesting an important role in insulin resistance (Shulman et al, 1990). Examination of nondiabetic first degree relatives of type II diabetic subjects has shown that they share this defect in nonoxidative glucose metabolism (Eriksson et al, 1989).

Mitrakou et al (1990) found evidence that excessive hepatic glucose output was a more important contributor to hyperglycaemia than was reduced muscle glucose uptake. The relative contribution of these two processes has been vigorously debated (Gerich, 1991; DeFronzo, 1992; DeFronzo et al, 1992) and it has also been suggested that the impact of each may alter with progression of hyperglycaemia and diabetes, hepatic glucose output taking on a greater role with increasing glucose concentrations (DeFronzo et al, 1989). Problems of methodology which may account for the discrepancies in results reported have been discussed by Beck-Nielsen et al (1994).

Lipid metabolism

In its role as a hormone promoting the use of glucose as the major metabolic fuel the effects of insulin on lipid metabolism are predominantly anabolic. Uptake of fatty acids from the circulation by adipose tissue and subsequent esterification for lipogenesis is enhanced, while fatty acid release and oxidation is suppressed. Fatty acid metabolism is extremely sensitive to the effects of insulin and this appears to be independent of effects on glucose metabolism. It has long been recognised that very low concentrations of insulin are needed to suppress peripheral free fatty acid output, lower than the concentration required to stimulate glucose uptake (Zierler and Rabinowitz, 1964). These findings, evidence of the sensitivity of lipolysis to suppression by insulin, have been confirmed by others (Bakir and Jarrett, 1981; Nurjhan et al, 1986; Jensen et al, 1989) and shown to remain true in type II diabetic subjects (Swislocki et al, 1987), although impairment of insulin action is observed in this group indicating insulin resistance.

With a continued flux of free fatty acid into and out of the cell, assessment directly of the influence of insulin on reesterification of fatty acids is difficult and has produced conflicting findings (Coppack et al, 1989; Groop et al, 1991). Adipocytes from

different sites have been shown to have different metabolic activities, those from abdominal fat being more active than femoral fat cells (Smith et al, 1979).

The relative circulating concentrations of insulin and glucagon are more important to hepatic lipid metabolism than absolute concentrations of either hormone. A relative excess of glucagon inhibits the hepatic conversion of acetyl CoA to malonyl CoA, thus limiting inhibition by the latter of the enzyme carnitine acyltransferase I on the mitochondrial membrane surface. Malonyl CoA inhibits fatty acid oxidation and consequent ketogenesis. Insulin enhances malonyl CoA synthesis and thus limits the use of acetyl CoA in ketogenesis (McGarry and Foster, 1980). Insulin can also be shown to reduce uptake of ketones and release of NEFA and glycerol by adipose tissue (Coppack et al, 1989).

In contrast to the tight regulation of glucose metabolism, fatty acid turnover is very loosely governed by interaction between a number of factors, chiefly insulin concentration and adrenergic activity (Coppack et al, 1994). Insulin increases synthesis of adipose tissue lipoprotein lipase thus enhancing removal of lipoprotein triglyceride from the circulation (Eckel, 1989). It is a potent inhibitor of hormone sensitive lipase which hydrolyses stored triglyceride to NEFA and glycerol; these two enzymes thus maintain a balance between fatty acid retention for esterification and release for oxidation (Coppack et al, 1994).

Protein metabolism

Insulin promotes protein synthesis in muscle by a direct ribosomal action. It inhibits proteolysis along with its action inhibiting gluconeogenesis. Its importance is most easily observed in insulin-deficient states (eg. diabetes or starvation), where proteolysis may be prominent resulting in loss of muscle mass. As previously indicated

insulin can modify gene transcription of a number of proteins, both enzymes and others, in this context notably including glucagon and the insulin-like growth factor binding protein IGFBP-1. It can enhance synthesis of ribosomal RNA and appears to increase amino acid uptake in cells, probably by a gene-related mechanism. It is thought that many of insulin's actions on protein synthesis are mediated by phosphorylation/dephosphorylation reactions on specific proteins but detailed mechanisms remain uncertain (Kimball et al, 1994).

Studies of amino acid flux in vivo have shown evidence of both inhibition of proteolysis and enhancement of tissue uptake by insulin. During euglycaemic hyperinsulinaemia net uptake of phenylalanine and leucine is enhanced (Bennet et al, 1990) and release into plasma, especially of branched-chain amino acids which appear most sensitive to the influence of insulin, is reduced (Fukagawa et al, 1986). A dose-response effect can be shown for a progressive reduction in leucine flux by increasing concentrations of insulin, implying reduced proteolysis limiting entry of leucine into the plasma (Fukagawa et al, 1985). In this studies insulin was also observed to increase de novo alanine synthesis although the source of alanine was not established. In a further study the same group was able to show that, despite evidence of an age-related decline in insulin sensitivity of glucose metabolism, leucine disposal remained sensitive to insulin in the elderly (Fukagawa et al, 1989).

The relative importance of insulin to protein metabolism under physiological conditions has been difficult to define with certainty; much of its contribution seems to be as a stabilising influence inhibiting excessive catabolism rather than any major anabolic effect per se (Gelfand and Barrett, 1987). As a result studies of insulin action in isolation have often tended to suggest little or no role for insulin although its absence is clearly detrimental and, as outlined above, specific actions can be determined.

Integrated metabolism

Metabolic processes cannot be considered in isolation, and the pathways outlined above interact with one another, not only within but often between tissues, especially liver, muscle and fat. The regulation of these systems depends on both feedback control and the ability to vary the direction and degree of flux of enzyme systems with great sensitivity. The concept of substrate cycles was proposed several years ago to explain this and has received widespread experimental support (Newsholme and Crabtree, 1976). Such cycles may exist on any scale, from a single enzyme reaction to the involvement of several reactions, sometimes across a number of tissues.

The observation that fatty acid levels were often elevated in diabetes led to the concept of the glucose-fatty acid cycle (Randle et al, 1963) whereby stable plasma glucose concentrations may be maintained by a balance between (muscle and adipose) tissue uptake of glucose inhibiting release of fatty acids into the circulation, and availability of fatty acids restricting tissue glucose uptake. Insulin favours glucose uptake and the esterification of fatty acid and where insulin deficiency or resistance is present, as in diabetes, both blood glucose and circulating fatty acid levels are elevated. Extensive experimental work has supported the existence of this cycle and emphasised its significance, as well as suggesting a role for defective fatty acid metabolism in the genesis of insulin resistance (Ferrannini et al, 1983; Vouillamoz et al, 1987; Groop et al, 1989; Chambrier et al, 1990; Boden and Jadali, 1991). Fatty acids can also be shown to enhance gluconeogenesis (largely from lactate) although in normal man there appear to be protective mechanisms to prevent a rise in hepatic glucose output (Clore et al, 1991).

The glucose-lactate cycle of Cori is well-defined and is probably important in regulating both gluconeogenesis and glycogen synthesis via the indirect pathway.

Glucose taken up in muscle can be converted to lactate which is then released for the synthesis of glucose again in liver. A similar process has been postulated for alanine (Felig et al, 1970), whose importance as a gluconeogenic precursor is probably second only to that of lactate, and which accounts with glutamine for some 50% of amino acid-derived gluconeogenesis. Insulin lowers plasma alanine concentrations at the muscle level, thus making it unavailable to the liver. In a study including examination of the role of glycerol in gluconeogenesis, evidence for competitive inhibition among gluconeogenic substrates has been found in normal volunteers, although feedback inhibition was not addressed (Jahoor et al, 1990).

Thus insulin, acting in a number of tissues by several separate but interrelated effects, mainly anabolic or anticatabolic, contributes to glucose homeostasis and regulates availability of alternative metabolic fuels. Clearly insulin deficiency, as in type I diabetes, will derange all of these processes, while insulin insensitivity or resistance to one or more of these effects will, directly or indirectly, have deleterious consequences on several other systems.

Assessment of insulin secretion, action and sensitivity

Historical Background

Since the introduction of insulin preparations into clinical use variation between individuals in response to given doses has been apparent. Himsworth (1936) was able to divide patients into insulin-sensitive and insulin-insensitive categories on the basis of the dose of injected insulin required to abolish glycosuria and/or provoke a fall in measured blood glucose. He described an insulin-glucose test (sometimes called an insulin sensitivity test) whereby intravenous insulin and oral glucose were given according to body surface area in fasting subjects with blood glucose estimations over

60 or 90 minutes. This resulted in a clear separation between subjects who showed a fall in blood glucose in the first 30 minutes and those in whom blood glucose continued to rise without any apparent effect of insulin (Himsworth and Kerr, 1939a). Using this method in healthy subjects they (Himsworth and Kerr, 1939b) showed a tendency for insulin sensitivity to decline with age, a finding confirmed by Lister et al (1951) in a study of 100 diabetic subjects. In this latter study it was concluded that the effect of age may have been confounded by increasing weight, and a curve of approximately Gaussian distribution could be constructed for insulin sensitivity over the whole group without clear separation between insulin-sensitive and -insensitive categories.

Prior to 1960 estimation of insulin concentration in plasma could only be inferred from indirect measures such as the degree of hypoglycaemia provoked in laboratory animals or effects on isolated tissue preparations. The work of researchers such as Grodsky and Forsham (1960) and more particularly Yalow and Berson (1960a) for the first time enabled direct measurement of plasma insulin concentration and permitted examination of relationships between concentration and effects on metabolic processes.

Yalow and Berson (1960b) used their new method to demonstrate that following a 100g oral glucose load, while fasting plasma insulin levels were comparable between diabetic and non-diabetic subjects, the former tended to show higher insulin concentrations one and two hours later despite also having higher blood glucose levels.

Martin et al (1968), in a study of healthy non-obese siblings of juvenile-onset diabetics, showed a significant positive correlation between 2hr blood glucose and 2hr plasma insulin after a 50g oral glucose load and, following this with a short insulin

tolerance test (100mU/kg insulin injected intravenously with a single blood glucose measurement 15 minutes later) also showed an inverse association of 2hr plasma insulin with insulin sensitivity. Fasting insulin concentration was associated with neither blood glucose nor insulin sensitivity.

Franckson et al (1966) had used a number of dynamic tests (IVGTT, 10mU/kg insulin tolerance test with blood glucose measured for 30 mins, glucose-insulin tests and tolbutamide response test) to compare healthy and obese non-diabetic subjects and found a reduction in the peripheral action of (exogenous) insulin on the glucose utilisation rate in obese subjects, noting only a modest effect of ageing in both groups.

A classification of tests of insulin resistance in modern use is given in table 1.1, and the major techniques are reviewed below.

Basal insulinaemia and plasma insulin-glucose relationships

Especially in large-scale studies a simple index of insulin secretion and sensitivity is preferable and intuitively a fasting plasma insulin estimation might appear to provide this. However, in isolation this has important limitations in terms of estimating both insulin secretory capacity and insulin sensitivity. It has been widely recognised that over time in subjects progressing from normal to impaired glucose tolerance insulin levels tend to rise before falling again with the onset of frank diabetes. A similar problem arises in subjects destined to develop type I diabetes in whom insulin secretion, whether acutely or subacutely, declines. Furthermore, it can be shown (Lang et al 1979, 1981; Matthews et al, 1983) that insulin is secreted in a pulsatile fashion in the resting state and this pulsatility is deranged in NIDDM. Nonetheless it is worth noting that a linear correlation has been demonstrated between fasting insulin

levels and insulin resistance as measured by the insulin suppression test (Olefsky et al, 1973).

In an attempt to take account of, and estimate the contributions of, both β cell function and insulin sensitivity to plasma insulin and glucose concentrations Turner and colleagues devised (Turner et al, 1979) and refined (Matthews et al, 1985) a mathematical model which they termed HOMA (homeostasis model assessment). This technique allows a quantitative estimation of both insulin secretory function and relative insulin sensitivity using a computer model based on the interaction between fasting plasma glucose and insulin concentrations. The authors were able to compare the technique (Matthews et al, 1985) with other established methods of assessing insulin secretion and sensitivity and its use has been favoured by some investigators although it is less widely accepted than the glucose clamp or the IVGTT. Recently a simplified version of the insulin sensitivity equation, with a small modification, has been put forward (Duncan et al, 1995) which may encourage its wider use.

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) is used as the standard for determining glucose tolerance status and thus in the diagnosis of diabetes. Aspects of insulin secretion, insulin action and glucose clearance are thus integral to the test.

A relationship can be shown between plasma insulin response and oral glucose tolerance in different pathological states (Berson and Yalow, 1965; Perley and Kipnis, 1966; Reaven and Miller, 1968). However the test itself can be criticised because of the degree of intra-individual variation on repeated testing, particularly noted in the in-between category of impaired glucose tolerance (McDonald et al, 1965; Yudkin et al, 1990) and it has been found unhelpful in quantitative estimation of insulin

resistance (Hollenbeck et al, 1984). Nonetheless it has recently been suggested that the 30-minute plasma insulin value derived during the OGTT may be a useful index of insulin secretion, particularly in population studies (Phillips et al, 1994a; Wareham et al, 1995). Differences in test standards between the USA (50g or 100g glucose load) and elsewhere (75g glucose), and the general absence in its application of any adjustment to glucose load for body weight, hamper the validity of the test in assessment specifically of insulin secretion or action.

Intravenous glucose tolerance test (IVGTT)

The principle of measuring the effects of an acute intravenous glucose load date back at least to 1923. Early studies examined changes in blood glucose concentration under a variety of protocols and extensive debate took place about the most suitable calculations to perform in order to extract meaningful information from the data obtained. Even in recent times a lack of a universal standard protocol has hindered comparisons over time and between centres (Bingley et al, 1992), and there is as yet no consensus on the most appropriate calculations to apply (Smith et al, 1988; Colman et al, 1992). Furthermore, the reproducibility of the test remains disputed (Rayman et al, 1990; Koschmann et al, 1992).

The two most studied effects of an intravenous bolus are the rapid rise followed by a more gradual decline in blood glucose concentration and the stimulation of insulin secretion which in normal man is biphasic (Porte and Pupo, 1969). Most studies assessing insulin secretion concentrate mainly on the first phase response which is lost in NIDDM and in certain study populations helps predict subjects at risk of IDDM.

Table 1.1. Tests of insulin resistance in vivo

Static tests

Fasting plasma insulin concentration

Homeostasis model assessment (HOMA)

Dynamic tests*(a) tests of endogenous insulin activity*

oral glucose tolerance test (OGTT)

intravenous glucose tolerance test (IVGTT)

frequently sampled IVGTT (FSIGT) with minimal modelling*

continuous infusion of glucose with modelling analysis (CIGMA)

hyperglycaemic clamp

(b) tests of exogenous insulin action

euglycaemic hyperinsulinaemic clamp

insulin suppression test (IST)

somatostatin-modified IST

graded and low-dose insulin infusions:

(i) with clamped glucose levels

(ii) with glucose levels allowed to fall

short insulin tolerance test (ITT)

*FSIGT may be modified by injection of exogenous insulin

The use of the intravenous glucose tolerance test (IVGTT) in assessing insulin sensitivity or resistance has been developed and promoted by Bergman and his colleagues (Bergman et al, 1979 and 1981) who have devised a mathematical model (minimal model) to describe the insulin and glucose dynamics following a glucose load and thus estimate quantitatively the contribution of endogenous insulin to metabolic glucose clearance. A single value (insulin sensitivity index) can be calculated from the plasma insulin and glucose concentrations obtained, using the model to predict the effect of insulin on glucose disappearance. Following criticism of poor comparability with euglycaemic clamp results (Donner et al, 1985) the original protocol has been modified by the use of an injection of tolbutamide some 20 minutes after the glucose load which further stimulates insulin secretion (Beard et al, 1986) and has since allowed claims of equivalence with clamp data (Bergman et al, 1987). Further modification by using insulin instead of tolbutamide permits the use of the technique in diabetic subjects with little or no endogenous insulin response (Welch et al, 1990). Other refinements have been proposed to improve the model's reliability including the use of somatostatin (Yang et al, 1987) and radiolabelled glucose infusion (Cobelli et al, 1986) although more recently Caumo et al (1991) have cast doubt on the validity of some of the assumptions made in the model, which may help explain some of the discrepancies in comparison studies with clamp methods.

Although its proponents claim simplicity, the protocol is long and intensive, requiring approximately 30 samples at precise time points over three hours (hence FSIGT - frequently sampled IVGTT) and is not adaptable for large-scale studies outside a research centre. Galvin et al (1992) have proposed a simplified IVGTT protocol allowing measurement of both insulin secretion and sensitivity (by estimating glucose disappearance per change in insulin level) over the first 40 minutes after a

glucose bolus. However they advise caution in extrapolating this method to subjects who may be particularly insulin resistant.

The application of the minimal model to assessing β cell function was developed alongside its use in insulin sensitivity studies (Toffolo et al, 1980; Bergman et al, 1981) but the modifications made to enhance its applicability in the latter have resulted in much of its value in the former being lost. Weber et al (1989) have proposed adaptations to retain its usefulness in assessment of insulin secretion. However simpler and shorter IVGTT protocols are more generally favoured for this purpose (Bingley et al, 1992; Koschmann et al, 1992).

Bergman (1989) has reviewed in detail the development and principles of the minimal model technique.

Continuous glucose infusions

In contrast to the highly dynamic changes associated with an intravenous bolus of glucose in an IVGTT continuous infusions of glucose can explore effects more closely corresponding to steady state conditions. Such techniques have been in use for many years both in the form of the hyperglycaemic clamp (DeFronzo et al, 1979) and without clamping. Reaven and Farquhar (1969) used a protocol which enabled them to demonstrate that hyperglycaemia in type II diabetes was not merely a result of peripheral hypoinsulinaemia but that it occurred despite an apparently appropriate pancreatic secretory response thus implicating insulin resistance in the pathogenesis of the condition.

The hyperglycaemic clamp is more often used as an alternative to the IVGTT in assessing β cell function but has recently been used to estimate insulin resistance (Hosker et al, 1985; Mitrakou et al, 1992). This potential to examine both insulin

secretion and sensitivity simultaneously may offer an advantage in versatility over the euglycaemic clamp; Mitrakou et al (1992) showed a close correlation between estimates of insulin sensitivity generated from hyperglycaemic and euglycaemic clamp procedures.

A more sophisticated technique, incorporating a simpler experimental protocol, for measuring both β cell function and insulin resistance has been proposed by Hosker et al (1985). Developed alongside the same group's HOMA method, CIGMA (continuous infusion of glucose with model assessment) uses a computer-derived model to estimate β cell response and insulin resistance from the plasma insulin and glucose data produced during a one hour fixed-rate infusion of glucose. The authors validated this method against both the hyperglycaemic clamp and IVGTT. The hyperglycaemic clamp is restricted in use to subjects whose basal plasma glucose does not exceed the level at which the clamp is to be set (most often 10mmol/L) and all of these techniques require that urinary glucose losses should be negligible.

Euglycaemic hyperinsulinaemic clamp

The glucose clamp developed by Andres and his colleagues has acquired the status of the 'gold standard' against which other techniques of assessing insulin sensitivity are now usually measured. Early reports of its use came in the context of modelling insulin and glucose kinetics in vivo (Sherwin et al, 1974; Insel et al, 1975) but the description of both the hyperglycaemic clamp to assess β cell responsiveness and more especially the euglycaemic insulin (hyperinsulinaemic) clamp to assess insulin resistance (DeFronzo et al, 1979) contributed significantly in bringing the use of clamp studies to their present pre-eminence.

Although labour-intensive the principles of the technique are essentially simple: by using a variable infusion of intravenous glucose, with (euglycaemic or hypoglycaemic clamps) or without (hyperglycaemic clamp) infusion of a predetermined dose of insulin, the plasma glucose concentration may be fixed or 'clamped' at any desired level. A quantitative measure of insulin sensitivity can be derived using the euglycaemic hyperinsulinaemic clamp from the amount of glucose infusate required to maintain the desired blood glucose level and counter the effect of the infused insulin (i.e. the more glucose infused under stable conditions the more insulin-sensitive the subject).

Insulin suppression test and variants

The insulin suppression test (IST) was developed specifically to examine insulin resistance (or 'impedance') by Shen et al (1970). The technique employs a fixed infusion of insulin and glucose, along with adrenaline to suppress endogenous insulin secretion, and propranolol both to suppress insulin secretion and inhibit any tendency of the adrenaline to restore hepatic glucose output suppressed by the insulin infusion. Stable conditions are usually achieved within 90 minutes of starting the test and subsequent values can then be averaged to yield figures for steady-state plasma glucose and insulin concentrations. By this means endogenous glucose production is suppressed and so tissue glucose uptake is equal to glucose infused; thus a higher steady state plasma glucose concentration would imply a greater degree of resistance to insulin-stimulated glucose uptake. The technique has been compared favourably with the euglycaemic hyperinsulinaemic clamp (Greenfield et al, 1981).

The need for propranolol and adrenaline renders the procedure somewhat cumbersome, and in order to address this drawback the substitution for these of somatostatin was proposed (Harano et al, 1977 and 1978; Nagulesparan et al, 1979).

This modification is probably now more widely used than the original method. Further modification is sometimes employed by omitting somatostatin from the infusate (modified Harano method). Some minor variations exist between protocols regarding whether to vary infusion of glucose (IST), insulin (Nagulesparan's method) or both (Harano technique) according to body weight.

Graded and low-dose insulin infusions

Both the insulin suppression test and the euglycaemic clamp produce steady-state plasma insulin concentrations at or above the limit of the physiological range (about 100 ± 20 mU/L or 600-800 pmol/L). Although these concentrations are clearly adequate to ensure that hepatic glucose production is suppressed, allowing attention to be directed more specifically at peripheral insulin sensitivity (Groop et al, 1989), other aspects of insulin action become more difficult to study with these techniques. It has long been recognised that other effects of insulin on peripheral tissues occur independently of and at lower concentrations than effects on glucose uptake (Zierler and Rabinowitz, 1964; Schade and Eaton, 1977; Brown et al, 1978). In order then to study the effects of insulin on these aspects of intermediary metabolism procedures more closely reproducing physiological conditions are required. Dose-response studies for insulin actions can be performed with or without clamping blood glucose level and insulin infusion can be set to achieve low-, mid-, or high-physiological or supraphysiological plasma concentrations allowing comparison of effects over the range studied. The techniques used have been successfully combined with indirect calorimetry techniques to assess glucose oxidation and storage, or have incorporated infusion of isotopic glucose tracer to measure glucose turnover.

Study methods which involve clamping blood glucose follow the basic procedure of the euglycaemic clamp of Andres (DeFronzo et al, 1979) but usually with stepwise

increments in the insulin dose infused (Rizza et al, 1981; Thiebaud et al, 1982; Proietto et al, 1982; Nurjhan et al, 1985; Groop et al, 1989).

Protocols which do not involve clamping of blood glucose concentration permit more direct comparison of the effects of insulin on glucose metabolism with those on other metabolites. Most of these feature stepwise increments in the dose of insulin infused, allowing the blood glucose level to fall naturally. The two main disadvantages of this approach are, firstly, concern about the extent to which the level of glycaemia itself influences glucose uptake and thus possibly affects results (DeFronzo and Ferrannini, 1982; Proietto et al, 1983), and secondly, the risk of inducing hypoglycaemia - an unwanted outcome both for investigator, in view of the confounding effects of counterregulatory hormones, and also for the subject. A third disadvantage is shared with glucose clamp studies where graded insulin infusions are used in that continuous perturbation of the steady state is taking place resulting in greater uncertainty about the validity of conclusions reached (Cobelli et al, 1987).

A number of protocols have been described using either fixed (Bakir and Jarrett, 1981) or incremental (Schade and Eaton, 1977; Saccà et al, 1979; Hale et al, 1986) infusion of insulin according to the effects under investigation. The low-dose incremental insulin infusion technique of Hale and Natrass (Hale et al, 1986) has been used extensively in this laboratory to examine aspects of insulin resistance in a variety of physiological and pathological conditions (Hale et al, 1985; Singh et al, 1987; Singh and Natrass, 1989; Krentz et al, 1991a and 1991b; Robertson et al, 1992).

Short insulin tolerance test

The rate of disappearance of glucose from plasma following an intravenous bolus of insulin can be used to estimate insulin sensitivity. Greater sensitivity to insulin will

result in more rapid glucose clearance. The technique has been in use for many years (Alford et al, 1971; Harrison et al, 1976), and with some modification has more recently been shown to give estimates of insulin resistance comparable with clamp data (Bonora et al, 1989; Akinmokun et al, 1992). It is often favoured in circumstances where more intensive techniques are impracticable.

Conclusions from earlier studies may be of questionable value because of the confounding effects of counterregulatory hormones arresting the fall in plasma glucose some 15-30 minutes after the onset of the hypoglycaemic stimulus; this has been addressed more recently by studying only the glucose disappearance rate in the first 15 minutes after insulin injection.

The test is clearly unphysiological and the dose of insulin commonly used (0.1U/kg) represents a supraphysiological stimulus. In common with other simple methods of measuring insulin resistance it provides limited information and is likely to have most value in large-group studies and in situations where longer and more intensive protocols are impractical.

Other modifications

Further information regarding insulin action, glucose clearance and specific sites of metabolic activity may be combined with many of the above techniques by the use of additional measurement devices. Thus forearm muscle metabolite exchange, whole body glucose turnover and indirect calorimetry have often been incorporated into studies using these methods.

Other tests of insulin secretion

In addition to tests of β -cell responsiveness to glucose other agents can be used to measure insulin secretion. Responses to certain amino acids, β -adrenergic agonists, glucagon and other hormones, and sulphonylureas have all been assessed. Most commonly used are tests employing arginine, tolbutamide or glucagon. First phase insulin secretion in response to arginine can be shown to persist even in diabetic subjects whose response to glucose has been lost (Palmer et al, 1976). Tests using these agents require pharmacological doses to exert their effect on the β -cell and for more physiological studies glucose is usually preferred.

Insulin resistance

Concepts of insulin resistance

Himsworth (1936) was probably the first observer to characterise a difference between diabetic individuals requiring only small doses of exogenous insulin to abolish glycosuria and those in whom glycosuria persisted despite much larger doses of insulin. Evidence that diabetes was not solely a result of absent or defective insulin secretion came from the work of Yalow and Berson (1960) who found fasting plasma insulin concentrations in their diabetic subjects to be at least equal to those of nondiabetic controls; this led to the conclusion that the tissues of diabetic subjects were less sensitive to the influence of endogenous insulin. The appreciation of differences between individuals in their responses to endogenous or exogenous insulin has therefore existed for many years. Understanding of these differences in sensitivity or resistance to insulin requires a clear definition of the concept, and this has proved more difficult.

Reaven and his colleagues (Shen et al, 1970; Reaven, 1983; Reaven, 1988) have preferred to confine their use of the term insulin resistance to describe impairment of insulin-stimulated glucose uptake; this takes a somewhat restricted view of insulin action with no account of effects on other aspects of intermediary metabolism, but does offer a rational starting point. Other authors have referred to insulin resistance or insensitivity without further clarification.

Kahn (1978) offered a broad definition in which "insulin resistance may be said to exist whenever normal concentrations of hormone produce a less than normal biological response". He further argued for clarity of terminology in distinguishing decreased sensitivity to insulin (shift of the dose-response curve to the right but with maintained maximal effect) from decreased responsiveness (normal dose-response but decreased maximal effect) and intermediate or combined states. In this definition assessment of dose-response characteristics is crucial. This is probably the best available definition of insulin resistance although in practice the distinctions recommended are infrequently observed.

The range of insulin sensitivities (for glucose metabolism) across even a group of normal glucose tolerant individuals is considerable (Hollenbeck et al, 1987) and it is probably simplest and most useful to assess insulin resistance or sensitivity as a concept relative either to a suitable control or between intervention categories rather than against a more elusive definition of normality.

The ability to measure plasma insulin concentrations directly has made it apparent that insulin resistance is not limited to glucose-intolerant individuals. Indeed, the degree of compensatory hyperinsulinaemia is critical in determining clinical consequences such that some individuals may maintain normo- or near-

normoglycaemia (and not develop diabetes) while others may be severely hyperglycaemic despite large doses of exogenous insulin (Moller and Flier, 1991).

States of severe insulin resistance

Syndromes of severe insulin resistance represent an extreme end of the clinical spectrum. These rare syndromes are usually recognised initially by their massive insulin requirements or by their associated clinical features. They will be considered here only briefly; their major importance in this context is to highlight mechanisms of impairment of insulin action. These conditions are summarised in table 1.2.

This subject has been reviewed in detail by Goldstein (1994) who proposed that in diabetic patients daily insulin requirements exceeding 1.5-2U/kg would warrant clinical assessment of possible extreme insulin resistance.

Three major categories of extreme insulin resistant conditions have so far been described: those with a defined genetic insulin receptor defect (Type A and related conditions eg. Rabson-Mendenhall syndrome, leprechaunism), those associated with insulin receptor antibodies (Type B and related conditions, usually associated with systemic autoimmune disease), and those of unknown aetiology (pseudoacromegaly and lipodystrophic types, both congenital and acquired). Numerous mutations of the insulin receptor have been identified (Taylor et al, 1990; Krook and O'Rahilly, 1996) and are associated with variable degrees of insulin resistance; with continuing developments in molecular biology it is likely that further reclassification of these disorders will result from elucidation of post-receptor events and defects therein.

Many of these syndromes are characterised by extreme hyperinsulinaemia to the extent that some of the associated features may reflect insulin action at IGF-1 or hybrid receptors (King et al, 1980; Taylor, 1985; Geffner and Golde, 1988).

Table 1.2. Syndromes of severe insulin resistance

(a) with defined insulin receptor defect:	Type A syndrome (some phenotypes only) Rabson-Mendenhall syndrome leprechaunism NIDDM (occasional case reports)
(b) with insulin receptor antibodies:	Type B syndrome ataxia telangiectasia systemic autoimmune disease
(c) pathogenesis undefined:	Type C syndrome congenital lipodystrophies - Köbberling-Dunnigan and Seip-Berardinelli syndromes acquired lipodystrophies pseudoacromegaly

Pathological conditions associated with insulin resistance

A number of pathological conditions are recognised in which insulin resistance may develop. Often this will go unnoticed clinically as compensatory hyperinsulinaemia supervenes but in some situations insulin resistance may exacerbate the clinical disorder or defective glucose tolerance may result when hyperinsulinaemia fails to compensate adequately. An extensive list of conditions associated with insulin resistance is shown in table 1.3, and some of these are reviewed further below.

Endocrine conditions

In view of the antagonistic relationship between insulin and several other hormones it is to be expected that many of these will reduce sensitivity to insulin under conditions of deranged homeostasis and this is indeed the case.

Glucagon, as a counter-regulatory hormone protecting against hypoglycaemia, antagonises the effects of insulin on hepatic glucose metabolism, although its stimulatory effect on insulin secretion and other feedback adjustments mean that this effect is shortlived (Ferrannini et al, 1982). Chronic hyperglucagonaemia also appears to induce insulin resistance and may reduce peripheral insulin-mediated glucose uptake (Kotzmann et al, 1995).

Noradrenaline impedes both insulin-mediated glucose uptake (Lembo et al, 1994) and suppression of lipolysis (Marangou et al, 1988). Similar effects of adrenaline have been shown both in nondiabetic (Capaldo et al, 1992) and in insulin-dependent diabetic subjects (Walters et al, 1992), and have been reported in phaeochromocytoma.

Physiological concentrations of growth hormone can be shown to enhance lipolysis and ketogenesis, thus antagonising the effects of insulin (Gerich et al, 1976) and to shift to the right the dose-response curves for suppression of hepatic glucose output and stimulation of glucose uptake (Rizza et al, 1982a). Studies of forearm muscle glucose metabolism in acromegaly confirm this impairment of insulin action prior to any deterioration in glucose tolerance (Foss et al, 1991).

Similar findings have been reported from related studies on the effects of adrenal glucocorticoids (Rizza et al, 1982b) and pharmacological doses of steroids have been found to impair insulin sensitivity when measured by insulin tolerance test (Yasuda et al, 1982).

The effects of thyroid disease on insulin sensitivity are less clear. While it is widely accepted that glucose tolerance tends to deteriorate in hyperthyroidism, many of the changes in carbohydrate metabolism may be linked to changes in insulin secretion and effects on insulin sensitivity may differ between substrates and also between tissues. Pharmacologically induced tri-iodothyronine excess has been found to promote both hepatic glucose output and forearm muscle glucose uptake in both nondiabetic (Sandler et al, 1983) and type II diabetic subjects (Bratusch-Marrain et al, 1985). The effects on forearm muscle have been confirmed in spontaneously hyperthyroid subjects (Foss et al, 1990). In their study Bratusch-Marrain's group, using clamp techniques, found evidence of impaired insulin sensitivity as did Shen et al (1988) in a study of Chinese subjects. However others (Randin et al, 1986) have claimed that insulin sensitivity is not influenced by hyperthyroidism although insulin clearance is increased. Harris et al (1993) have reported no differences between euthyroid and hypothyroid subjects in insulin sensitivity for glucose or lipid metabolism.

Primary hyperparathyroidism has been reported to show an association with hyperinsulinaemia and insulin resistance which was reversible with surgical treatment of the primary lesion (Kim et al, 1971; Ginsberg et al, 1975).

Relationships between insulin resistance and hyperandrogenism are complex. The two frequently coexist, most notably in polycystic ovary syndrome, and are often found in conjunction with obesity and acanthosis nigricans (Flier et al, 1985) although obesity is not a prerequisite (Chang et al, 1983). There has been controversy as to which is the primary defect (Stuart et al, 1986; Barbieri et al, 1988) but support for the contention that insulin resistance contributes more to hyperandrogenism than vice versa has come from the finding that suppression of hyperandrogenism does not restore insulin sensitivity (Dunaif et al, 1990) while suppression of hyperinsulinaemia may improve hyperandrogenism (Nestler et al, 1989).

Other conditions

Both chronic liver disease (Cavallo-Perin et al, 1985) and chronic renal disease (DeFronzo et al, 1978) are recognised as causing insulin resistance. DeFronzo et al (1978) found the defect in uraemic subjects to be greatly improved following dialysis.

A number of degenerative neuromuscular disorders are associated with insulin resistance (Krentz et al, 1991) and the resulting defects in muscle metabolism may contribute adversely to the course of the underlying clinical condition.

Several drugs, including corticosteroids (Yasuda et al, 1982), oral contraceptives (Singh and Nattrass, 1989), β -adrenergic antagonists (Tötterman et al, 1984) and the lipid-lowering agent nicotinic acid (Kahn et al, 1989) have also been reported to induce insulin resistance in man.

Physiological influences on insulin sensitivity

It can be shown that insulin sensitivity may vary by a factor of 2.5 even in healthy glucose tolerant subjects (Hollenbeck et al, 1987) and a number of factors have been examined as contributors to this variability.

Higher fasting and stimulated plasma insulin levels have been found in women than in men during an oral glucose tolerance test (Boyns et al, 1969; Welborn et al, 1969) and using a low dose insulin infusion Hale et al (1985) reported lower insulin sensitivity in healthy women than in men. However Yki-Järvinen (1984) found no differences in either fasting plasma insulin concentrations or in euglycaemic clamp-derived measures of insulin resistance, and further suggested that weight for weight muscle metabolism may be more insulin-sensitive in women than in men.

More controversy surrounds the effects of age. During puberty Amiel et al (1986) reported a fall in insulin-stimulated glucose uptake both in type I diabetic subjects and in a control group of nondiabetic members of their families, when compared with prepubertal subjects. Later in life glucose tolerance is generally acknowledged to decline but the role of insulin resistance is less clear. Rowe et al (1983) described a shift to the right in the insulin dose-response curve in older subjects, after controlling for lean body mass; these findings were supported by Fink et al (1983) whose older subjects had lower lean body mass than younger comparisons. Although there remains support for an effect of ageing per se in increasing insulin resistance more recent studies have increasingly emphasised the importance of changes in body fat distribution and in particular increasing abdominal adiposity with age (Coon et al, 1992; Kohrt et al, 1993; Boden et al, 1993).

Table 1.3. Other conditions having reported associations with insulin resistance

Endocrine disorders

Diabetes mellitus	NIDDM IDDM (long duration) diabetic ketoacidosis impaired glucose tolerance
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acromegaly

glucocorticoid excess - endogenous or exogenous

hyperandrogenism - polycystic ovary syndrome, 'HAIR-AN' syndrome

primary hyperparathyroidism

hyperthyroidism

phaeochromocytoma

glucagonoma

insulinoma

Degenerative conditions of other organ systems

cirrhosis and other chronic liver disease

chronic renal failure

degenerative neuromuscular diseases - eg. Friedreich's ataxia, motor neurone disease

other inherited disorders (usually associated with hypogonadism) - eg. dystrophia myotonica, progeria

Other disorders

obesity

hypertension

hypertriglyceridaemia

hyperuricaemia

drug-induced (eg. steroids, oral contraceptives, β -blockers, nicotinic acid)*Physiological factors*

sex differences

ageing

pregnancy

menstrual cycle

physical activity

fasting/starvation

racial origin

Effects of exercise on insulin resistance show consistent benefits. Highly trained athletes have lower basal and stimulated plasma insulin concentrations than controls for similar blood glucose levels achieved during an IVGTT, implying enhanced insulin sensitivity (Lohmann et al, 1978). Although this advantage is lost after as little as 14 days abstinence (King et al, 1988), improvements in insulin sensitivity have been shown even after a single episode of exercise in lean and obese diabetic and nondiabetic subjects (Devlin and Horton, 1985; Devlin et al, 1987).

The effect of race on insulin resistance is complex and to some extent confounded by other influences such as obesity, but patterns emerge from studies of declining glucose tolerance from normal to diabetic in defined populations. Among Pima Indians the degree of insulin resistance correlates only to a limited extent with glucose tolerance or obesity (Bogardus, 1993). In a black American population Banerji and Lebovitz (1989) have identified two distinct subgroups: one with insulin-resistant type II diabetes and one with an insulin-sensitive and relatively insulin-deficient state. How representative this study population is remains questionable, but using subjects carefully matched for degree and distribution of obesity Dowling and colleagues (Dowling and Pi-Sunyer, 1993; Dowling et al, 1995) were able to report greater insulin sensitivity in black than in white women. McKeigue et al (1988 and 1991) have described higher stimulated plasma insulin levels (using this as a surrogate for insulin resistance) in South Asians living in Britain than in white controls, allowing for body fat distribution.

Along with race genetic factors are clearly of note although no gene for insulin resistance has yet been identified. Evidence of insulin resistance, using various measures, has been described among non-diabetic first degree relatives of NIDDM subjects in comparison with controls (Haffner et al, 1988; Gelding et al, 1995; Migdalis et al, 1996; Vaag et al, 1996) although here again obesity and body fat

distribution may be important confounders (Osei et al, 1991). Among Pima Indians insulin resistance is clearly familial, and predates the onset of NIDDM (Bogardus, 1993).

To some extent it might be expected that as insulin sensitivity declines so will glucose tolerance but many instances can be found of comparable degrees of insulin resistance in glucose tolerant and diabetic subjects (Hollenbeck and Reaven, 1987). The converse, that declining glucose tolerance may exacerbate insulin resistance, is lent some support by demonstrations that, in comparison with healthy controls, subjects with impaired glucose tolerance (Krentz et al, 1991) and type II diabetes (Swislocki et al, 1987) have defective insulin action on intermediary metabolism. In type I diabetes these defects are more apparent with greater duration of disease (Singh et al, 1987).

Obesity, body fat distribution and insulin resistance

Obesity per se, whether measured by reference to actuarial ideal body weight or by body mass (Quetelet) index (Keys et al, 1972), has a well recognised association with insulin resistance and many studies have examined this relationship with respect to glucose metabolism (Kolterman et al, 1980; Prager et al, 1986; Laakso et al, 1990), lipid metabolism (Bakir and Jarrett, 1981; Meylan et al, 1987; Hale et al, 1988) and with regard to broader aspects of intermediary metabolism (Robertson et al, 1991 and 1992). Kolterman et al (1980) reported a predominant receptor defect in both liver and peripheral tissues (insulin dose-response curve shifted to the right) but found evidence of a mixed receptor and postreceptor defect at the periphery with increasing obesity. However this study and that of Laakso et al (1990) in particular can be criticised for failing to exclude subjects with abnormal glucose tolerance in the obese group. This may account for the contrary findings reported by others (Hollenbeck et

al, 1984; Firth et al, 1987; Ludvik et al, 1995) with the conclusion that any effect of obesity is negligible in comparison with that of diabetes. Bogardus et al (1985), in a study of Caucasian and Pima Indian men, reported a non-linear association between obesity (measured by percent body fat) and insulin resistance but more recent data (Campbell and Gerich, 1990) have suggested that a threshold (BMI 27kg/m^2 or $>120\%$ of IBW) exists above which a deterioration in insulin sensitivity may be detected.

Studies from this department (Hale et al, 1988; Robertson et al, 1991 and 1992), with careful regard to the confounding effects of abnormal glucose tolerance, have consistently been able to show an important role for obesity. Hale et al (1988) showed improvements in insulin sensitivity following significant weight reduction, exposing different rates of change in different metabolic processes.

While the impact of android (male pattern or upper body) versus gynoid (female pattern or lower body) fat distribution on health has been recognised for many years (Vague, 1956), only in recent times has its relation to insulin resistance come under scrutiny, partly in an attempt to explain differences between individuals with comparable overall obesity.

In obese subjects an upper body fat distribution (usually measured by waist-hip ratio or skinfold ratios) has been shown to be associated with reduced glucose tolerance, hyperinsulinaemia and hypertriglyceridaemia (Kissebah et al, 1982; Krotkiewski et al, 1983) and subsequently with increased insulin resistance in premenopausal women (Evans et al, 1984; Peiris et al, 1988), postmenopausal women (Landin et al, 1989) and in men (Haffner et al, 1994). The work of Landin et al (1989, 1990) and Peiris et al (1988) has suggested that in women at least overall leanness may protect from the adverse effects of an upper body fat distribution but this may not be true in men. The



effect of body fat pattern in combination with defective glucose tolerance is as yet unknown. More recent attention has focused on the role of visceral adipose tissue in modulating insulin sensitivity, and in this regard the validity of the indices of obesity and body fat distribution in conventional clinical use has been questioned as these measure predominantly superficial fat. As yet though, visceral fat mass can only be accurately estimated using magnetic resonance imaging whose availability is limited and costly.

Hypertension and cardiovascular disease

The preceding section has attempted to review aspects of insulin resistance in terms of factors which may influence insulin resistance in both pathological and physiological situations. Great efforts have been addressed to the question of whether and how insulin resistance may affect health, especially cardiovascular disease. Much of the literature in this area is hampered by blurring of the distinctions between glucose intolerance, hyperinsulinaemia and insulin resistance, which although clearly related are not synonymous.

Hugh-Jones (1955) observed high rates of hypertension in Jamaican diabetic patients and this relationship has been confirmed in other populations. Modan et al (1985) further demonstrated a link with hyperinsulinaemia. Lind et al (1993) showed that insulin resistance correlated better than hyperinsulinaemia with hypertension and other metabolic abnormalities. Evidence has been proposed for a genetic or at least familial component with the implication that insulin resistance precedes hypertension (Ferrari et al, 1991). Although hyperinsulinaemia is not a prerequisite for the development of hypertension (Zavaroni et al, 1992) overall insulin sensitivity within a population does appear to decline in association with raised blood pressure (Pollare et al, 1990). However the nature of the relationship has been complicated by observations firstly,

that hyperinsulinaemia may only be a feature of hypertension in diabetic (NIDDM) subjects (Mbanya et al, 1988), although this finding has not been supported elsewhere, and secondly that in insulinoma, despite hyperinsulinaemia and insulin resistance, hypertension is not a feature (Pontiroli et al, 1992; Vettor et al, 1994). Furthermore, in a large study of Pima Indian subjects Saad et al (1990) found associations of hypertension with glucose tolerance and obesity but not with fasting insulin concentration; in a further study the same group (Saad et al, 1991) demonstrated higher plasma insulin levels and reduced glucose disposal with increasing blood pressure in white subjects but no association in black or Pima Indian subjects. A recent epidemiological study (Muller et al, 1993) reported no association between hypertension and hyperinsulinaemia after adjustment for age, body fat and fat distribution.

Dyslipidaemia

The commonest lipid abnormalities in type II diabetes are raised serum triglyceride concentration and reduced HDL-cholesterol level (Gibbons, 1985). The association of these abnormalities with hyperinsulinaemia has been noted in both diabetic and nondiabetic subjects (Laakso et al, 1987). Treatment of type II diabetes with insulin improves lipid profiles, a finding somewhat at odds with the association of endogenous hyperinsulinaemia with lipid disorders (Taskinen et al, 1987; Lindström et al, 1990) unless this is explained by the improvement in insulin sensitivity observed with insulin treatment (Scarlett et al, 1982). An association between hypertriglyceridaemia and insulin resistance has been recognised for many years (Olefsky et al, 1974; Bernstein et al, 1978; Steiner et al, 1980) and there is evidence that the metabolic defect is primarily peripheral rather than hepatic in both diabetic (Widén et al, 1992) and nondiabetic subjects (Bernstein et al, 1978; McKane et al, 1990). Hypertriglyceridaemia appears to be closely linked to defective antilipolytic

action of insulin and a causal relationship has been proposed although which is the primary factor remains unclear (Yki-Järvinen and Taskinen, 1988). As with hypertension, hypertriglyceridaemia is said not to be a feature of the insulin resistance associated with insulinoma (Vettor et al, 1994).

If hypertriglyceridaemia causes or exacerbates insulin resistance then studies addressing the effects of its treatment assume great importance. The fibrate group of lipid-lowering drugs, comprising clofibrate, bezafibrate and gemfibrozil among others, has been studied most extensively because of the prominent triglyceride-lowering component of these agents' action. When used to lower triglyceride levels in type II diabetic subjects no significant effect on glucose tolerance has been found with these drugs (Marks and Howard, 1982; Garg and Grundy, 1989; Vinik et al, 1993). In a predominantly nondiabetic, although rather poorly defined, group a fall in plasma insulin response to oral glucose challenge was found only in those whose plasma triglycerides fell by more than 1.7mmol/l with gemfibrozil (Steiner, 1991). However neither gemfibrozil in diabetic subjects (Vuorinen-Markkola et al, 1993) nor bezafibrate in diabetic and nondiabetic subjects (Riccardi et al, 1989; Karhapää et al, 1992) has been shown to influence insulin sensitivity despite producing significant reductions in serum triglyceride concentrations. Shen et al (1991) found no overall effect of gemfibrozil on glycaemic control or insulin sensitivity in NIDDM but reported significant reduction in hepatic glucose output in the subgroup with fasting blood glucose above 9mmol/l although not in better-controlled subjects. This study group consisted of Chinese men on sulphonylurea treatment and these findings may be difficult to extrapolate. Interestingly, benfluorex, an unrelated agent with lipid-lowering properties derived from the amphetamine family of drugs, has been reported to improve insulin sensitivity in comparison with placebo (Bianchi et al, 1993), but as the achieved reduction in triglyceride levels was not significantly different from that of

placebo, its mode of action on insulin sensitivity may not be related to its lipid-lowering effect.

Reaven and his coworkers (Olefsky et al, 1974; Reaven, 1988) have maintained that hypertriglyceridaemia is secondary to insulin resistance, resulting from increased hepatic VLDL-triglyceride synthesis in the presence of hyperinsulinaemia; the lack of evidence favouring a primary role for hypertriglyceridaemia may give credence to this view.

Coronary heart disease

Links between defective glucose tolerance and coronary heart disease have been well-defined in impaired glucose tolerance (the Whitehall Study - Fuller et al, 1980) and in diabetes (the Framingham Study - Kannel and McGee, 1979). The Whitehall Study reported a doubling in 7½ year coronary mortality in IGT compared with euglycaemic subjects among middle-aged British male civil servants; the Framingham Study found an incidence of coronary heart disease doubled in men and trebled in women with diabetes compared with nondiabetics. Data from the Paris Prospective Study (Fontbonne et al, 1988) suggested that fasting plasma insulin concentration was a better indicator of coronary risk than was glucose intolerance, at least in obese subjects (BMI>26). A report on the men screened for the MRFIT Study concluded that diabetes conferred an additional risk to the effects of smoking, blood pressure and serum cholesterol (Stamler et al, 1984). Despite this, insulin resistance per se has not so far been shown directly to correlate with incidence of coronary heart disease, although Martin and Hopper (1987) have made a limited attempt to address this issue in type I diabetes.

Various lipid abnormalities have been well-defined as coronary risk factors although controversy persists as to which lipid or lipoprotein components operate as independent risk factors in different populations. Pocock et al (1989), in a study of middle-aged British men, found total cholesterol and low HDL-cholesterol levels to be independent predictors of coronary heart disease but found triglyceride level not to be an independent risk factor after taking HDL-cholesterol into account. While the independent and inverse association of HDL-cholesterol with CHD has been supported by the PROCAM Study (Assmann and Schulte, 1992) and by follow-up work from the Lipid Research Clinics Prevalence Study (Jacobs et al, 1990), the effect of triglycerides has remained much more contentious with findings from the Framingham Study indicating no independent role (Kannel, 1985) but an important contributory role in women, and also in men with high (>4.5) total/HDL- cholesterol ratios (Castelli, 1986). The Lipid Research Clinics Follow-up Study found no independent role for triglyceride levels when HDL-cholesterol was taken into account (Criqui et al, 1993) and the PROCAM Study found an association only when LDL/HDL-cholesterol ratio exceeded 5.0 (Assmann and Schulte, 1992).

However a British study (Bainton et al, 1992) regarded triglyceride concentration as a better predictor of CHD than total cholesterol was, and in a subgroup with abnormal glucose tolerance the Paris Prospective Study found a strong association for triglyceride in both univariate and multivariate analyses (Fontbonne et al, 1989). Bengtsson et al (1993) have reported that triglyceride concentration is, and total cholesterol level is not, an independent risk factor for coronary heart disease in women.

In view of the consistently strong inverse relationship found between HDL-cholesterol and triglyceride concentrations (Davis et al, 1980) it may be disingenuous to seek independent roles for these risk factors. In this respect a retrospective analysis

of the Helsinki Heart Study data has concluded that a very good index of coronary risk can be obtained by combined assessment - the greatest risk being found in subjects with LDL/HDL-cholesterol ratio >5 and triglyceride level $>2.3\text{mmol/l}$ (Manninen et al, 1992).

In the context of diabetes, Rosengren et al (1989), using a rather weak definition of diabetes (self-reported by subjects), found serum cholesterol and smoking habit to be independent risk factors for myocardial infarction, although in contrast Uusitupa et al (1990) found no association between plasma insulin or any other expected risk factor with myocardial infarction.

Clustering of metabolic coronary risk factors

The coexistence in individual subjects of some or all of the abnormalities of blood pressure, obesity, and lipid and carbohydrate metabolism indicated above has led to the definition of a number of closely overlapping cardiovascular risk syndromes differing mainly in their emphasis on obesity (Kaplan, 1989), hyperinsulinaemia (Modan et al, 1985; Ferrannini et al, 1991) or insulin resistance (Reaven, 1988; DeFronzo and Ferrannini, 1991) as the favoured unifying or primary factor. Glucose intolerance, hypertension, hypertriglyceridaemia and hyperinsulinaemia are usually included in these definitions; insulin resistance may be excluded only by not being measured, with hyperinsulinaemia taken as a surrogate. By contrast, however, the role of obesity has been strongly contested (Reaven, 1994).

Nonetheless, perhaps the most coherent and widely acknowledged attempt to draw together a metabolic syndrome of cardiovascular risk has been that of Reaven (1988) who proposed a 'Syndrome X' (also referred to as the (pluri)metabolic syndrome, insulin resistance syndrome or Reaven's syndrome) comprising resistance to insulin-

stimulated glucose uptake as its core feature with consequent hyperinsulinaemia, glucose intolerance, hypertension, increased VLDL-triglyceride concentration and reduced HDL-cholesterol level. Although clear cutoff points for definition of these variables have been elusive, widespread support has been offered for the existence of such a syndrome (Zavaroni et al, 1989; Haffner et al, 1992). Various new features have been proposed for addition to the definition of the syndrome; these have most plausibly included small dense LDL particles, raised PAI-1 activity, impaired urate metabolism and truncal or upper body obesity. A number of issues remain unresolved regarding the clinical relevance and validity of such a syndrome, including its applicability to different populations. It has not yet been tested in a prospective study predicting coronary risk so that questions such as how many features must be present to define an individual as having the syndrome, whether risk accumulates with an increasing number of features, and whether the individual factors described can be considered as independent enough of each other to warrant separate inclusion, remain as yet without clear answers. The absence of a direct link between insulin resistance and coronary artery disease remains a problem. Furthermore, it is not clear whether insulin resistance itself is a risk factor in all or only in some populations; most notably the frequently cited highly insulin resistant Pima Indians have much lower rates of coronary artery disease than do white Americans (Sievers, 1967; Ingelfinger et al, 1976). The main disadvantage of using such a syndrome to assess coronary risk may be to detract attention from smoking and cholesterol as important modifiable risk factors for coronary heart disease.

Other hypotheses have also been proposed. In 1962 Neel (Neel, 1962) proposed the thrifty genotype hypothesis - that an increased metabolic efficiency advantageous to our hunter-gatherer ancestors during periods of famine might now predispose to NIDDM in a modern world with more plentiful and reliable sources of food. With increasing interest in the role of hyperinsulinaemia and insulin resistance the concept

has been revived (Zimmet, 1993; Swinburn, 1996) and remodelled, notably with the suggestion that the phenotypic expression of the thrifty genotype may come in the form of insulin resistance (in muscle) leading to obesity and NIDDM (Wendorf and Goldfine, 1991). Support for this view has waxed and waned, and there has been a lack of substantial supporting evidence; it has been widely recognised that European subjects seem to have a low prevalence of the genotype, whether through selection or a constitutional low risk, and may therefore be an inappropriate group to study in this context.

From almost the opposite standpoint, using epidemiological data from Britain, has come the 'thrifty phenotype' hypothesis, arguing that fetal undernutrition at crucial stages of in utero development may have a major influence on susceptibility to disease in later life, notably including coronary heart disease, hypertension, NIDDM and dyslipidaemia (Barker, 1995). Barker and his colleagues have published data showing associations of low birthweight with increased frequency of certain features of Reaven's syndrome X (Barker et al, 1993) in adult life and also of small babies (specifically thin babies) with subsequent insulin resistance (Phillips et al, 1994b). From these data increased fatness in utero and in later life appear to have contrasting effects on the development of insulin resistance. This has led these authors to suggest that insulin resistance may be a consequence of a failure of prenatal muscle development.

More recently has come the suggestion that the clustering of metabolic abnormalities observed arises not because of the effect of one factor on the others but from sharing common genetic and environmental influences. This has become known as the 'common soil' hypothesis (Stern, 1995) and takes account of the findings of Barker and his colleagues indicated above, as well as epidemiological data supporting the existence of a metabolic or insulin resistance 'syndrome' (Zavaroni et al, 1989; Haffner

et al, 1992). The common soil hypothesis also offers a means of resolving the controversy over the possible atherogenic effect of exogenous insulin in NIDDM (Stout, 1979 and 1987; Jarrett, 1988 and 1992) and may help to explain the absence alluded to above of any measured direct association between insulin resistance and coronary heart disease. Indeed, part of the intrinsic appeal of this model lies in its avoidance of a commitment to support any single factor as pre-eminent or causative.

Summary and Objectives

A clear excess morbidity and mortality from coronary artery disease is well defined among diabetic subjects. To what extent insulin resistance contributes to this excess is less clearly defined. Further, it is not clear whether other known coronary risk factors make a major contribution to the excess risk among diabetic subjects, or whether the diabetic state itself is the most important risk factor. There is no *a priori* reason to suppose that contributory factors to coronary risk in diabetic subjects differ from those in non-diabetic individuals, or are intrinsically less modifiable.

The work described herein attempts to address a number of these issues, and developed largely by a process of evolution. The starting point for the work in this thesis came with a study designed to examine the relationship between hypertriglyceridaemia and insulin resistance (chapter 5). If these two disorders are closely linked, then would reducing circulating triglyceride concentrations improve insulin resistance in subjects with NIDDM? In addition, might improvements in these components of the metabolic syndrome be associated with any changes in other components of the syndrome?

From this starting point came the ideas for the studies described in chapters 3 and 6. While epidemiological data exist regarding the metabolic syndrome in the general population, there are no reports in diabetic subjects. Can the metabolic syndrome be said to exist in a diabetic population? Is it truly a syndrome, and if so which features

predominate or are necessary to diagnose it in an individual? The exclusion of obesity from Reaven's description of the metabolic syndrome seems surprising, and it features in the clusters described by most other authors. The relationship of obesity with other features of the metabolic syndrome is addressed in chapter 3; in addition I attempt to examine more closely the relationships between obesity and insulin resistance in diabetic subjects in chapter 6.

The study of a new insulin secretagogue developed separately, and is linked to the other studies mainly by the exploration of changes in intermediary metabolite concentrations during the intravenous glucose tolerance test, and by a brief assessment of insulin resistance measuring glucose disappearance rates (chapter 4).

Finally, I have made use of the data derived from the small cohort of diabetic subjects studied in chapter 6 to compare different methods of assessing insulin resistance, and in particular to try and validate the low-dose incremental insulin infusion technique against alternative methods in this population.

Each chapter ends with a brief summary; I have attempted to draw together the findings and review them in the context of the existing literature in a final chapter (chapter 8).

CHAPTER 2

Methods

General Introduction

In this chapter are outlined the procedures followed in the studies and experiments described in the succeeding chapters. Where specific deviations from the procedures described below have taken place, these are explained in the relevant chapters.

Subjects

All subjects were recruited from the Diabetic Clinic at the General Hospital and Selly Oak Hospital, Birmingham with the exception of a small number of subjects with newly-diagnosed diabetes who were recruited, with the permission of their general practitioners, into the study examining effects of A4166 (chapter 4). All subjects were diagnosed as having diabetes mellitus according to conventional clinical practice whereby the diagnostic criteria of the World Health Organisation (1980) for definition of diabetes were followed, i.e fasting venous plasma glucose concentration ≥ 7.8 mmol/L and/or random (or 2 hours after 75g oral glucose load) venous plasma glucose concentration ≥ 11.1 mmol/L.

Ethical Considerations

Protocols for all studies were submitted for, and received, approval by the Research Ethics Committee of South Birmingham Health Authority prior to commencement. All subjects received written and verbal explanations of the procedures involved and gave signed informed consent to take part.

The recommendations of the Declaration of Helsinki by the World Medical Assembly (reprinted in Randle et al, 1978) were complied with. In addition,

particularly in those studies which involved the participation of a pharmaceutical company, guidelines on good clinical practice (Hutchinson, 1993) were also followed.

Protocols

For all procedures subjects attended following an overnight fast (minimum 8-10 hours) so that all studies were conducted under postabsorptive conditions. Subjects were asked not to consume alcohol on the day prior to attending and were requested not to take any medications on the morning of the study.

Intravenous glucose tolerance test (IVGTT)

After any preliminary assessments (eg. weight, resting blood pressure) an 18G cannula was inserted into an antecubital vein for sampling. A 20G cannula was inserted into a contralateral forearm vein for infusion of glucose. After 30 minutes rest two basal blood samples were withdrawn via the antecubital cannula. Following this a single tablet of either active drug (60mg A4166) or placebo was given at time -20 minutes (relative to start of glucose infusion). Further blood samples were taken at times -10 and 0 minutes. At time 0 an infusion was started of 50% glucose (Baxter Healthcare, Thetford, UK) given via an IMED 960 volumetric infusion pump (IMED Corp., San Diego, USA) to provide a bolus of 0.3g/kg glucose over 3 minutes. Blood samples were taken at 3, 5, 7, 10, 15, 20, 30, 40, 50 and 60 minutes. Infusion cannulae were flushed with 0.9% saline to prevent phlebitis. Subjects were allowed to drink water during the procedure and were offered a meal at the end. After an observation period of one hour subjects were permitted to return home.

Low-dose incremental insulin infusion

Following any preliminary measurements an 18G canula was inserted into an antecubital vein for sampling. A 20G cannula was inserted into a contralateral forearm vein for infusing insulin. After 30 minutes rest the first of four sequential hourly infusions was started. These contained, in order, 0.9% saline (no insulin), insulin 0.005 IU/kg, insulin 0.01 IU/kg and insulin 0.05 IU/kg. Infusions were prepared using Human Actrapid Insulin U100 (Novo Nordisk Pharmaceuticals Ltd., Crawley, UK) diluted to the required concentration in 0.9% saline. To each infusate 2.5ml Human Albumin Solution 20% (Immuno Ltd., Sevenoaks, UK) was added to prevent adsorption of insulin to syringes or plastic tubing. Infusions were administered continuously at a controlled rate via a Braun Perfusor VI infusion pump (B. Braun Melsungen AG, Melsungen, Germany).

Blood samples were withdrawn via the antecubital cannula 30, 40, 50 and 60 minutes after the start of each infusion. Blood glucose levels were monitored throughout using capillary testing strips and a glucose meter, and the test was terminated either at the end of the sampling schedule (i.e. the end of the fourth infusion period) or when symptoms or biochemical signs of hypoglycaemia (defined as blood glucose <3.3 mmol/L) appeared, whichever was the earliest. Subjects were permitted to drink water throughout the procedure and were offered a meal at its end. After an observation period of one hour subjects were allowed home.

For studies in which comparison was made between low-dose intravenous insulin infusion and euglycaemic hyperinsulinaemic clamp techniques the above procedure was modified as follows. Instead of the sampling cannula being placed in an antecubital vein the cannula was sited in a vein on the dorsum of the hand to allow samples to be taken from arterialised blood. The cannula, once inserted, was

connected to a 7cm extension with 3-way tap so that blood could be sampled without removing the hand from a temperature-controlled hot box (on loan from Amylin Europe Ltd., Oxford, UK) and to enable continuous slow infusion of 0.9% saline (Baxter Healthcare Ltd., Thetford, UK) to maintain cannula patency.

Infusions were carried out as above except that the initial infusion of saline alone ran for only 30 minutes. Blood was sampled at 20 and 30 minutes after commencement of this infusion. During infusion of insulin at concentrations of 0.005 and 0.01 units/kg/hr samples were taken at 30, 45 and 60 minutes; during the final infusion of 0.05 units/kg/hr samples were taken at 30, 40, 50 and 60 minutes. Blood glucose concentrations were monitored using a YSI Stat Plus glucose analyser (Yellow Springs Instrument Co. Inc., Yellow Springs, USA) and the test terminated according to the criteria outlined above.

Euglycaemic hyperinsulinaemic clamp

After preliminary measurements a 20G cannula was inserted into a forearm vein for infusing insulin and glucose. A flexible 20G cannula was inserted retrogradely into a vein on the dorsum of the contralateral hand for arterialised blood sampling. The retrograde cannula was connected to a 7cm extension with 3-way tap attachment to allow blood sampling without withdrawal of the hand from a temperature-controlled hot box (on loan from Amylin Europe Ltd., Oxford, UK) and to enable continuous slow infusion of 0.9% saline (Baxter Healthcare Ltd., Thetford, UK) to maintain cannula patency. Insulin infusion was prepared by diluting 30 Units Human Actrapid Insulin U100 (Novo Nordisk Pharmaceuticals Ltd., Crawley UK) in 58ml 0.9% saline with 2ml of the subject's own blood to prevent adsorption of insulin to syringe or plastic tubing.

After 30 minutes rest two basal blood samples were withdrawn and the insulin infusion commenced. Insulin infusion rate was determined by reference to standard protocols and calculated according to body weight. Thus infusion was started at $[0.75 \times \text{body weight}]$ ml/hr for two minutes, followed by infusion (in ml/hr) at $0.33 \times \text{wt}$, $0.30 \times \text{wt}$, $0.28 \times \text{wt}$, $0.26 \times \text{wt}$, each for two minutes, and then a maintenance rate of $[0.24 \times \text{body wt}]$ ml/hr for the duration of the procedure. Infusion rate was controlled using a Graseby MS2000 syringe pump (Graseby Medical Ltd., Watford, UK).

In order to maintain euglycaemia at a constant 5mmol/L, blood glucose was measured every five minutes using a YSI Stat Plus glucose analyser (Yellow Springs Instrument Co. Inc., Yellow Springs, USA). From the glucose concentrations obtained the rate of infusion of glucose 20% (Baxter Healthcare Ltd., Thetford, UK) via an IMED 960 volumetric infusion pump (IMED Corp., San Diego, USA) could be adjusted appropriately. Further blood samples were taken 30 and 60 minutes after starting insulin infusion. Total infusion time was approximately 180 minutes to allow a minimum of 30 minutes stable euglycaemia (60 minutes where possible). Three further blood samples were taken in the final 30 minutes of the procedure.

At the end of the test insulin infusion was stopped but glucose infusion was continued to provide as much glucose as had been required in the previous hour of the procedure. When this additional infusion was complete the procedure was terminated. Subjects were permitted to drink water throughout the procedure and were offered a meal at its end. After an observation period of one hour subjects were allowed home.

Sampling

Free-flowing blood samples (i.e. without the use of a tourniquet) were obtained at all times. For dynamic metabolic studies as described above approx. 8-10ml of blood

was withdrawn at each testing point. Of this sample 1.5-2.0ml was added to a 10ml glass tube (L.I.P. Ltd., Shipley, UK) which had been prepared in-house with 5ml 0.77M perchloric acid and pre-weighed using digital scales accurate to 0.01g (Sartorius GmbH, Göttingen, Germany). After the addition of the blood sample the tube was again weighed to allow an accurate estimate of blood volume added so that after analysis correction could be made for the volume of perchloric acid in the sample assayed.

The remainder of the blood obtained was added to a 10ml tube commercially prepared with lithium heparin (Laboratory Services Ltd., UK) to allow plasma separation.

Samples were stored immediately at 4°C and separated from cells within one hour using a refrigerated centrifuge (Denley BR401, Denley Instruments Ltd., Billingshurst, UK); samples were spun for 12-15 minutes at 4°C.

After centrifugation supernatant was immediately drawn off and labelled aliquots were stored at -20°C for later analysis. Storage of samples for metabolite analysis broadly followed the recommendations of Lloyd et al (1978) whereby samples may be stored for several months with low risk of deterioration prior to measurement of glucose, lactate, alanine or 3-hydroxybutyrate; acetoacetate was always measured within 3 days of sampling and pyruvate within 28 days.

Arterialised versus venous blood sampling

Advantages and disadvantages of venous and arterial or arterialised blood sampling continue to be debated. It has generally been felt that arterial blood samples reflect whole body metabolism whereas venous blood samples are necessarily influenced by tissues draining into the sampling vein. In view of the natural preference of both

researchers and volunteers to avoid direct arterial cannulation where possible, the use of arterialised venous blood sampling (by retrograde cannulation of a hand vein in a heated box) has become widely accepted and in clamp studies is now almost universally used. Studies of forearm muscle metabolism with simultaneous sampling from arterial, venous and arterialised venous sites have confirmed the anticipated arteriovenous differences and the appropriateness of arterialised venous blood as a substitute for direct arterial sampling (Jackson et al, 1973; McGuire et al, 1976; Abumrad et al, 1981). More recently, however, arguments have been proposed in favour of the use of mixed venous blood for estimating glucose requirements in clamp studies (Andrews et al, 1984; Wahab et al, 1992) and also for IVGTT with minimal modelling (Godsland et al, 1993).

In the studies undertaken and described here these issues have not been systematically addressed and a pragmatic approach has been adopted. For single postabsorptive samples venous blood has consistently been used. For the IVGTT studies and insulin infusion studies assessing the effects of Gemfibrozil on insulin sensitivity, venous blood has been used, making the assumption that intra-individual variability will be too small to have a material effect on paired tests. No data exist for the low-dose insulin infusion to indicate the extent of intra-individual variation with this test, nor to compare venous and arterialised venous blood samples. In the study employing the euglycaemic clamp method arterialised venous blood samples were used according to convention; hence in order to make comparison with clamp data from the same subjects arterialised venous sampling was also used for insulin infusions in this study.

Laboratory Assays

Glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glycerol and alanine were all measured in whole blood extracts deproteinated by the use of 0.77M perchloric acid. All assays were performed using a Cobas Bio centrifugal analyser with a fluorescence capability following the methods described by Stappenbeck et al (1990). The laboratory methods employed all make use of enzymatic reactions of the analyte which depend on the formation or utilisation of the reduced co-enzymes NADH or NADPH. Each reagent system is either a single enzyme system requiring the co-conversion of NAD(P) to NAD(P)H, or the reverse, or a coupled system which involves the metabolite as the initial substrate in a series of reactions, one of which involves an NAD(P)H-dependent enzyme.

The rate of formation or utilisation of NADH, or NADPH, can be monitored by taking advantage of the fact that on spectrophotometry both of these reduced co-enzymes have an absorption peak at 340 nanometres; this property is not shared by the oxidised forms NAD or NADP.

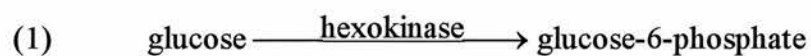
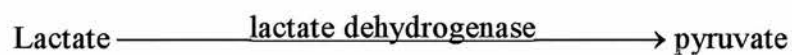
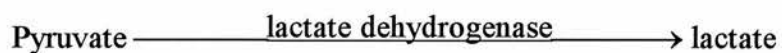
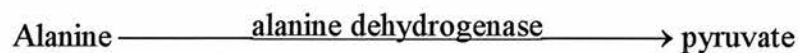
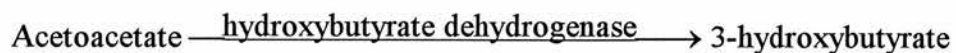
Those metabolites present in relatively high concentration (i.e. glucose and lactate) can be measured by spectrophotometry; for the others, the concentrations of NAD(P)H present are not adequate for sensitive detection by this means and fluorimetry is required, using the same excitation wavelength of 340nm and the emission wavelength of 470nm.

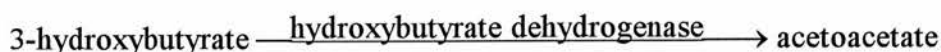
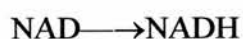
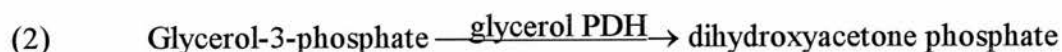
For certain reactions the same enzyme may be used to measure both substrates of a reversible process if reaction conditions are altered appropriately. This applies to lactate-pyruvate and 3-hydroxybutyrate-acetoacetate interconversions.

Individual reactions are described below and precision data shown in table 2.1.

Table 2.1. Analytical precision for metabolite and hormone assays

<i>Analyte</i>	<i>Units</i>	<i>Mean concentration</i>	<i>CV (%)</i>
Glucose	mmol/L	1.0	1.2
Lactate	μmol/L	1000	2.5
Pyruvate	μmol/L	50	3.4
Alanine	μmol/L	101	3.1
Acetoacetate	μmol/L	53	3.0
3-hydroxybutyrate	μmol/L	81	2.1
Glycerol	μmol/L	42	1.9
NEFA	mmol/L	1.07	1.3
Total Insulin	pmol/L	369	6.2
C-peptide	pmol/L	511	8.4

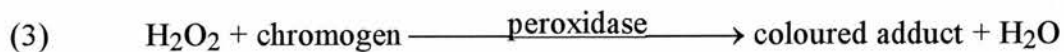
GlucoseLactatePyruvateAlanineAcetoacetate

3-hydroxybutyrate (β -hydroxybutyrate)Glycerol

(glycerol PDH = glycerol phosphate dehydrogenase)

Non-esterified fatty acids (NEFA)

NEFA concentrations were measured in heparinised plasma using a commercial enzymatic colorimetric assay (Wako NEFA-C test kit; Wako Chemicals GmbH, Neuss, Germany). A three-step reaction takes place as follows:



The coloured adduct has an absorption peak of 550nm; this is measured by spectrophotometry.

Hormone Assays

Insulin and C-peptide were measured by commercial radioimmunoassay (Pharmacia AB, Uppsala, Sweden and Guildhay, UK respectively) using a double antibody technique. Hormone in plasma samples competes with a fixed quantity of radiolabelled hormone for binding sites on the antibody. After using an immunoabsorbent to separate free from bound hormone the radioactivity of the sample can be measured and is then inversely proportional to the original plasma concentration of hormone.

Where alternative units of measurement are required for certain calculations (eg. for HOMA-R values from insulin and glucose concentrations) a standard procedure has been followed. Insulin concentrations (pmol/L) are converted to mU/L by division by 7.46; C-peptide concentrations (pmol/L) can be converted to ng/L by division by 331.

Proinsulin was measured using a Europium immunofluorometric assay developed by Dr PM Clark in conjunction with Professor CN Hales (Cambridge). This assay measures specific proinsulin with approximately 95% recovery.

Lipids

Cholesterol and triglyceride concentrations were measured using an Instrumentation Laboratory ILAB900 Clinical Chemistry Analyser; HDL-cholesterol and Lipoprotein (a) were measured on a Cobas Bio analyser.

Both cholesterol and triglyceride assays employ enzyme reactions generating hydrogen peroxide, which in the presence of peroxidase reacts with 4-aminoantipyrine and phenol to produce a quinoneimine with absorption peak at 510nm.

HDL-cholesterol concentration was measured by a modification of the method for total cholesterol after precipitation of LDL-cholesterol from serum samples.

Lipoprotein (a) concentration was measured by an immunoturbidimetric assay. Antiserum to Lp(a) was added to the serum samples for analysis and Lp(a) concentration determined by the turbidity of the reaction mixture formed by the resulting antigen-antibody complexes.

Glycosylated haemoglobin

HbA1 was measured by an electroendosmosis method to separate haemoglobin fractions on agar gel after stabilisation of HbA by haemolysis of the whole blood sample. The gel can then be scanned densitometrically at 420nm and the HbA1 content expressed as a percentage of total haemoglobin. This analysis was used for all studies except for the study of body fat distribution and insulin resistance; when this study was carried out the HbA1 assay had been superseded by a new HbA1c assay. This measures HbA1c by latex-enhanced immunoturbidimetry on a Roche Cobas Mira analyser. Blood samples were haemolysed and antibodies raised against glycated N-terminal amino acids on the β -chain of the haemoglobin molecule. After total haemoglobin has been measured on the same haemolysed sample, HbA1c is again expressed as a percentage. (An approximate estimate of HbA1c can be obtained by subtracting 1.85% from measured HbA1 values).

Other analytes

Uric acid (urate) concentration was determined by a standard method again using hydrogen peroxide to generate a coloured product (in this case indimine) with characteristic absorption at 540nm.

Fructosamine was measured by reaction of serum samples with nitroblue tetrazolium, under alkaline conditions, to produce formazan at a rate directly related to the fructosamine concentration.

Statistical Analyses

All statistical analyses were carried out by me either manually or using the analytical tools available on computer software (Microsoft Excel version 4.0 for Windows). The additional procedures performed on SPSS for Windows were carried out by me with the assistance of Vicki Allgar. Methods followed those described by Armitage and Berry (1994).

For the purposes of analysis missing data in dynamic studies have been treated by inserting the mean concentration of analyte of the two values immediately adjacent for that subject (i.e. immediately before and immediately after the missing value). I have regarded this method as having greater biological plausibility than the usually recommended procedure of inserting the mean for that time point of all subjects in the group. Missing values for single fasting samples have not been replaced.

Where correlation coefficients have been calculated these have been applied to ranked data (except where indicated) in order to avoid the distortions produced by non-normally distributed variables (notably plasma insulin, serum triglyceride and ketone concentrations, and HOMA-R values). Spearman's rank correlation coefficient

has then been used, with significance testing on the *t* distribution. Where multiple correlation coefficients were obtained appropriate correction has been made in assessing statistical significance, using the Bonferroni correction or the equivalent method of multiplying *p* values by the number of comparisons made (Bland and Altman, 1995). For calculating regression lines I have used actual data, log transformed where appropriate.

Multiple regression analysis was used in the analysis of data in chapter 3. The calculations were carried out using the computer software package SPSS version 5 for Windows. Up to 20 variables were included in the analyses (of data from 231 subjects) and models were derived which allowed for the inclusion of variables at significance levels up to $p=0.1$, while variables with $p>0.15$ were automatically excluded.

The Kolmogorov-Smirnov test used in chapter 3 to assess for normality of distribution of the number of features of Syndrome X was also performed using SPSS.

Factor analysis, or more correctly principal components analysis, again in chapter 3, was also performed using SPSS for Windows. The methods used follow closely those described by Meigs et al. (1997). Here variables of interest are examined to see whether their intercorrelations form a single group or whether the variance in the data can be better explained by separate subsets of variables, which may overlap. The procedure attempts to identify a minimum number of components to explain the maximum amount of variance in the original data; if only one component is identified then in this instance the metabolic syndrome may be considered as an essentially homogeneous entity in the population studied.

Components were identified and defined by Eigenvalues which relate correlations between original variables and the components identified, and represent the proportion of variance attributable to a given component. An Eigenvalue of 1 in this study reflected a component accounting for approximately 10% of total variance; only components yielding Eigenvalues of one or more were therefore included. To identify the most important variables contributing to each component, only those variables with loadings >0.3 were included in the final model. Loadings are derived mathematically from correlations between individual components and their constituent variables.

The model derived from these calculations gives a description of the major features of interest and their interrelationships, and helps to identify clustering of particular variables within the original group of variables selected for analysis.

More detailed explanation of this technique can be found in Armitage and Berry (1994) and Meigs et al (1997).

Analysis of data generated from the IVGTT studies was carried out by two way analysis of variance. Individual subjects were treated as replicates. Data were grouped by treatment (A4166 before diet, A4166 after diet, placebo before diet and placebo after diet) and by time (timepoints at which blood samples were taken relative to onset of glucose infusion). Taking the mean of the two baseline blood samples at each visit, there were therefore four treatment groups and thirteen timepoints for analysis. Where overall variance ratios were statistically significant comparison of pairs of treatments was made using Scheffe's test to assess significance. The use of this test allows for multiple comparisons and reduces the likelihood of significant differences arising by chance; significance (p value) is tested on the F distribution.

Data from low-dose insulin infusions were analysed by calculating regression lines for each metabolite on insulin concentrations. For the studies of gemfibrozil in hypertriglyceridaemia (chapter 5) and of body fat (chapter 6), regression lines were calculated for metabolite concentrations on log [mean insulin] for each group in order to obtain a straight line relationship. For comparison of the low-dose insulin technique with the euglycaemic clamp (chapter 7) regression lines were calculated for individuals on their own plasma insulin concentrations (with log transformation of insulin values).

The regression lines obtained were then subjected to analysis of covariance in order to examine the relationships between groups by comparison of the parallel regression lines produced. Significance testing was carried out on the corrected mean values of y obtained and follows the approach described by Armitage and Berry (1994). Although this has certain disadvantages it offers a convenient way of expressing differences between the regression slopes, comparing derived values of y at the same x (in this instance the overall mean plasma insulin concentration for all groups). Again, where overall variance ratios were statistically significant, differences between groups were examined using Scheffe's test.

CHAPTER 3

Metabolic characteristics of subjects with newly-diagnosed
non-insulin-dependent diabetes

Introduction

Subjects with non-insulin-dependent diabetes (NIDDM) are recognised to have an increased risk of cardiovascular disease in comparison with the general population. Extensive interest has been generated in studying and attempting to limit this excess risk.

The development of concepts of syndromes of metabolic risk (Reaven, 1988; Kaplan, 1989; Ferrannini et al, 1991; DeFronzo and Ferrannini, 1991) and the related 'common soil' hypothesis (Stern, 1995) have focused attempts to evaluate and quantify cardiovascular risk.

With particular reference to Reaven's syndrome X, if such a syndrome truly exists, then it should be possible to identify individuals with the syndrome, compare their characteristics with subjects not having the syndrome, and intervene to reduce risk.

Reaven's description of syndrome X contains the following features: glucose intolerance, hyperinsulinaemia, hypertension, hypertriglyceridaemia and low circulating HDL-cholesterol concentrations, with insulin resistance present as the unifying or primary factor. Reaven and other authors have since proposed additional factors for inclusion in the syndrome, among the most plausible being hyperuricaemia and upper body obesity. The role of obesity, and which index of obesity is most useful as an indicator of coronary risk, still seems somewhat controversial.

This study set out to examine the characteristics at diagnosis of diabetes of a group of adults with particular reference to cardiovascular risk factors, to assess whether any of these factors co-segregated more than would be expected by chance, and to try to establish whether in this group of subjects a subgroup could be defined as having 'syndrome X'.

Newly-diagnosed diabetic subjects were chosen because they form part of a high risk group, because of the ready availability of a suitable cohort of subjects, and because at the time of diagnosis of diabetes, although many risk factors may be present, confounding features attributable to diabetes itself may be less well established than in a group with longer duration of NIDDM.

The study was carried out as a cross-sectional survey.

Subjects and Methods

Subjects were recruited from the Diabetic Clinic at The General Hospital, Birmingham between September 1993 and June 1995.

Subjects were eligible for inclusion if they fulfilled the following criteria:

1. diagnosis of diabetes within the preceding three months
2. aged over 18 years
3. not already treated with insulin nor considered (by the clinic physician) to need insulin treatment at the time of presentation to the diabetic clinic
4. no identified secondary cause of diabetes (pancreatitis, endocrine disease, etc.)
5. not known to be pregnant

Subjects with a diagnosis of impaired glucose tolerance were also excluded.

Subjects who later started treatment with insulin were excluded from data analysis if they were known to have started insulin during the recruiting period or within the six months thereafter; this was regarded as indicating insulin-dependent diabetes. This exclusion was not applied to any subjects who were known to have been treated

temporarily with insulin and then reverted to diet or oral hypoglycaemic therapy (one subject).

Procedure

All eligible subjects attending the Diabetic clinic for the first time were invited to attend for assessment. Assessment usually took place within 5 days of clinic attendance. A printed information sheet was supplied and a verbal explanation given; all participating subjects gave signed consent on the day of assessment. Subjects already taking oral hypoglycaemic agents at the time of assessment (43 of those studied; see table 3.1) were asked to omit these for 72 hours prior to assessment; where this was not possible all subjects omitted such medication for 24 hours. Any other medication was continued as normal but omitted on the morning of the study.

All subjects attended for assessment after overnight fast. All subjects were asked to confirm that they had fasted and the assessment was rescheduled if anything other than water had been consumed.

Details of name, date of birth, sex, hospital number, country of birth and religious/ethnic group were obtained, along with duration since diagnosis of diabetes (to nearest whole month) and current treatment, family history of diabetes, other drug treatment, details of alcohol consumption and cigarette smoking, and personal history of hypertension, ischaemic heart disease (defined as a diagnosis by a doctor of myocardial infarct or angina), hyperlipidaemia, gout and peripheral vascular disease.

Height, weight, and waist and hip circumferences were measured. The latter were measured with the subject standing and outer clothing removed; waist circumference was taken at the horizontal level of the umbilicus and hip circumference at the level of the greater trochanters.

Blood pressure was measured twice, at least 5 minutes apart, after a minimum of 5 minutes rest in a semi-recumbent position. All blood pressure readings were carried out on the subject's right arm. Where identifiable Korotkov phase IV was used for diastolic pressure.

After the above procedures had been completed, approx. 50ml of free-flowing venous blood (i.e. taken without a tourniquet) was withdrawn to be separated, frozen and later analysed for glucose, insulin, NEFA, 3-hydroxybutyrate, urate, total and HDL-cholesterol, triglycerides, lipoprotein (a), HbA1 and fructosamine, along with electrolytes, creatinine, calcium, protein and liver enzymes.

Definitions

Racial groups were determined by skin colour and self-reported country of origin. 'Caucasian' therefore included all white subjects reporting a European (including Eastern Mediterranean) or Australasian background; 'Asian' included all subjects of Indian, Bangladeshi and Pakistani origin, including those born in East Africa. 'Black' included individuals from both African and Caribbean backgrounds. Probably because of the predominant age group studied the 'Asian' group included only four subjects, and the 'Black' group three, who were born in the United Kingdom. The designation 'Other' covered seven subjects studied (as well as one who did not attend for assessment, from Yemen). These seven were four Arab subjects, all from Yemen, and three of mixed race - one Afro-Chinese, one Indo-African, and one mixed-race Mauritian.

Cutoff points for determining the presence or absence of features of Reaven's syndrome were taken as follows: hypertension - defined as mean diastolic pressure >95mmHg and/or on treatment for known hypertension; triglycerides >2.30mmol/L;

HDL-cholesterol $<0.91\text{mmol/L}$ for men and $<1.16\text{mmol/L}$ for women; hyperinsulinaemia was determined by the highest quartile for the study population, i.e. fasting plasma insulin concentration $> 20.83\text{mU/L}$ (155.4pmol/L). These definitions are broadly in accord with those applied in other epidemiological studies (Uusitupa et al, 1985; Ferrannini et al, 1991; Haffner et al, 1992; Criqui et al, 1993). The definition for hyperinsulinaemia is necessarily arbitrary, especially in the absence of a control population, although the insulin concentrations observed in the highest quartile are broadly comparable with those observed in a mixed diabetic and non-diabetic population studied by Solymoss et al (1995). Insulin resistance was defined as a HOMA-R value of ≥ 3.5 ; this allows for some natural variation within and between normal subjects, although again this cutoff must be accepted as arbitrary. When examining features of Syndrome X, all subjects were regarded as glucose intolerant by definition based on their diagnosis of diabetes; in addition, and for examination of the cluster analysis data the alternative option of considering only those with fasting blood glucose $\geq 6.7\text{mmol/L}$ (i.e. reflecting the cutoff for WHO definition of diabetes) was used, allowing further examination of the distribution of abnormalities present. For examining features of the components derived from cluster analysis, the following additional cutoffs were used: high BMI was defined as $\text{BMI} > 30$; high waist-hip ratio was defined as >0.9 for women and >1.0 for men; and high HDL-cholesterol taken as the highest quartile, i.e. >1.16 for women and >1.05 for men.

Statistical Analyses

For all calculations log transformation was made of insulin and triglyceride concentrations and HOMA-R and HOMA-S values to approximate normal distributions.

Comparison of variables between attenders and non-attenders was made by Student's t-test.

A correlation matrix was obtained for the variables of interest and individual correlation coefficients calculated. Significance tests were then calculated on the t distribution and are expressed as p values after applying appropriate correction for multiple comparisons. Because of the complexity of assessing hypertension (using both previous diagnosis and current measurement, and also the confounding effect of treatment) it was not analysed in this form. Correlation coefficients are given for relationships between absolute values, log transformed where appropriate. Correlation coefficients from ranked data yielded very similar results (not shown).

The data were then examined using multiple regression analysis. A stepwise method was applied to develop a series of models. These were used to determine the major contributors to the number of features of syndrome X: (a) the full model including all variables studied; (b) the 'clinical model' excluding HOMA-R from the independent variables, and (c) an 'additional features' model which excluded those features defining syndrome X from the analysis. The computer programme was run to permit inclusion of variables of interest in the model up to a maximum significance level of $p = 0.1$; variables were automatically excluded if p exceeded 0.15. Following on from this factor analysis (principal component analysis) was applied to construct a model of risk factors to examine whether a single syndrome truly describes any clustering of cardiovascular risk factors. The details of this procedure are described in Chapter 2, and follow closely the method described by Meigs et al (1997).

Table 3.1. General characteristics of study group and comparison with subjects excluded or not assessed.

		<u>Study group</u>	<u>Excluded</u>	<u>Not assessed</u>
Total		231	22	66
Age	mean (range)	56 (20-79)	46 (19-80)	53 (23-85)
Sex	male	143	17	43
	female	88	5	23
Racial group	Caucasian	136	16	37
	Asian	65	2	24
	Black	23	4	4
	other	7	0	1
Duration of diabetes				
	<1 month	106	13	23
	1-2 months	88	6	35
	2-3 months	37	3	7
Treatment*	diet	188	18	49
	sulphonylurea	35	4	14
	metformin	4	0	2
	both drugs	3	0	1
Other drugs	yes	103	9	26
	no	128	13	40
Hypertension	yes	73	1	13
	no	158	21	†52
BMI	mean (range)	29.57	25.58	31.53
		(18.8-47.4)	(17.5-39.2)	(21.6-48.9)
Fructosamine	mean (range)	363.6	467.3	344.9
		(212-688)	(278-663)	(183-618)

* one participant in the study group was taking an unidentified oral hypoglycaemic agent

† not known for one subject

Table 3.2. Characteristics of whole study population (231 subjects).

	mean	median	range	interquartile range
Waist-hip ratio	0.99	1	0.77-1.18	0.94-1.03
waist circumference (cm)	98.6	97	68-143	92-105
mean systolic BP (mmHg)	150.3	147	102-217	132-168
mean diastolic BP (mmHg)	92.3	92	56-125	85-100
HbA1 (%)	11.57	11.3	5.6-18.4	9.5-13.4
Total cholesterol (mmol/L)	5.88	5.76	2.80-9.59	4.93-6.60
Triglycerides (mmol/L)*	1.91	1.80	0.49-21.05	1.27-2.53
HDL-cholesterol (mmol/L)	0.96	0.92	0.19-2.15	0.80-1.10
Urate (mmol/L)	0.30	0.30	0.13-0.58	0.25-0.34
Glucose (mmol/L)	8.8	8.4	3.8-21.0	6.6-10.4
Insulin (pmol/L)*	104.5	97.0	29.1-510.3	69.8-155.4
NEFA (mmol/L)	0.74	0.72	0-1.77	0.55-0.90
HOMA-R*	5.19	5.11	1.01-26.75	3.29-8.45
HOMA-S (%)*	62.8	59.1	9.2-680	33.7-123.7

* geometric mean

Table 3.3 (a). Mean and range of values for racial groups

	<u>Caucasian</u>	<u>Asian</u>	<u>Black</u>
Total numbers	136	65	23
Age (years)	60.1 27-79	46.3 24-70	55.1 20-77
Treatment (numbers)			
diet	112	53	18
sulphonylurea	18	12	3
metformin	4	0	0
both	2	0	1 [†]
On no other drugs	83	49	12
Known hypertension	47	13	11
Coronary heart disease	23	3	1
Measured systolic BP (mean and range)	155.3 108-216	137.7 102-213	158.7 120-217
Measured diastolic BP	93.1 64-125	88.4 56-116	98.7 82-116
BMI (kg/m ²)	30.05 18.8-47.4	28.31 20.8-45.3	29.81 24.1-37.8
Waist (cm)	100.3 68-143	96.2 77-131	94.3 74-119
Waist-hip ratio	0.985 0.77-1.17	1.007 0.86-1.18	0.969 0.79-1.08

	<u>Caucasian</u>	<u>Asian</u>	<u>Black</u>
Total cholesterol (mmol/L)	6.00 2.92-9.59	5.79 2.8-9.2	5.42 3.1-8.3
*Triglycerides (mmol/L)	2.04 0.69-8.59	1.99 0.75-21.1	1.15 0.49-4.50
HDL-cholesterol (mmol/L)	0.936 0.19-2.15	0.96 0.34-1.46	1.13 0.63-1.84
*Lipoprotein (a) ($\mu\text{mol/L}$)	161.5 47-1136	176.6 47-1251	578.7 203-1453
HbA1 (%)	11.51 5.6-18.4	12.00 7.7-17.4	11.06 6.0-17.4
Fructosamine ($\mu\text{mol/L}$)	359.4 212-603	369.2 232-657	381.8 217-688
Glucose (mmol/L)	8.83 3.8-21.0	9.04 4.6-16.0	8.04 4.3-15.8
*Insulin (pmol/L)	101.3 29.1-510	120.6 39.5-464	79.2 36.6-289
*HOMA-R	5.05 1.01-26.8	6.24 1.73-25.8	3.51 1.05-8.92
*HOMA-S (%)	60.5 9.2-680	65.9 11.3-249	61.86 10.0-485
*3-hydroxybutyrate ($\mu\text{mol/L}$)	137.8 5-3639	73.9 8-1421	276 16-1350
NEFA (mmol/L)	0.752 0-1.59	0.688 0.14-1.38	0.818 0.30-1.77
Urate (mmol/L)	0.311 0.13-0.54	0.285 0.17-0.58	0.314 0.17-0.48
Creatinine ($\mu\text{mol/L}$)	94.3 59-214	86.3 62-119	103.9 72-216

* geometric mean

† unknown drug for one subject

Table 3.3 (b). Mean and range of values for all male vs. all female subjects.

	<u>Male</u>	<u>Female</u>
Total numbers	143	88
Age (years)	54.6 20-79	57.3 27-79
Treatment (numbers)		
diet	108	80
sulphonylurea	29	6
metformin	3	1
both	2 [†]	1
On no other drugs	81	47
Known hypertension	44	39
Coronary heart disease	16	12
Measured systolic BP (mean and range)	149.1 102-217	152.3 106-216
Measured diastolic BP	92.4 56-119	92.1 56-125
BMI (kg/m ²)	28.96 20.8-46.3	30.55 18.8-47.4
Waist (cm)	99.3 70-143	97.4 68-138
Waist-hip ratio	1.008 0.79-1.17	0.962 0.77-1.18

	<u>Male</u>	<u>Female</u>
Total cholesterol (mmol/L)	5.78 3.1-9.2	6.04 2.8-9.6
*Triglycerides (mmol/L)	1.92 0.62-21.1	1.89 0.49-5.07
HDL-cholesterol (mmol/L)	0.92 0.19-1.69	1.02 0.55-2.15
*Lipoprotein (a) ($\mu\text{mol/L}$)	180 47-1453	187.5 47-988
HbA1 (%)	11.33 5.6-18.4	11.97 6.5-17.4
Fructosamine ($\mu\text{mol/L}$)	358.2 217-688	372.6 212-657
Glucose (mmol/L)	8.6 3.8-21.0	9.1 4.3-15.6
*Insulin (pmol/L)	99.4 29.1-510	113.5 32.8-483
*HOMA-R	4.8 1.01-26.8	5.9 1.27-22.9
*HOMA-S (%)	63.5 9.2-680	61.6 11.3-485
*3-hydroxybutyrate ($\mu\text{mol/L}$)	118.8 8-3639	103.6 5-1757
NEFA (mmol/L)	0.673 0.06-1.77	0.847 0-1.59
Urate (mmol/L)	0.317 0.16-0.58	0.283 0.13-0.54
Creatinine ($\mu\text{mol/L}$)	97.7 73-216	83.8 59-140

* geometric mean

† unknown drug for one subject

Results

Study population

A total of 319 subjects who were considered to fulfil the inclusion criteria were seen in the Diabetic Clinic during the study period. Sixty-six of these (21%) either declined to participate or did not attend for assessment ('non-attenders'). Available relevant data for these subjects at clinic attendance was obtained and compared with results for the study group to ensure that no systematic bias was present (table 3.1).

A further 22 subjects were excluded after assessment; in 16 cases this was as a result of starting insulin treatment during or within 6 months of completion of the study period. One subject who started insulin treatment was also thought to have diabetes secondary to pancreatitis. It was not possible to obtain blood samples in the fasting state from three subjects, and two further subjects were considered on review of casenotes to have probable or possible secondary diabetes (one pancreatitis, one steroid treatment). One other subject may have had diabetes for longer than three months and was therefore also excluded. Comparable data available for all three groups are summarised in table 3.1. No clear systematic differences were observed between the attenders and the non-attenders; t-statistics and p values are not shown. Formal comparison was not made with the group excluded retrospectively (22 subjects), since these subjects may be expected to belong to a different population.

Data from 231 subjects were therefore available for analysis. Descriptive data are shown in table 3.2, with breakdown of data by racial group in table 3.3 (a) and by sex in table 3.3 (b).

Only two subjects reported taking any lipid-lowering treatment (one bezafibrate, one simvastatin) and no special account has been taken of these in data analysis. Twenty-

eight subjects (12%) reported a previous diagnosis of coronary heart disease (CHD). Comparing this group with those not having such a diagnosis showed no differences other than older age among those with CHD (mean age 65.2 vs. 54.3 years; unpaired Student's t-test $p=0.0001$), and the CHD group were also more overweight (mean BMI 31.97 vs. 29.23; $p=0.008$). Those with existing CHD were also more likely to be on drug treatment of any kind (27/28 subjects vs. 76/203; for χ^2 test $p<0.0001$). There were no differences in plasma insulin, blood glucose or serum triglyceride concentration, or presence of hypertension.

Frequency of features of the metabolic syndrome

Prevalence figures (percent) for the individual features of syndrome X are shown in table 3.4. Numbers of features for groups (classified by race and sex) within the study population are shown in table 3.5 (a), and illustrated for all subjects in figure 3.1, showing the effect of the two definitions of glucose intolerance used. For the population studied an assessment of the distribution of features was made. This gave a mean number of features of 3.4 with standard deviation of 1.28. Kolmogorov-Smirnov test for normality of distribution suggested that there may be skewness present but this was felt to be a result of the small number of components (i.e. 6) possible within the distribution curve.

Table 3.5 (b) shows the number of additional features of Syndrome X present in association with other features of the syndrome. Thus it can be seen that insulin and triglycerides show the greatest degree of skewness, i.e. are more likely to be associated with several other features, while the pattern for the other features follows a more normal distribution. The bottom row of table 3.5 (a) can be seen as a similar summary for the associations of glucose intolerance (here regarding all subjects as glucose intolerant).

Table 3.4. Prevalence (%) of individual features of Syndrome X.

(a) whole group, and arranged by sex

	all	male	female
total subjects	231	143	88
glucose intolerance	100	100	100
hypertension	51.9	47.6	59.1
hypertriglyceridaemia	31.2	32.9	28.4
hyperinsulinaemia	25.1	22.4	29.5
low HDL-cholesterol	59.3	50.3	73.9
insulin resistance	72.3	67.1	80.7

(b) arranged by racial group

	Caucasian	Asian	Black	Other
total subjects	136	65	23	7
glucose intolerance	100	100	100	100
hypertension	55.9	36.9	69.6	57.1
hypertriglyceridaemia	36.0	29.2	8.7	28.6
hyperinsulinaemia	24.3	32.3	8.7	28.6
low HDL-cholesterol	61.8	56.9	43.5	85.7
insulin resistance	71.3	81.5	43.5	100

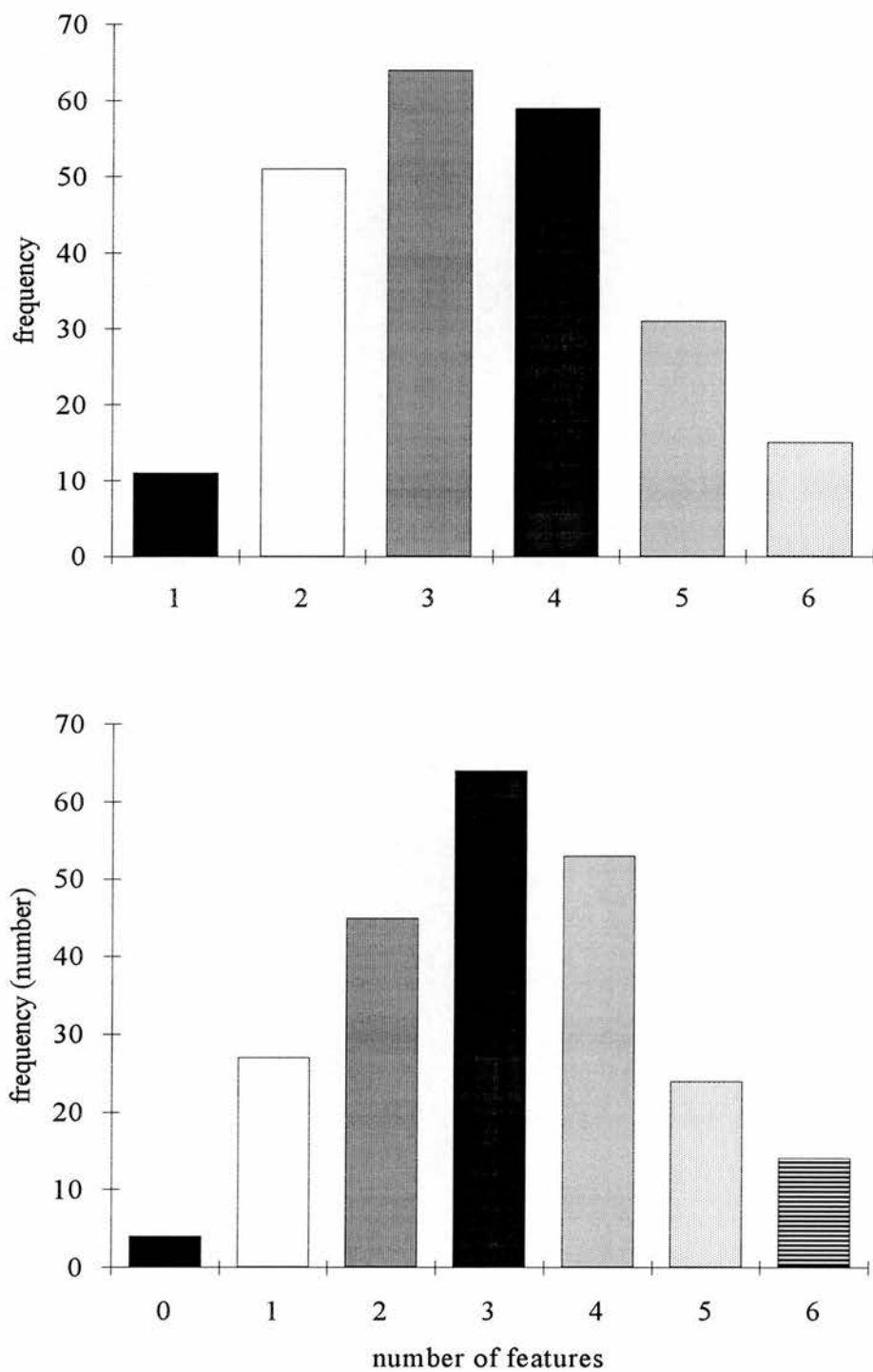


Figure 3.1. Frequency distribution for features of syndrom X: top - including all subjects as glucose intolerant; bottom - glucose intolerant defined to include only subjects with fasting blood glucose ≥ 6.7 mmol/L.

Table 3.5 (a). Frequency (absolute numbers) of features of syndrome X; any combination of features is included here.

		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>total</u>
Caucasian	F	0	7	10	21	7	3	48
	M	9	19	21	19	12	8	88
Asian	F	0	5	6	10	3	3	27
	M	1	11	15	5	5	1	38
Black	F	0	2	5	0	2	0	9
	M	1	7	5	1	0	0	14
Other		0	0	2	3	2	0	7
total		11	51	64	59	31	15	231

Table 3.5 (b). Number of *additional* risk factors occurring in the presence of any individual feature of Syndrome X (glucose intolerance always included).

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
Hypertension	18	34	35	18	15
Hypertriglyceridaemia	0	8	26	23	15
Low HDL-cholesterol	17	30	46	29	15
Hyperinsulinaemia	0	5	15	23	15
Insulin resistance	16	50	55	31	15

Relationships between variables studied

Correlation

Each of the main features of Syndrome X was closely related to number of features of the syndrome; correlation coefficients for number of features against insulin, HOMA-R, triglycerides and HDL-cholesterol all $r > 0.45$, $p < 0.00001$. Relationship with fasting glucose was not significant.

As would be expected, since insulin concentration is a factor in calculating the insulin resistance value, insulin and insulin resistance (HOMA-R) were closely related ($r = 0.85$; $p < 0.00001$), and both also correlated closely with triglycerides ($r > 0.35$; $p < 0.00001$) but not with HDL-cholesterol ($p > 0.1$). Triglyceride and HDL-cholesterol concentrations were inversely related ($r = 0.36$; $p = 0.00001$).

Serum urate concentrations did not correlate to any significant extent with any of the above features, but did correlate with BMI ($r = 0.31$; $p = 0.0003$) and waist circumference ($r = 0.32$; $p = 0.0001$).

The three indices of obesity studied (BMI, waist circumference and waist-hip ratio) all correlated together, the weakest correlation being between BMI and waist-hip ratio ($r = 0.38$; $p < 0.00001$). All three showed a positive correlation with number of features of Syndrome X but only waist circumference was related to all four of the individual features studied (plasma insulin, HOMA-R, serum triglycerides and serum HDL-cholesterol concentration); BMI and waist-hip ratio showed no significant association with either triglycerides or HDL-cholesterol.

Multiple regression analysis

Four models were analysed, each using 'number of features of syndrome X' as the dependent variable. The following twenty factors were included in the main group of

independent or predictor variables: age, sex, smoking, BMI, waist circumference, waist-hip ratio, hypertension, history of coronary heart disease, glucose, (log) insulin, (log) HOMA-R, HbA1c, fructosamine, cholesterol, (log) triglycerides, HDL-cholesterol, LDL-cholesterol, (log) Lp(a), NEFA and urate. The data are summarised in table 3.6.

In the full model, where all variables studied were included, only log HOMA-R, log triglycerides, presence of hypertension, low HDL-cholesterol, *lower* fasting blood glucose, and female sex proved to be important, accounting for 79.25% of the variance in number of features present (table 3.6a). An identical set of independent variables proved predictive when the model was restricted only to the 188 subjects who had not previously been exposed to oral hypoglycaemic drugs, again accounting for 79% of variance, $p < 0.01$ (data not shown).

When HOMA-R was removed, since it is a derived variable, log insulin substituted as the most important predictor, along with log triglycerides, hypertension, low HDL-cholesterol, and female sex. Fasting blood glucose was also included, just reaching statistical significance; here a positive association was seen with higher blood glucose. This suggests that the data from the first model including HOMA-R were distorted by an interaction between glucose and log HOMA-R. This second model again accounted for 79% of overall variance (table 3.6b).

A third model was constructed excluding both HOMA-R and plasma insulin; here BMI substituted in importance for insulin, although ranking third in importance after triglycerides and hypertension. In this model glucose concentration was no longer a significant predictor. This model, however, only explained 69% of total variance (data not shown).

Finally, in an attempt to see whether a model could be constructed which might predict the number of features of Syndrome X without measuring known contributors, and which might suggest additional features which should be included in definitions of the syndrome, all known features were excluded. In this model waist circumference, total cholesterol concentration, low calculated LDL-cholesterol, female sex and lower log Lp(a) were of predictive value but these factors explained only a third of overall variance (table 3.6c). The contradictory trends for total and LDL-cholesterol suggest an interaction as may be expected from the method of calculating LDL-cholesterol values.

Factor analysis

Factor analysis was applied to create two models. The first model included all factors indicated by Reaven to be components of Syndrome X (Reaven, 1988), with the exception of HOMA-R as this is derived directly from insulin and glucose values. This model also included BMI and WHR since these appeared to be important in the findings described above, and their inclusion would also aid comparison with the data in the study of Meigs et al (1997). The model therefore comprised (log) insulin, glucose, (log) triglycerides, HDL-cholesterol, BMI, WHR, and measured systolic and diastolic blood pressures. In order to avoid excessive complexity in the calculations no adjustment was made for use of antihypertensive treatment. The component matrix derived, showing loadings for each factor contributing to the component and the resulting Eigenvalues and contributions to overall variance, is shown in table 3.7a.

A second model was constructed to try and examine the role of all potentially relevant variables and thus included those variables indicated for model 1 above, with the addition of age, sex, HbA1 and (log) HOMA-R; its component matrix is shown in table 3.7b.

Using only those factors with loadings >0.3 , the three component model obtained in Model 1 is described pictorially in figure 3.2. From this, and using the original definition for abnormal values, I have again calculated the frequency of each factor (for components 1 and 2, and also for component 3 but including only those with fasting blood glucose $\geq 6.7\text{mmol/L}$). This is tabulated in table 3.8, and shown graphically for the whole population studied in figure 3.3 (cf. table 3.5 (a) and figure 3.1).

Conclusions

Features of Syndrome X, using these definitions, are present to a varying degree in subjects with newly-diagnosed NIDDM, and the number of features present appears to follow a broadly normal distribution in this population. This finding is somewhat at variance with some published data from non-diabetic populations.

The use of multiple regression techniques and factor analysis offers an additional dimension to the interpretation of these data. The findings from multiple regression analysis show that the known features of the metabolic syndrome are all major contributors to it and appear to contribute independently. The application of factor analysis methods, however suggests that the metabolic syndrome per se may only be an umbrella for a group of more restricted risk factor clusters. This appears to be the case both in this diabetic population as well as in the general population reported by others, and again components in this population appear to be broadly normally distributed.

Table 3.6. Multiple regression models. For all of these female sex was assigned 0 and male 1. Variables included after log transformation are indicated by *.

(a) independent variables predicting 'number of features of syndrome X'

<i>variable</i>	<i>multiple R</i>	R^2	<i>adjusted R²</i>	<i>SE(R)</i>	<i>slope (SE)</i>	<i>T</i>
*HOMA-R	0.658	0.433	0.430	0.947	2.36 (0.19)	12.7†
*Triglycerides	0.770	0.594	0.589	0.804	2.54 (0.26)	9.9†
Hypertension	0.843	0.711	0.706	0.680	0.88 (0.09)	10.3†
HDL-chol.	0.869	0.756	0.751	0.626	-1.16 (0.18)	-6.4†
Glucose	0.885	0.783	0.777	0.592	-0.08 (0.02)	-5.1†
Sex	0.890	0.792	0.786	0.580	-0.27 (0.09)	-3.0#

† $p < 0.0001$; # $p = 0.003$

(b) as for model (a) after exclusion of HOMA-R from the model

<i>variable</i>	<i>multiple R</i>	R^2	<i>adjusted R²</i>	<i>SE(R)</i>	<i>slope (SE)</i>	<i>T</i>
*Insulin	0.654	0.427	0.424	0.952	2.41 (0.19)	12.5†
*Triglycerides	0.797	0.636	0.632	0.760	2.51 (0.26)	9.7†
Hypertension	0.858	0.736	0.732	0.650	0.87 (0.09)	10.1†
HDL-chol.	0.879	0.772	0.767	0.605	-1.17 (0.18)	-6.4†
Sex	0.887	0.786	0.780	0.588	-0.29 (0.09)	-3.2#
Glucose	0.889	0.791	0.784	0.583	0.03 (0.02)	2.0§

† $p < 0.0001$; # $p = 0.002$; § $p = 0.047$

(c) model for predictors of number of features, after excluding known features

<i>variable</i>	<i>multiple R</i>	R^2	<i>adjusted R²</i>	<i>SE(R)</i>	<i>slope (SE)</i>	<i>T</i>
Waist	0.410	0.168	0.164	1.15	0.03 (0.006)	6.1†
Cholesterol	0.470	0.221	0.213	1.11	0.95 (0.21)	4.5†
LDL-chol.	0.540	0.291	0.280	1.06	-0.91 (0.25)	-3.7§
Sex	0.567	0.321	0.307	1.04	-0.47 (0.15)	-3.1#
*Lp(a)	0.581	0.337	0.320	1.03	-0.39 (0.18)	-2.1

† $p < 0.0001$; § $p = 0.0003$; # $p = 0.003$; || $p = 0.03$

Table 3.7. Factor analysis (factor loadings >0.3 highlighted in bold)

3.7(a). Component matrix for Model 1 showing factor loadings for main variables.

Variable	Component 1	Component 2	Component 3
*Insulin	0.74	0.05	-0.25
Glucose	0.12	-0.13	0.89
*Triglycerides	0.66	0.08	0.41
HDL-cholesterol	-0.56	0.39	0.02
BMI	0.67	0.31	0.01
WHR	0.65	0.13	-0.28
Diastolic BP	0.08	0.90	0.08
Systolic BP	-0.13	0.88	0.15
Eigenvalue	2.21	1.88	1.13
Contribution to variance	27.7	23.5	14.1

Table 3.7(b). Component matrix for Model 2 showing factor loadings for all major variables of interest.

Variable	Component 1	Component 2	Component 3	Component 4
*Insulin	0.78	0.26	-0.28	-0.33
Glucose	0.37	-0.17	0.77	0.23
*Triglycerides	0.63	0.11	0.14	0.19
HDL-cholesterol	-0.49	0.22	0.23	-0.35
BMI	0.55	0.44	0.10	-0.11
WHR	0.49	0.21	-0.41	0.41
Diastolic BP	-0.07	0.81	0.06	0.17
Systolic BP	-0.31	0.85	0.20	0.14
Age	-0.40	0.53	0.22	0.11
Sex	-0.08	-0.13	-0.44	0.75
HbA1	0.25	-0.18	0.77	0.31
*HOMA-R	0.89	0.15	0.15	-0.19
Eigenvalue	3.04	2.13	1.81	1.26
Contribution to variance	25.3	17.7	15.1	10.5

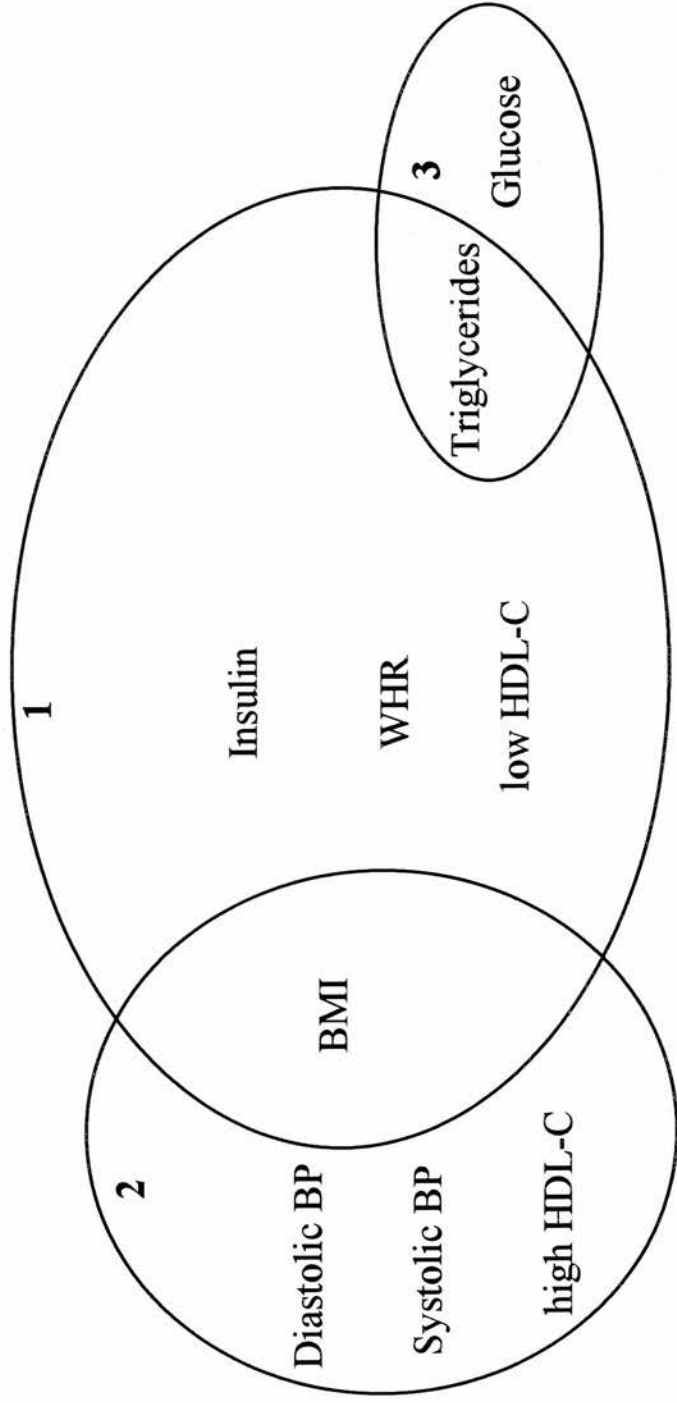


Figure 3.2. Pictorial representation of the main factors contributing to the three component model obtained from factor analysis.

Table 3.8. Frequency data for number of variables in each component

3.8 (a). Component 1 (insulin, triglycerides, low HDL-C, BMI, WHR)

		<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>total</u>
Caucasian	F	3	10	12	15	4	4	48
	M	19	19	13	15	12	10	88
Asian	F	0	0	11	8	6	2	27
	M	9	11	10	3	5	0	38
Black	F	1	2	4	1	1	0	9
	M	3	5	5	0	1	0	14
Other		0	0	3	1	3	0	7
total		35	47	58	43	32	16	231

3.8 (b). Component 2 (hypertension, BMI, high HDL-C)

		<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>total</u>
Caucasian	F	8	19	18	3	48
	M	25	34	25	4	88
Asian	F	9	9	7	2	27
	M	16	18	4	0	38
Black	F	1	3	2	3	9
	M	3	3	6	2	14
Other		2	2	3	0	7
total		64	88	65	14	231

3.8 (c). Component 3 (glucose, triglycerides). Here only fasting blood glucose ≥ 6.7 mmol/L has been included in the 'hyperglycaemic' category.

		<u>0</u>	<u>1</u>	<u>2</u>	<u>total</u>
Caucasian	F	7	30	11	48
	M	19	41	28	88
Asian	F	2	19	6	27
	M	6	22	10	38
Black	F	3	6	0	9
	M	7	6	1	14
Other		2	3	2	7
total		46	127	58	231

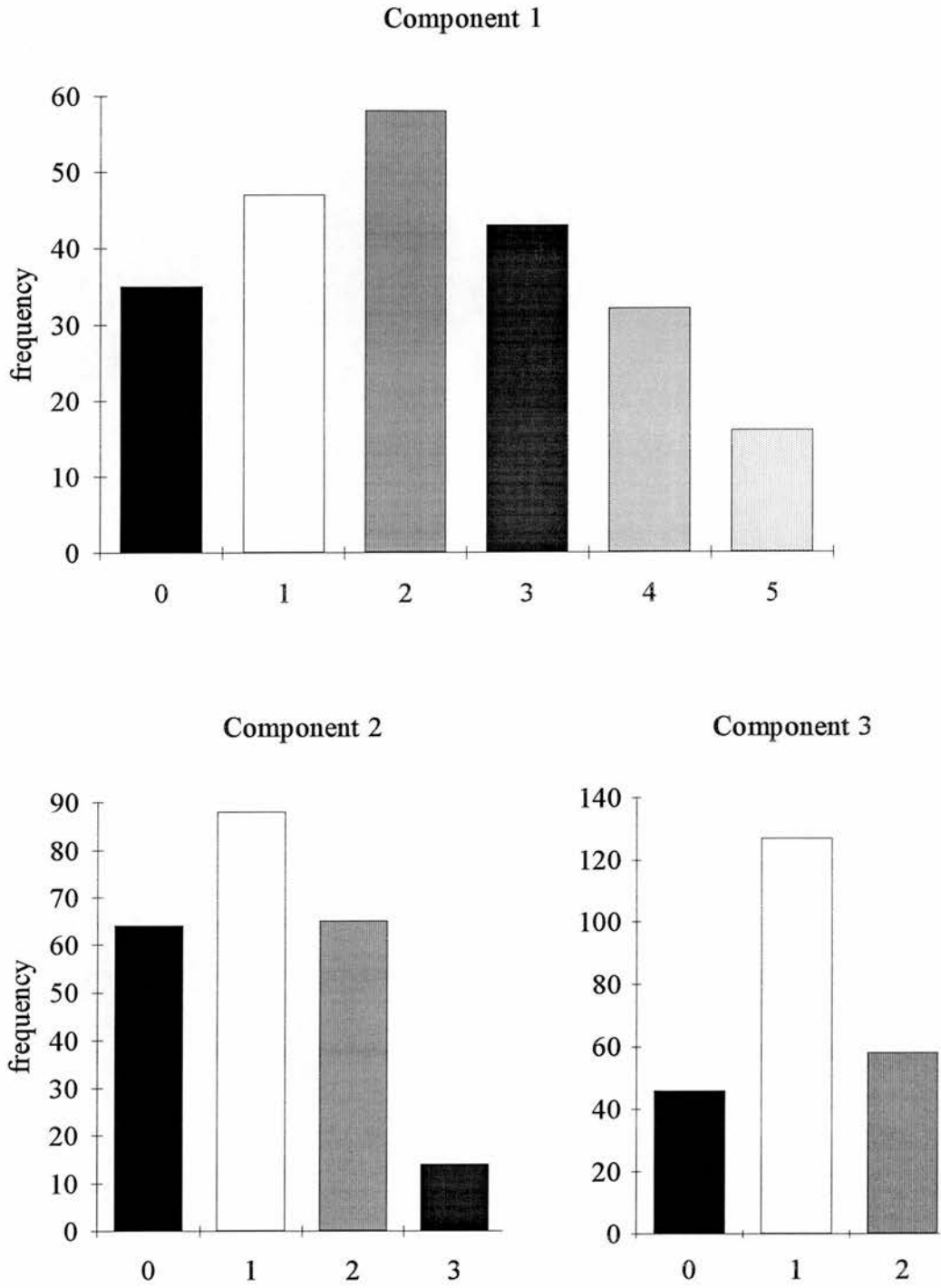


Figure 3.3. Frequency distributions for factors contributing to each component

CHAPTER 4

Effects of the new oral hypoglycaemic agent A4166 on
secretion and metabolic actions of insulin in NIDDM

Introduction

A number of oral hypoglycaemic agents are currently under development for use in the treatment of type II (non-insulin-dependent) diabetes mellitus. Among these are several structural relatives of meglitinide, the non-sulphonylurea moiety of glibenclamide (Malaisse, 1995a). In common with sulphonylureas this group of agents is thought to act via closure of ATP-sensitive K^+ channels (Malaisse, 1995b). The new phenylalanine derivative A4166 (N-[(trans-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine), which belongs to this group, has been shown in animal studies to improve glucose tolerance and to enhance insulin secretion (Sato et al, 1991), apparently without any adverse effect on islet protein synthesis (Viñambres et al, 1995). A4166 has been shown to have similar effects in healthy human volunteers (Sandoz Pharmaceuticals Corporation, data on file) although its mechanism of action has not yet been elucidated.

This study was designed to examine the effects of A4166 on endogenous insulin secretion and intermediary metabolite concentrations in subjects with NIDDM during an intravenous glucose tolerance test, and to assess whether this effect was sustained after a period of intensive dietary treatment of diabetes, by means of a randomised double-blind placebo-controlled trial.

Subjects and Procedures

Ten male caucasian subjects with non-insulin dependent diabetes were recruited to the study. All subjects gave signed informed consent after approval had been received from the Research Ethical Committee of the South Birmingham Health Authority.

Table 4.1. Subject characteristics

	<i>before diet</i>	<i>after diet</i>
	<u>Mean (range)</u>	<u>Mean (range)</u>
Age (years)	53 (41 - 65)	
Duration (months)	0 - 44 (7 x 0-1 month; 3 x 20-44 months)	
BMI (kg/m ²)	27.54 (23.91 - 32.82)	27.39 (24.44 - 32.95)
Fasting blood glucose (mmol/l)	9.1 (6.7 - 13.5)	8.3 (6.9 - 11.8)
HbA1 (%)	10.2 (5.6 - 15.2)	9.8 (7.8 - 13.2)
Fasting serum triglycerides (mmol/L)*	1.67 (0.97 - 3.31)	1.68 (1.02 - 3.95)
Fasting plasma insulin (pmol/L)*	76.7 (44 - 245)	79.7 (40 - 219)
Basal C-peptide (pmol/L)*	744 (463 - 1059)	574 (298 - 1258)
Basal proinsulin (pmol/L)*	18.5 (3.5 - 44.1)	18.6 (3.7 - 69.2)

*geometric mean

Subjects considered eligible for entry into the study were male caucasians aged between 40 and 65 years with non-insulin-dependent diabetes treated with diet alone and of duration less than four years. Where possible subjects with newly-diagnosed diabetes were preferred in order to maximise any differences in effects of the study drug between the pre-diet and post-diet assessments. Subjects were required to be moderately overweight (BMI 25-32 approx.) and to have fasting blood glucose at entry of approx. 7-12 mmol/L (i.e. clearly hyperglycaemic but not anticipated to require additional treatment with oral agents during the period of the study). Routine biochemical and haematological measures for all subjects were within normal limits both at entry and at the time of the first post-diet assessment. No subject was taking any regular prescribed medication, none had any complications of diabetes, nor any current or past medical conditions of any significance.

Two subjects (no.4 and no.11) withdrew from the study. Subject 4 withdrew at his request before undergoing any assessment; subject 11 was considered ineligible after undergoing one IVGTT because of high fasting blood glucose concentration. Although all data shown here relates only to the ten subjects who completed the study, original subject numbers have been retained (figs. 4.7 and 4.8).

All subjects received a detailed dietary assessment and advice by the same dietitian immediately after the first pair of assessments; this was repeated four weeks later in an effort to achieve optimal diabetic control.

Each subject initially underwent two intravenous glucose tolerance tests (IVGTT) separated by 2-7 days. Twenty minutes prior to injection of glucose subjects received in random order either 60mg A4166 or matching placebo, taken orally. After an eight week period (± 2 weeks) of intensive dietary management of diabetes, including two assessments by a hospital dietitian, each pair of IVGTTs was repeated, again preceded

by either 60mg A4166 or placebo in random order. The IVGTT procedures were carried out according to the protocol described in chapter 2.

Venous blood samples were taken as previously described and prepared for subsequent analysis of total insulin, proinsulin and C-peptide concentrations, and glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glycerol and NEFA.

Statistical analysis

Data were analysed in three different ways. Overall effects on metabolite and hormone concentrations were analysed by two-way analysis of variance (2-way ANOVA) with four groups - (1) IVGTT with A4166 prior to diet treatment, (2) IVGTT with placebo before diet, (3) IVGTT with A4166 following diet treatment, and (4) IVGTT with placebo post-diet treatment. Differences between groups were then assessed for each metabolite. In view of the large number of potential comparisons to be made Scheffé's test was used to reduce the likelihood of significant differences arising by chance. In table 4.2 standard error of the mean (SEM) for each metabolite is shown; SEM for all groups is the same when this calculation is based on the within-groups mean square, with variances for all groups assumed to be equal and the number of samples in each group also equal.

First phase insulin secretion was calculated from areas under the insulin concentration curves over the first 10 minutes from onset of glucose injection in each of the four groups indicated above. Student's t-test was used to compare the effect of A4166 vs placebo.

Glucose disappearance (k_g) was calculated from log glucose concentrations from 20-60 minutes after onset of glucose injection using the equation $k_g = 0.693/t_{1/2}$ where

t is the calculated time required for blood glucose concentration to return to that at baseline, and 0.693 is a constant ($\log_e 2$) (Amatuzio et al, 1953; Marks and Marrack, 1962). The value of k_g is then expressed as percent per minute. Student's t-test was used to compare the effect of A4166 vs placebo.

Results

General

During the study period no significant changes (by paired Student's t-test) were observed in subjects' weight, fasting blood glucose concentrations, or overall diabetic control measured by HbA1 concentration (table 4.1). Mean fasting metabolite and hormone concentrations for each visit are shown in table 4.2.

Effects of A4166 and diet on overall metabolite and hormone concentrations

Data are summarised in table 4.3, with mean values and SEM for overall concentrations of each metabolite shown. There were no significant differences between visits for fasting concentrations of any of the hormones or metabolites, measured by one way ANOVA carried out separately from that used to examine overall changes. Despite the apparent fall in mean fasting C-peptide concentrations (shown in table 4.2) this change did not reach statistical significance ($p=0.20$).

Glucose

Trends in mean glucose concentrations for each group are shown in figure 4.1. Although this is suggestive of a lower blood glucose concentration at each time point with A4166 than with placebo, overall this trend did not reach statistical significance

(for A4166 vs placebo glucose concentrations before diet $p=0.11$, after diet $p=0.08$). There was no significant change in overall glucose concentration after the period of dietary treatment compared with before diet.

Insulin

In each pair of studies comparing A4166 against placebo, overall insulin concentration was significantly increased by A4166 (before diet $p=0.0001$; after diet $p=0.0005$) (figure 4.1). No effect of the period of diet was observed on overall insulin concentration.

C-peptide

C-peptide concentration (figure 4.2) was increased significantly with A4166 on both occasions (before diet $p=0.0001$, after diet $p=0.0001$). C-peptide concentrations, both stimulated (by A4166) and unstimulated (after placebo) showed significant decline ($p<0.001$) after dietary treatment. Although fasting values were lower after diet these differences were not statistically significant ($p=0.08$).

Proinsulin

Proinsulin concentrations were increased by A4166 before dietary treatment but this difference was halved and no longer statistically significant after diet (before diet $p=0.01$, after diet $p=0.12$). Diet itself had no effect on overall concentrations (figure 4.2).

Table 4.2. Mean fasting values for analytes at each visit (mean of two samples prior to taking A4166 or placebo) with interindividual ranges shown below.

	<i>before diet</i>		<i>after diet</i>	
	<u>placebo</u>	<u>A4166</u>	<u>placebo</u>	<u>A4166</u>
Glucose (mmol/L)	8.7	8.1	8.4	8.2
	6.0-13.6	6.6-12.5	6.7-11.6	6.7-12.0
Insulin (pmol/L)*	75.5	77.9	82.7	76.8
	44-221	46-238	40-194	48-201
C-peptide (pmol/L)*	729.7	757.7	598.4	551.8
	496-1010	579-976	314-1150	305-1192
Proinsulin (pmol/L)*	18.1	18.9	19.7	17.6
	3.9-42.7	4.1-37.8	6.2-68.5	3.9-45.0
Lactate (μ mol/L)	718.6	802.4	918.2	857.0
	336-986	533-1119	550-1841	570-1408
Pyruvate (μ mol/L)	85.0	86.4	95.1	99.1
	42-212	40-188	44-126	59-153
NEFA (mmol/L)	0.713	0.645	0.575	0.618
	0.20-1.15	0.36-1.02	0.09-1.11	0.19-1.11
Glycerol (μ mol/L)	51.0	46.3	49.7	56.8
	22-82	22-90	19-82	23-106
Ketones (μ mol/L)*	170.6	148.4	118.8	149.8
	56-464	65-426	44-328	62-408

*geometric mean

Table 4.3. Overall mean values of analytes

	<i>before diet</i>		<i>after diet</i>		<u>SEM</u>
	<u>placebo</u>	<u>A4166</u>	<u>placebo</u>	<u>A4166</u>	
Glucose (mmol/L)	12.96	12.26	12.96	12.15	0.25
Insulin (pmol/L)*	91.8	149.9	99.2	150.7	1.05
C-peptide (pmol/L)*	827.2	1128.7	642.1	870.5	1.03
Proinsulin (pmol/L)*	19.7	27.2	21.1	25.2	1.07
Lactate (μ mol/L)	784.0	894.3	935.8	958.8	29.66
Pyruvate (μ mol/L)	75.8	76.0	94.1	108.9	3.43
NEFA (mmol/L)	0.62	0.48	0.46	0.43	0.02
Glycerol (μ mol/L)	44.8	34.7	42.0	37.4	1.42
Ketones (μ mol/L)*	130.0	96.9	96.5	95.3	1.06

* geometric mean

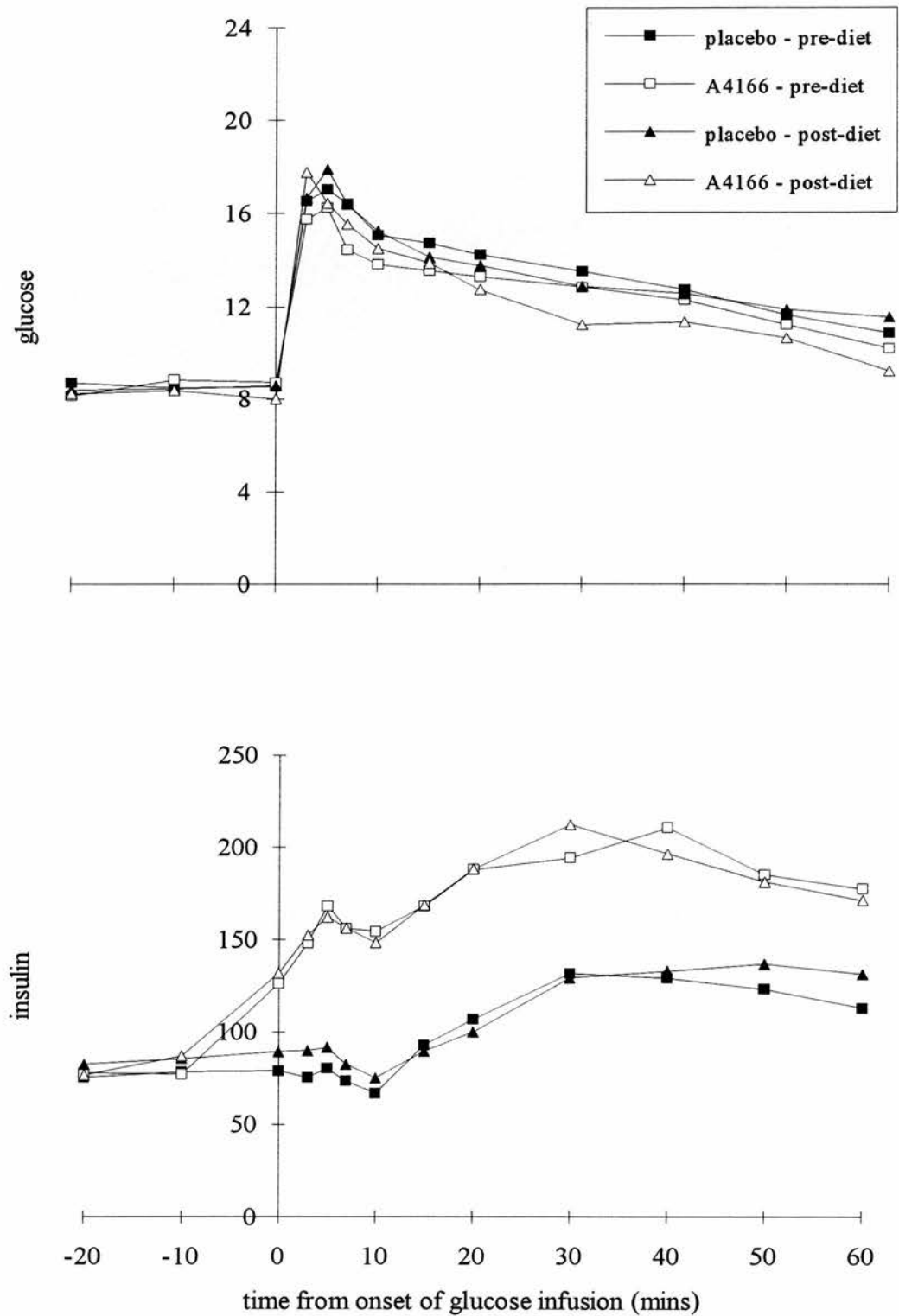


Figure 4.1. Mean blood glucose (mmol/L, top) and plasma insulin (pmol/l, bottom; geometric mean) concentrations achieved during IVGTT.

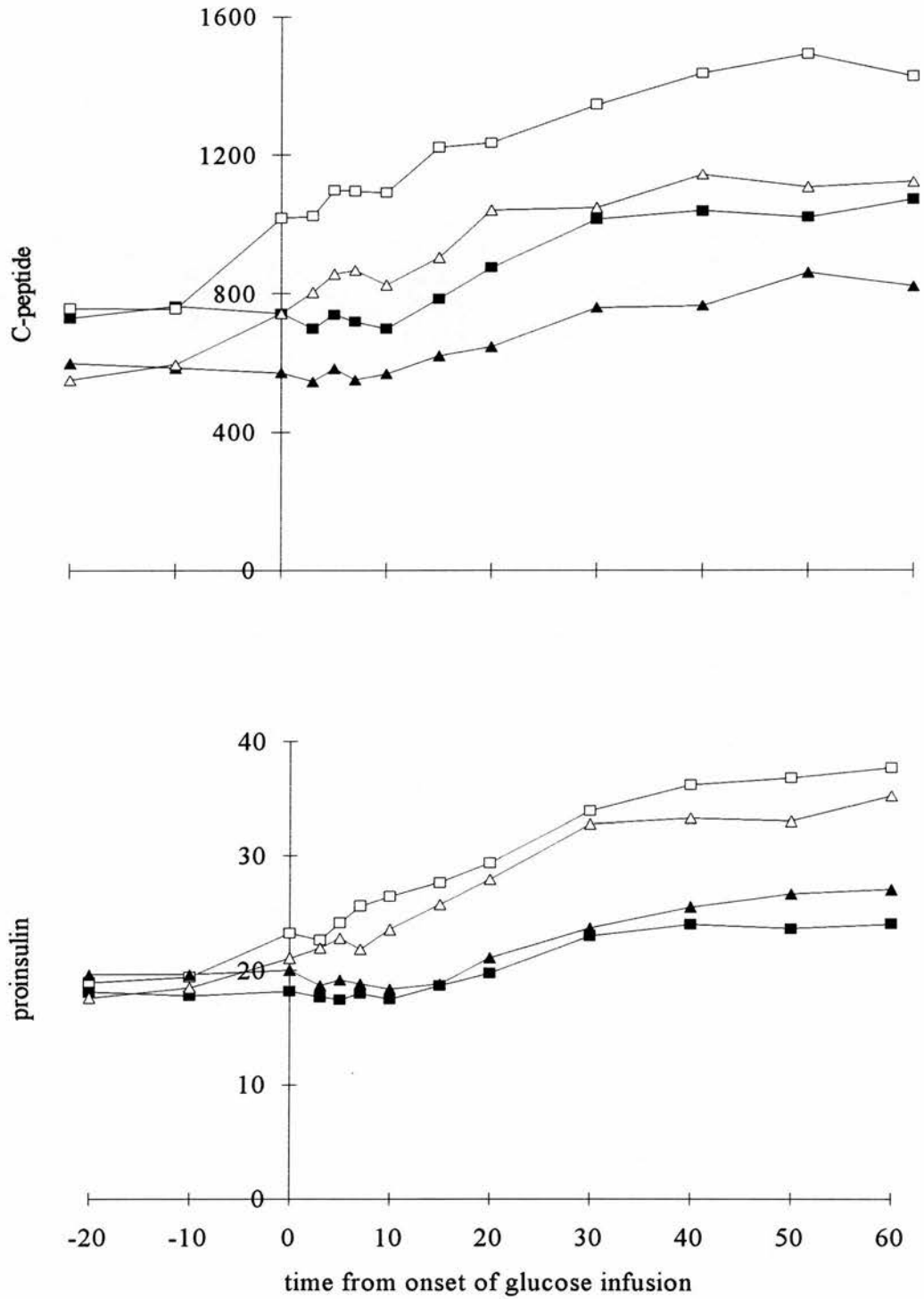


Figure 4.2. Geometric mean plasma C-peptide (pmol/L, top) and proinsulin (pmol/L, bottom) concentrations reached during IVGTT. (Legends for figs. 4.2-4.5 as shown in fig. 4.1.)

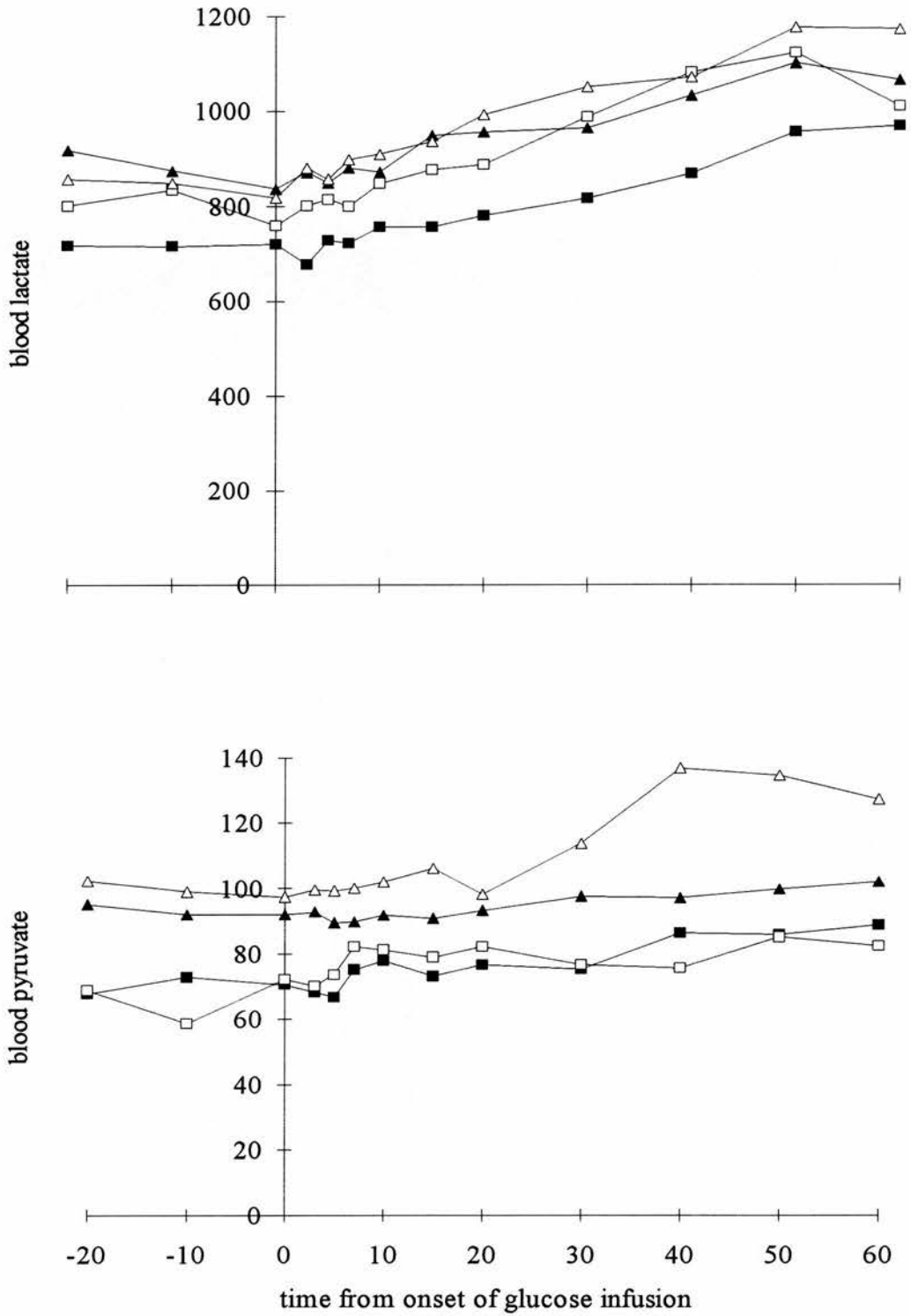


Figure 4.3. Mean blood lactate ($\mu\text{mol/L}$, top) and pyruvate ($\mu\text{mol/L}$, bottom) concentrations reached during IVGTT.

Lactate

Lactate concentrations were elevated after A4166 in comparison with placebo prior to dietary treatment ($p=0.05$). Diet itself had no consistent effect on lactate concentrations and the effect of A4166 was lost after the diet period ($p>0.1$) (figure 4.3).

Pyruvate

Pyruvate concentrations were not significantly influenced by A4166 in comparison with placebo at initial assessment ($p>0.1$). The effects of diet itself were prominent with a significant rise in pyruvate concentrations post-diet compared with pre-diet (comparing placebo vs. placebo $p=0.01$, and active vs. active $p=0.0002$). At the end of the diet period pyruvate concentrations were significantly higher with A4166 than with placebo ($p=0.02$) (figure 4.3).

NEFA

NEFA concentrations were significantly reduced by A4166 before diet ($p=0.002$). No consistent effects of diet were observed. After the period of dietary treatment this effect of A4166 was no longer apparent ($p>0.1$) (figure 4.4).

Glycerol

As with NEFA mean glycerol concentrations fell with A4166 before dietary treatment ($p=0.002$); diet itself caused no change but any effect of A4166 was no longer statistically significant at the end of the study ($p=0.08$) (figure 4.4).

Total ketone bodies

A4166 significantly reduced ketone body levels prior to diet treatment ($p=0.02$). No consistent trend was observed for changes as a result of dietary treatment. Again, at the end of the period of diet the initial effect of A4166 was no longer present ($p>0.1$) (figure 4.5).

Effects on first phase insulin secretion

First phase insulin response was restored on both occasions with significant differences regardless of whether area under the curve (AUC) was calculated for total insulin, incremental insulin, or incremental insulin/glucose during the first 10 minutes from onset of glucose administration (Koschmann et al, 1992). Comparisons of total 1st phase insulin secretion are illustrated in figure 4.6; before diet mean insulin AUC (\pm SEM) for A4166 was 1711 (230) pmol/L/10 minutes compared with 826 (135) pmol/L/10 mins for placebo ($p=0.002$); after diet 1993 (509) vs 958 (153) pmol/L/10 mins ($p=0.025$).

Effects on glucose clearance

Comparison of k_g values between A4166 and placebo showed no difference before diet (mean k_g (\pm SEM) = $1.49 \text{ \%/min} \pm 0.18$ vs. 1.49 ± 0.16 ; $p=0.98$) whereas after diet A4166 resulted in a significantly higher mean k_g than did placebo ($1.98 \text{ \%/min} \pm 0.32$ vs. 1.07 ± 0.10 ; $p=0.025$). Comparisons of differences in k_g for placebo before and after diet showed a trend towards a deterioration in glucose clearance after diet of borderline significance ($p=0.052$), while the apparently greater glucose clearance rate seen with A4166 after diet was not significantly different from k_g with A4166 before diet ($p=0.28$) (figure 4.7).

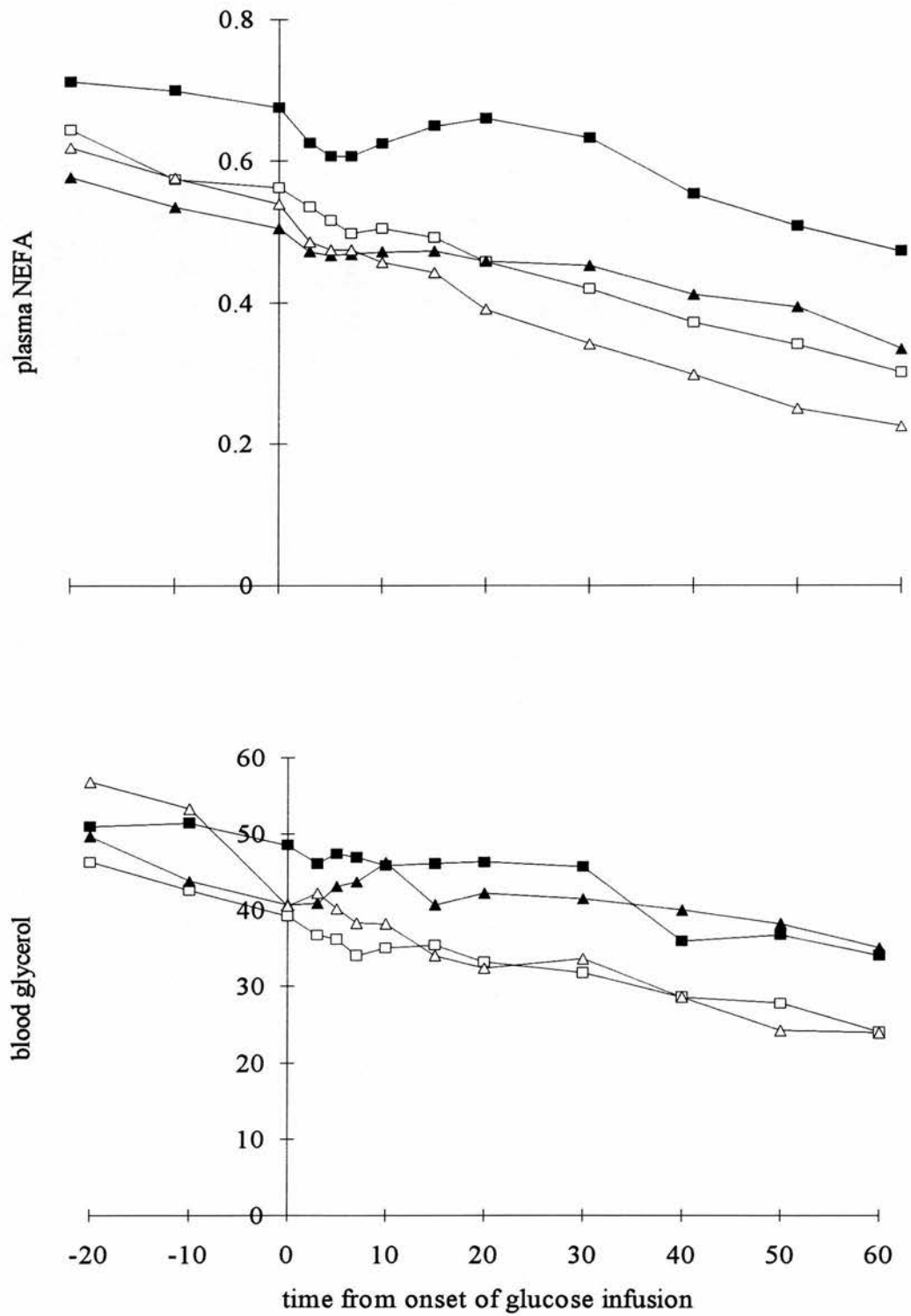


Figure 4.4. Mean plasma NEFA (mmol/L, top) and blood glycerol ($\mu\text{mol/L}$, bottom) concentrations reached during IVGTT.

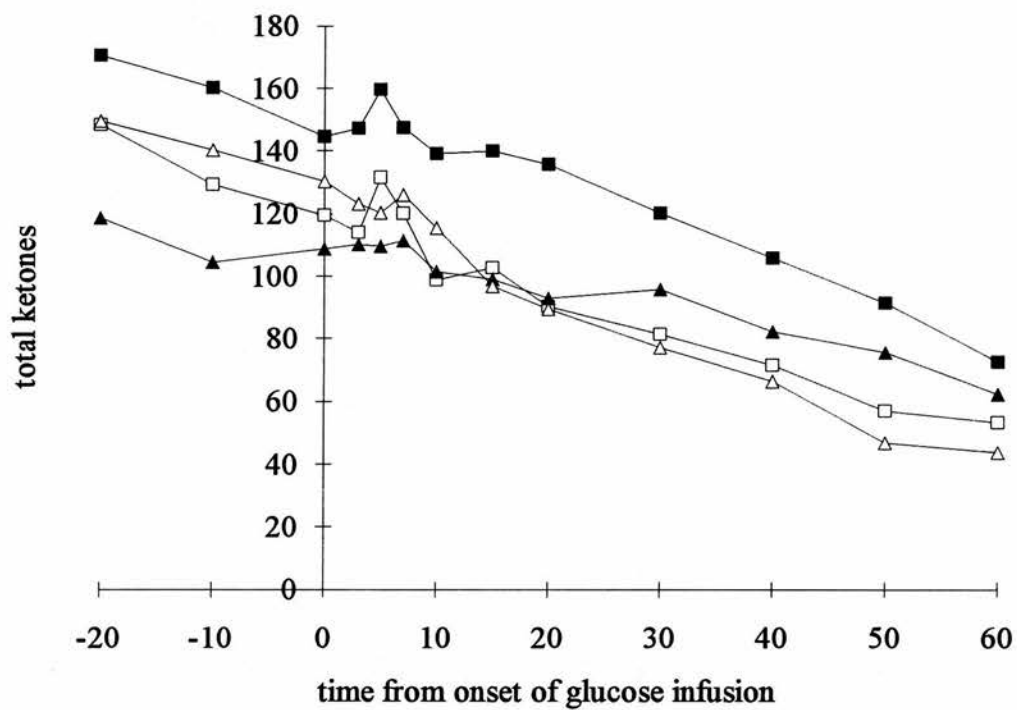


Figure 4.5. Geometric mean total ketone body ($\mu\text{mol/L}$) concentrations reached during IVGTT.

Plasma concentrations of A4166

Mean plasma concentrations of A4166 peaked 27-30 minutes after ingestion (i.e. 7-10 minutes after onset of the glucose infusion) at approx. 2.5µg/ml (range 0.16-6.63) with measurable concentrations of drug detectable within 10 minutes of ingestion, before any glucose had been given.

Considerable interindividual variation was observed in concentrations of A4166 achieved (figure 4.8). Prior to diet subjects 2 and 7 achieved peak plasma concentrations of A4166 of <1µg/ml and after diet subjects 1, 2 and 6 similarly failed to reach plasma concentrations of 1µg/ml. While subject 2 can therefore be regarded as a persistent non-responder, an explanation for the variation in absorption of drug by the other subjects is not clear.

Conclusions

The acute administration of 60mg A4166 results in a partial restoration of the first phase insulin response to intravenous glucose, and a significant increase in overall plasma insulin and C-peptide concentrations. Although no statistically significant reduction in blood glucose concentrations was observed there was a trend in this direction, and after diet glucose uptake was significantly increased in the presence of A4166. However blood glucose-lowering effect was not a primary objective of this study which may not have had adequate power to show a significant effect. Individual responses were related to plasma concentrations of A4166 achieved; failure to respond to the drug appears to be related to inadequate absorption but underlying causes remain unexplained.

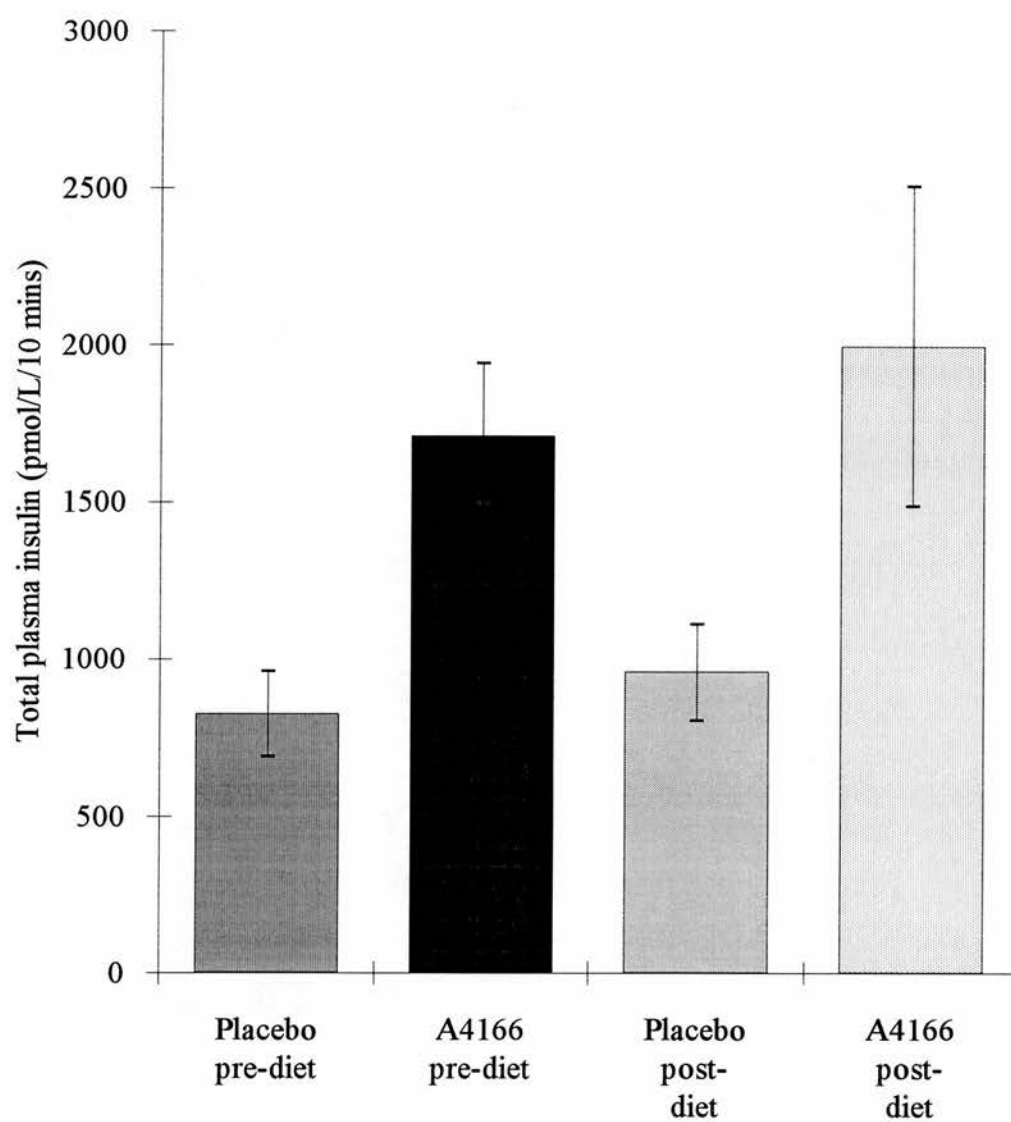


Figure 4.6. Mean (\pm SEM) total first phase insulin AUC.

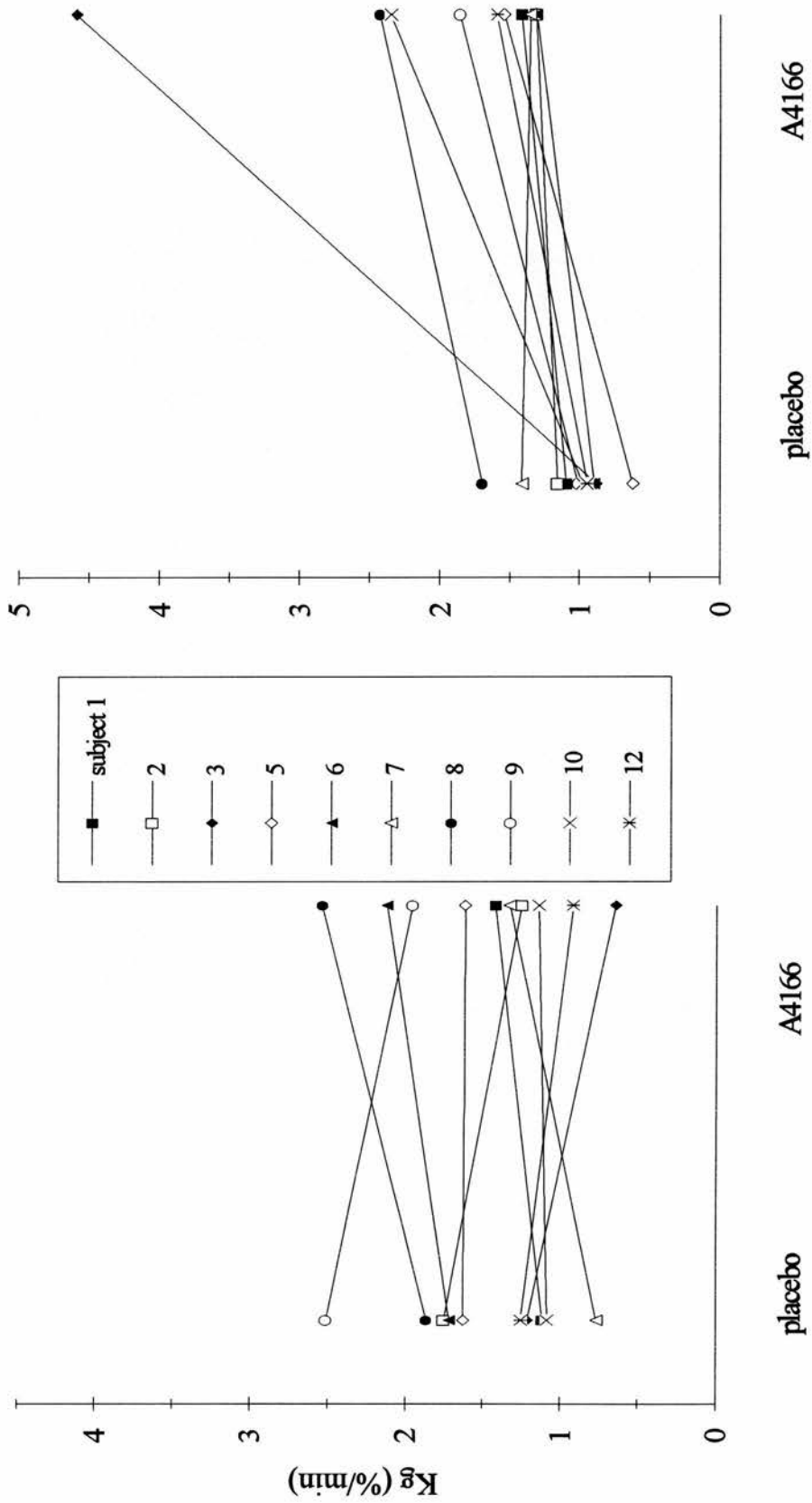


Figure 4.7. Glucose clearance (K_g) for placebo vs. A4166 before diet (left) and after diet (right).

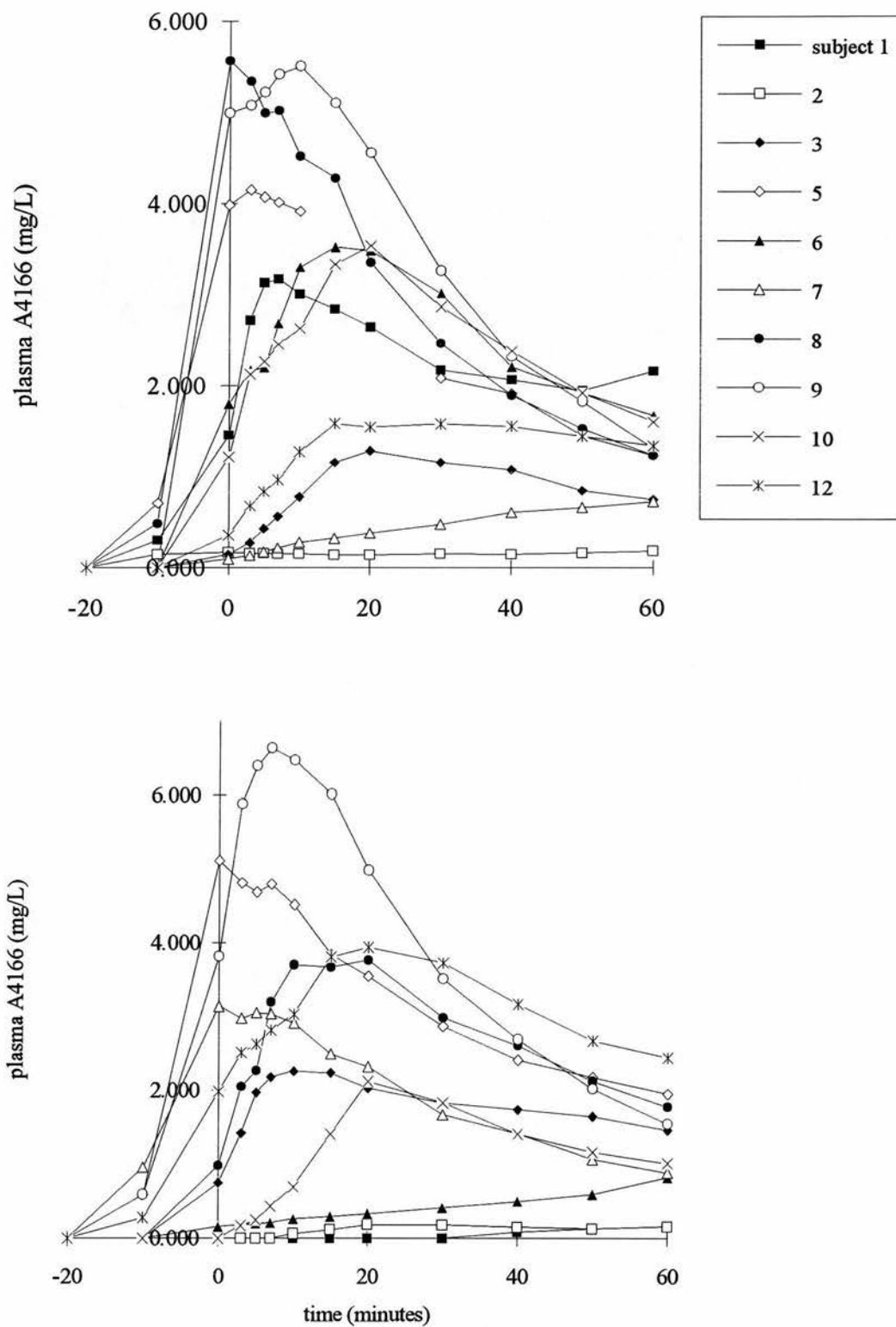


Figure 4.8. Individual A4166 concentrations reached before diet (top) and after diet (bottom).

CHAPTER 5

Effects of gemfibrozil on insulin resistance in subjects with
NIDDM and hypertriglyceridaemia

Introduction

Hypertriglyceridaemia is a common feature in non-insulin-dependent diabetes. Although its role as an independent risk factor for coronary heart disease remains contentious, it is known to be associated with other lipid abnormalities predisposing to coronary heart disease and is also included in proposed clusters of coronary risk factors (Reaven, 1988).

Hypertriglyceridaemia has notably been included in descriptions of a metabolic syndrome of coronary risk, one of whose core features is insulin resistance. Efforts to treat risk factors for CHD are often hampered by diverging effects on different risk factors. If risk factor intervention is to be truly effective it should have beneficial or at worst neutral effects on other risk factors.

Gemfibrozil (Lopid[®], Parke Davis Pharmaceuticals Ltd, Eastleigh, UK) is a lipid-lowering agent of proven effectiveness of the fibrate class of drug. It has both cholesterol- and triglyceride-lowering properties, and has been shown to be safe and effective in diabetic populations. Although for many years the mode of action of this class of agent has remained unclear, recent evidence points to an effect on the peroxisome proliferator activated receptors (PPAR) of the α subtype found in liver (Staels et al, 1997). Stimulation at PPAR α sites results in a number of effects limiting fatty acid synthesis and reducing triglyceride concentrations.

In a double-blind placebo-controlled study, with insulin resistance assessed by low-dose incremental insulin infusion, I set out to examine whether Gemfibrozil had any effect on insulin resistance, studying several aspects of intermediary metabolism in subjects with non-insulin-dependent diabetes and hypertriglyceridaemia. Following the observations by Steiner (1992) I also hoped to examine whether the degree of

hypertriglyceridaemia (or the magnitude of its reduction) was related to any change in insulin resistance.

Subjects and Methods

All subjects were recruited from the Diabetic Clinic of the General Hospital, Birmingham between June 1994 and January 1995. Ethical approval was granted by the local Research Ethics Committee; all subjects gave written informed consent and received a detailed written and verbal explanation of the study.

The study was designed in collaboration with Parke Davis Pharmaceuticals Ltd (Eastleigh, Hants, UK) who provided financial support and supplied both Gemfibrozil and matching placebo.

Entry Criteria

Adult subjects (aged 18-70) with NIDDM of duration in excess of two years, currently treated with diet alone or oral agents in stable dosage, were considered eligible for inclusion if they fulfilled the following additional criteria: BMI <33; stable diabetic control not expected to need insulin during the 6 month study period; urine albustix negative; no significant haematological abnormality on baseline testing; no evidence of significant hepatic or renal impairment on blood testing; no evidence of other causes of secondary hyperlipidaemia including normal thyroid function; no known pre-existing (or current treatment for) coronary heart disease; no other serious illness; not on any other drugs known to affect lipid metabolism (although antihypertensive medication was permitted so long as treatment and condition were regarded as stable).

Subjects were identified as possible candidates for inclusion on the basis of non-fasting lipid data available in the clinic, when results suggested that fasting cholesterol and triglyceride levels might fall within the required study range of total cholesterol 5.2 -10mmol/L and triglycerides 2.3 - 5.6mmol/L. Subjects included whose lipid values were outwith this range at visit 2 were ineligible to enter the treatment phase and were withdrawn at this point. This criterion accounted for the majority of withdrawals.

Protocol

The study protocol provided for the inclusion of 56 subjects, to be randomised to either active treatment with gemfibrozil or placebo. No formal power calculations were carried out, although data from previous studies using this technique to assess insulin resistance suggested that small numbers of subjects (6-12) should be adequate to find clear differences between two groups where such differences exist. There are few comparable data examining effects of gemfibrozil or other fibrates on insulin resistance. (The protocol for this study was written before publication of the findings of Vuorinen-Markkola et al (1993).) A larger cohort was provided for in the hope that it might be possible to stratify effects on insulin resistance by the magnitude of the fall in triglyceride concentration achieved, and clearly the subgroups derived would need to be large enough themselves to demonstrate any differences. (In practice the limited numbers proceeding to treatment precluded any attempt at this analysis.)

Each subject was required to attend on seven occasions over a six month period. Visit 1 was a recruitment and initial assessment visit, at which baseline height, weight, blood pressure and details of age, sex, duration of diabetes, and details of medication and other medical conditions were recorded. Fasting blood samples were taken for

glucose, HbA1, fructosamine, total and HDL-cholesterol, triglycerides, urate, thyroid function, biochemical screen (electrolytes, liver enzymes including γ -glutamyltranspeptidase) and full blood count. Subjects not excluded at this visit were given a dietary assessment and advice by a dietitian.

At visit 2, eight weeks after visit 1, weight and blood pressure were again measured, and blood taken for glucose, urate, fructosamine, lipid parameters and general biochemistry.

On the basis of the results from visit 2 subjects were assessed for eligibility to continue into the treatment phase of the study at visit 3 (two weeks after visit 2). Those continuing at this stage had the same clinical and laboratory assessments as at visit 2, with full blood count (safety measure) and HbA1 in addition. Subjects then underwent a low-dose incremental insulin infusion (described in Chapter 2). A dietary review was undertaken at this point, and supplies of medication were given out.

Visits 4 and 5 followed at 4-weekly intervals. At each of these visits treatment compliance and adverse effects of medication were checked. Weight and blood pressure were measured, along with fasting glucose and lipids, fructosamine, urate and a biochemical screen.

At visit 6, a further 4 weeks later, a second low-dose insulin infusion was performed, using the same protocol as for the first. Assessments as for visits 4 and 5 were also carried out, and subjects underwent a dietary review. All supplied tablets were returned, this visit being the end of the treatment phase.

Visit 7, the final visit, followed about 4 weeks later, when a full symptomatic and physical review was carried out; fasting blood samples were again taken. Subjects were discharged back to Diabetic clinic follow-up.

Conduct of the Study

Forty-seven potentially suitable candidates consented to take part. Thirty-three of these were withdrawn from the study prior to randomisation, i.e. before visit 3. Reasons for withdrawal were as follows: ineligible by lipid criteria (23, total cholesterol and/or fasting triglycerides below required intervention level of 5.2 and 2.3 mmol/L respectively), dipstick proteinuria (1), subject request or default (4), pre-existing coronary heart disease (2), abnormal baseline biochemistry (1, abnormal liver enzymes), body mass index outwith defined limits for study (1) and intercurrent illness making insulin infusion inadvisable (1).

Fourteen subjects proceeded to the treatment phase of the study. Two subjects were withdrawn during the treatment phase: subject 8 developed symptoms suggestive of new angina (visit 4), and subject 10 requested withdrawal having been unable to comply with the treatment schedule (visit 5).

All subjects in the treatment phase were supplied with either Gemfibrozil 600mg tablets or matching placebo to be taken as one tablet twice daily. Drugs were dispensed at visits 3, 4 and 5; surplus tablets from previous visits were returned at visits 4, 5 and 6. All other regular medications were continued unchanged.

Laboratory Analyses

Biochemical and haematological screening samples were analysed as part of routine laboratory testing on the same or next day. All lipid analyses from each visit were carried out using an identical method. Samples from the low-dose insulin infusions were stored at -20°C for later measurement of glucose, insulin, lactate, pyruvate, alanine, 3-hydroxybutyrate, acetoacetate, glycerol and NEFA. Details of laboratory methods for these analyses are described in Chapter 2.

Statistical Analyses

Comparison of changes over time in serum triglyceride (log transformed), total and HDL-cholesterol and fructosamine concentrations, and in weight, were carried out by one-way analysis of variance; treatment and placebo groups were analysed separately. Comparison between groups of mean fasting metabolite concentrations was also by one way ANOVA; here gemfibrozil and placebo groups (before and after treatment) were analysed together.

Regression slopes were calculated from the metabolite data (against log [mean insulin]) for each group, and analysis of variance applied to the regression lines obtained. In view of the large number of potential comparisons, Scheffe's test was applied when comparing differences between groups. Differences have been expressed as separations between parallel slopes at a common insulin concentration (i.e. differences between corrected analyte means). Missing data were treated in as biologically plausible a way as possible; values were taken as the mean of the immediately preceding and succeeding values for the individual subject concerned.

For the insulin resistance studies, differences were sought between regression lines before and after treatment for both gemfibrozil and placebo groups, and also between gemfibrozil group (before treatment) and placebo group (before), and gemfibrozil (after treatment) and placebo (after). Clearly the most important differences to observe would be those occurring between gemfibrozil (after vs. before) which were not seen in the placebo group (after vs. before), or vice versa if any native tendency to change might be limited by active treatment.

Insulin and ketone concentrations were log transformed prior to analysis because of their known non-normal distribution. Total ketone body concentrations were obtained by adding acetoacetate and 3-hydroxybutyrate concentrations together.

Table 5.1. Characteristics at baseline (visit 1)

Subject	Age	Sex	Duration	Treatment	BMI	hypertension
<i>Gemfibrozil</i>						
1	54	M	6	gliclazide	27.14	no
3	66	M	8	glipizide	26.26	yes
5	65	M	7	diet	24.24	no
6	67	M	8	glibenclamide	26.89	no
9	68	M	5	diet	27.28	yes
12	55	M	2	diet	31.30	no
14	64	F	16	glipizide	25.71	yes
*10	69	M	10	glipizide	29.41	yes
<i>Placebo</i>						
2	67	M	4	glibenclamide	28.40	no
4	65	M	8	glibenclamide	25.42	no
7	43	M	3	diet	27.73	no
11	55	F	2	diet	24.38	no
13	60	M	6	diet	32.15	yes
*8	60	M	6	diet	33.00	yes

*withdrew

Results

Baseline characteristics of each subject who proceeded to the treatment phase of the study are given in table 5.1. Seven subjects completing the study received gemfibrozil and five placebo. Of the two withdrawals during the treatment period (subjects 8 and 10) one received gemfibrozil and one placebo.

Changes from baseline during the study were calculated for total cholesterol, HDL-cholesterol, triglycerides, glucose, fructosamine and body mass index (BMI). For these calculations only data from the subjects completing the study were used. Only for triglycerides (geometric mean falling from 3.68 to 1.79mmol/L (51.4%) with gemfibrozil, $p=0.00003$; fall with placebo from 2.78 to 2.70mmol/L (2.9%), $p=0.92$) was a statistically significant change observed with gemfibrozil while none of these variables were altered by placebo. These data are summarised in table 5.2.

There were no statistically significant differences between groups for mean fasting metabolite concentrations (table 5.3); for all metabolites $p>0.5$.

Data showing changes in metabolite and insulin concentrations with time during the low-dose insulin infusions are shown in figures 5.1-5.9. After calculation of regression lines for metabolite concentration against log [mean insulin], slopes for these data were subjected to analysis of covariance. Comparisons of both separate and common slopes are shown in figures 5.10-5.15.

Mean blood glucose concentrations were not significantly altered by gemfibrozil or placebo. Difference between corrected means for parallel slopes before and after gemfibrozil was 0.10mmol/L, and for placebo 0.15mmol/L ($p>0.5$ for both).

Mean lactate concentrations were slightly higher following gemfibrozil treatment and fell slightly after placebo. None of these changes were statistically significant, and

it should be noted that the regression slopes for lactate, and also for alanine, did not reach statistical significance; i.e changes in x (plasma insulin concentration) did not produce statistically significant changes in y (lactate or alanine concentrations). Mean alanine concentrations fell after gemfibrozil but rose after placebo, neither change reaching statistical significance.

Blood glycerol concentrations fell after gemfibrozil but were unaltered by placebo, again these differences did not reach statistical significance. Difference between corrected means for parallel slopes before and after gemfibrozil treatment was $4.1\mu\text{mol/L}$ ($p=0.28$); difference for placebo $0.2\mu\text{mol/L}$ ($p=1$).

For both NEFA and log total ketones (TKB), a significant shift of the regression line downwards and to the left was observed in the gemfibrozil group which was not present in the placebo group. Taking differences between corrected mean values on the parallel slopes obtained by analysis of covariance, fall in mean NEFA concentration with gemfibrozil (after vs. before treatment) was 0.15mmol/L , $p=0.003$ by Scheffe's test; change with placebo (after vs. before treatment) was 0.08mmol/L , $p=0.10$. For mean TKB fall was $41.0\mu\text{mol/L}$, $p=0.002$ with gemfibrozil; $10.9\mu\text{mol/L}$, $p=0.43$ with placebo.

Changes in pyruvate concentrations were not calculated because full data sets were not available for all subjects.

Analysis by fall in triglyceride concentrations showed that all but two subjects in the gemfibrozil group had greater absolute or percentage falls in triglyceride levels than any of the placebo group; therefore reanalysis of the data comparing subjects with large triglyceride reductions against those with small or absent triglyceride falls yielded no additional information.

Table 5.2. Changes with treatment (mean values)

	<i>Gemfibrozil</i>		<i>Placebo</i>	
	<u>change(%)</u>	<u>p (ANOVA)</u>	<u>change(%)</u>	<u>p (ANOVA)</u>
Total cholesterol (mmol/L)	-0.84 (12.5)	0.14	-0.26 (4.1)	0.89
Triglycerides (mmol/L)*	-1.89 (51)	0.00003	-0.08 (2.9)	0.92
HDL-cholesterol (mmol/L)	+0.01 (1.1)	0.69	0	1
Glucose (mmol/L)	-0.29 (3.8)	0.95	-0.29 (3.8)	0.98
Fructosamine ($\mu\text{mol/L}$)	+23.4 (7.9)	0.81	+25.5 (9.1)	0.95
BMI (kg/m^2)	-0.17 (0.64)	1	+0.1 (0.36)	1

*comparison of geometric means; data log transformed for analysis

Table 5.3. Mean fasting metabolite concentrations

	<i>Gemfibrozil</i>		<i>Placebo</i>	
	pre	post	pre	post
Insulin (pmol/L)*	85.0	84.0	116.3	113.1
Glucose (mmol/L)	6.85	7.16	6.56	6.81
Lactate (μ mol/L)	736.0	858.4	787.8	799
Alanine (μ mol/L)	342.9	308.4	345.8	344.8
Glycerol (μ mol/L)	49.2	47.8	55.2	57.2
NEFA (mmol/L)	0.79	0.64	0.76	0.69
TKB (μ mol/L)*	134.1	88.7	156.1	167.2

*geometric mean

Conclusions

This study was hampered by the high dropout rate during the dietary run-in phase, largely due to the improvement in fasting triglyceride levels brought about by diet alone leading to many subjects being rendered ineligible. Nonetheless, despite the small numbers of subjects completing the study, gemfibrozil reduced triglyceride concentrations to a significant degree, and was shown to bring about significant overall reductions in NEFA and ketone concentrations. It seems most likely that this is a direct effect of the drug on fatty acid synthesis rather than any indirect effect mediated by enhanced insulin sensitivity. The small size of this study makes it impossible to exclude a type 2 error in analysis, particularly with respect to effects on glycerol metabolism, although the consistency with other published data suggests that these findings are likely to be true.

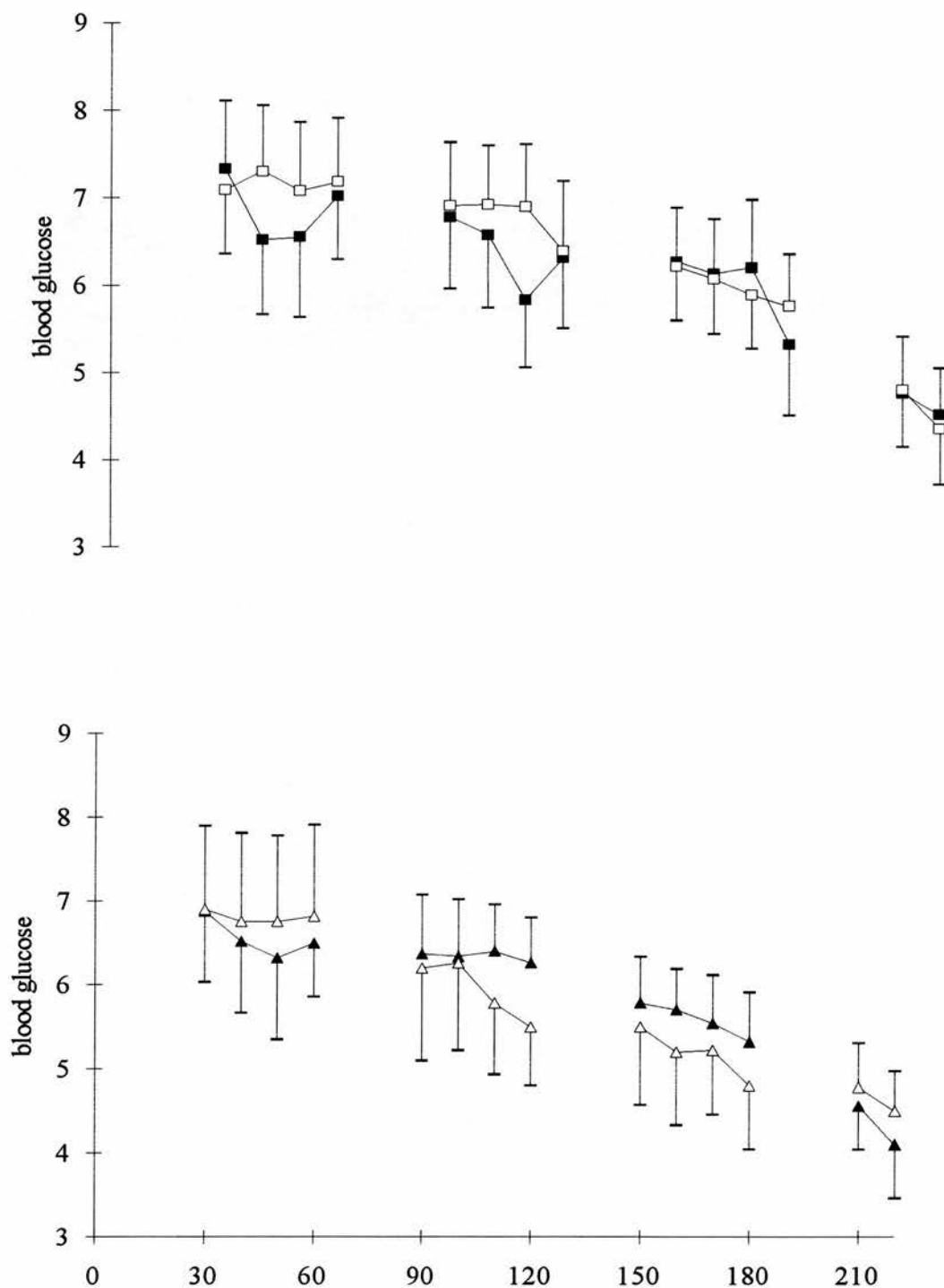


Figure 5.1. Changes in blood glucose concentration (mmol/L; mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

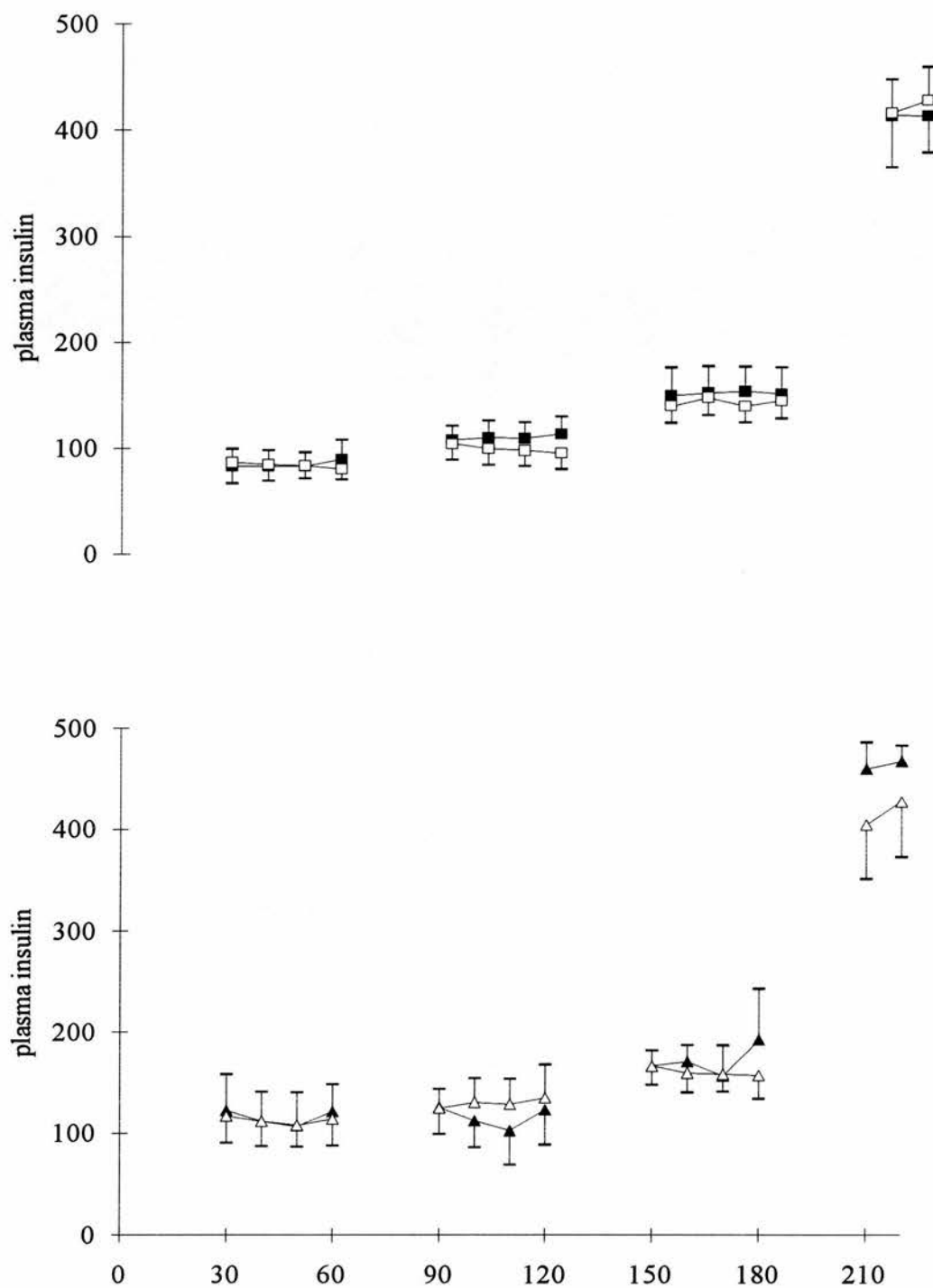


Figure 5.2. Changes in plasma insulin concentration (pmol/L; geometric mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

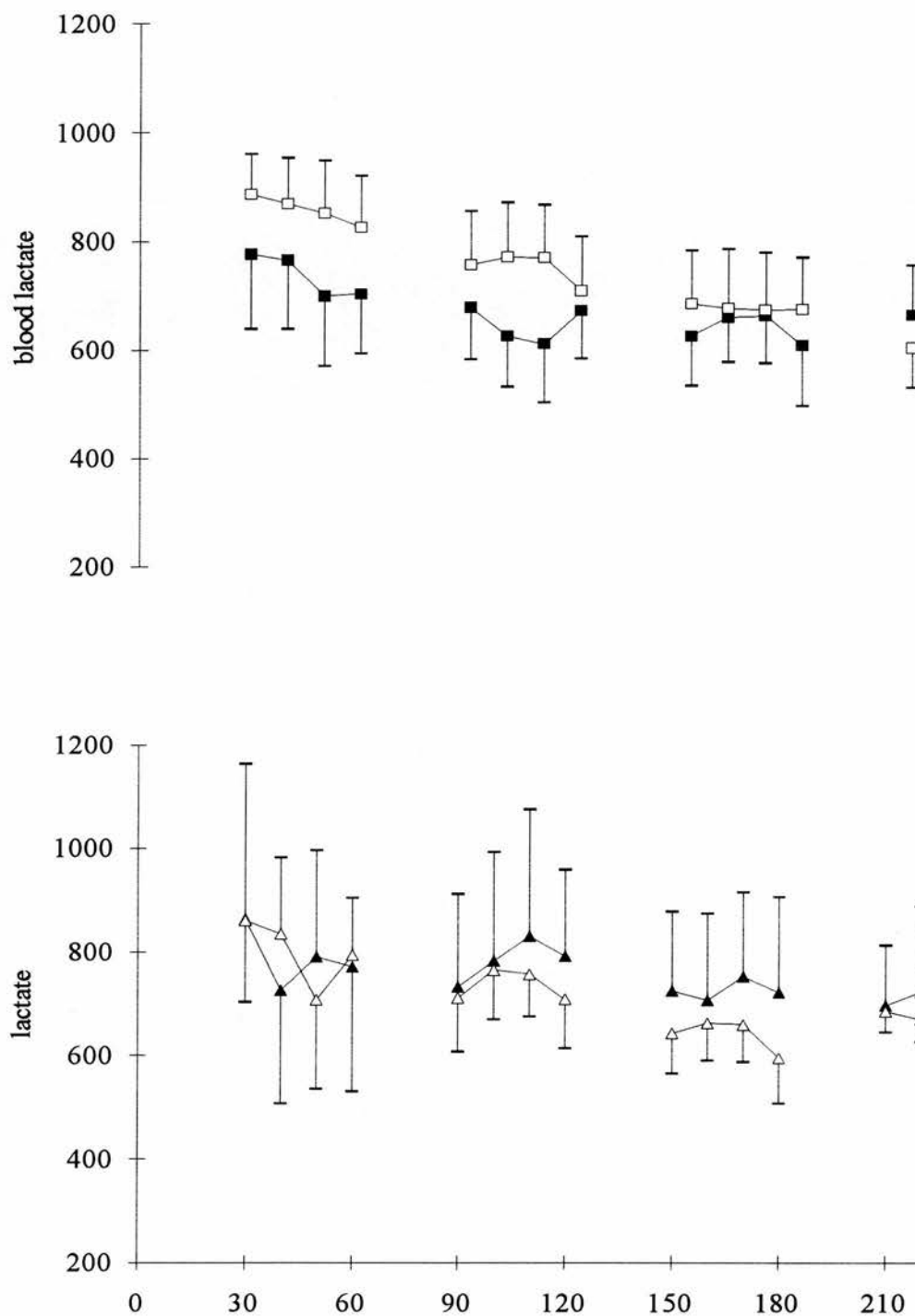


Figure 5.3. Changes in blood lactate concentration ($\mu\text{mol/L}$; mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

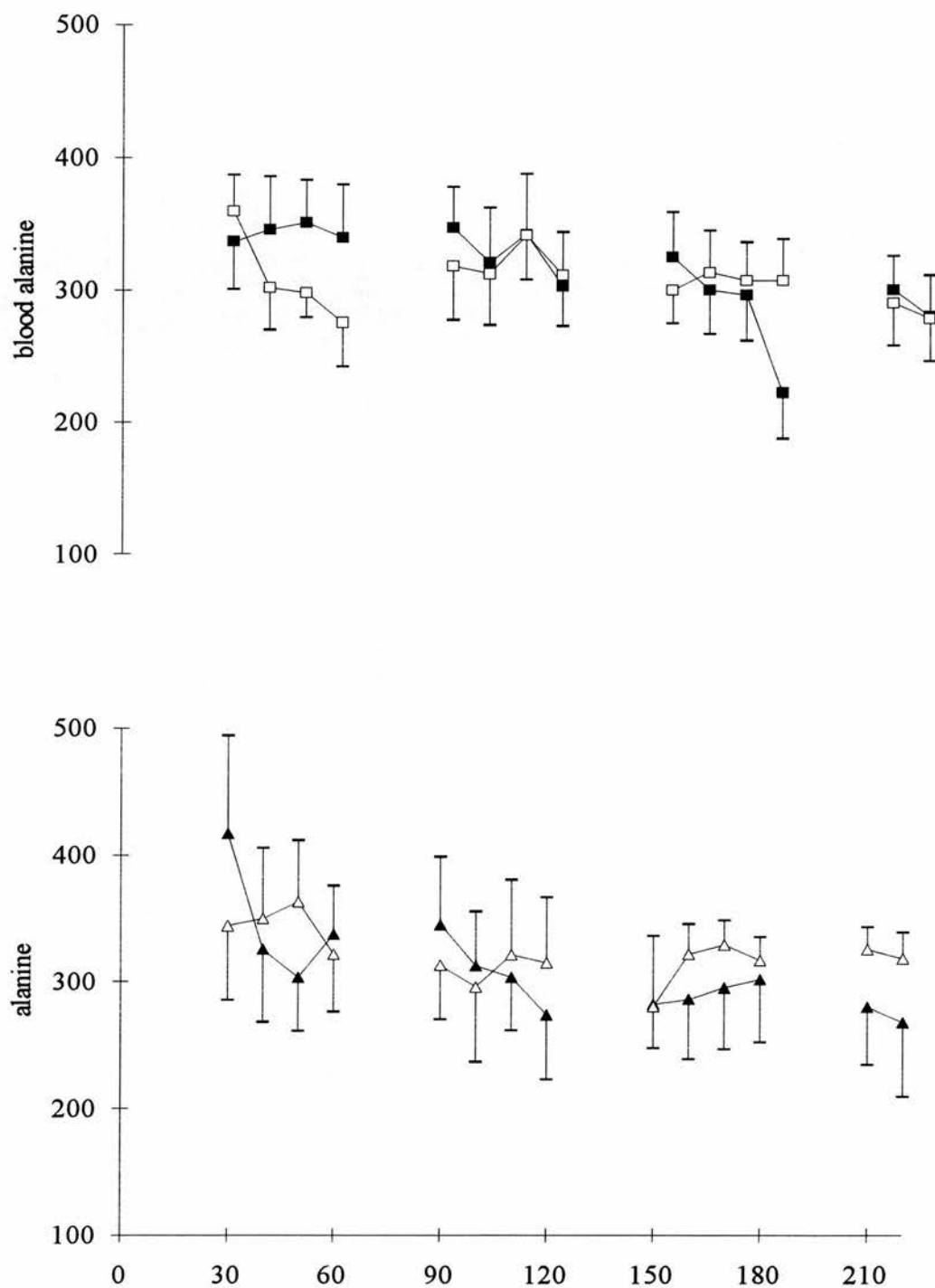


Figure 5.4. Changes in blood alanine concentration ($\mu\text{mol/L}$; mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

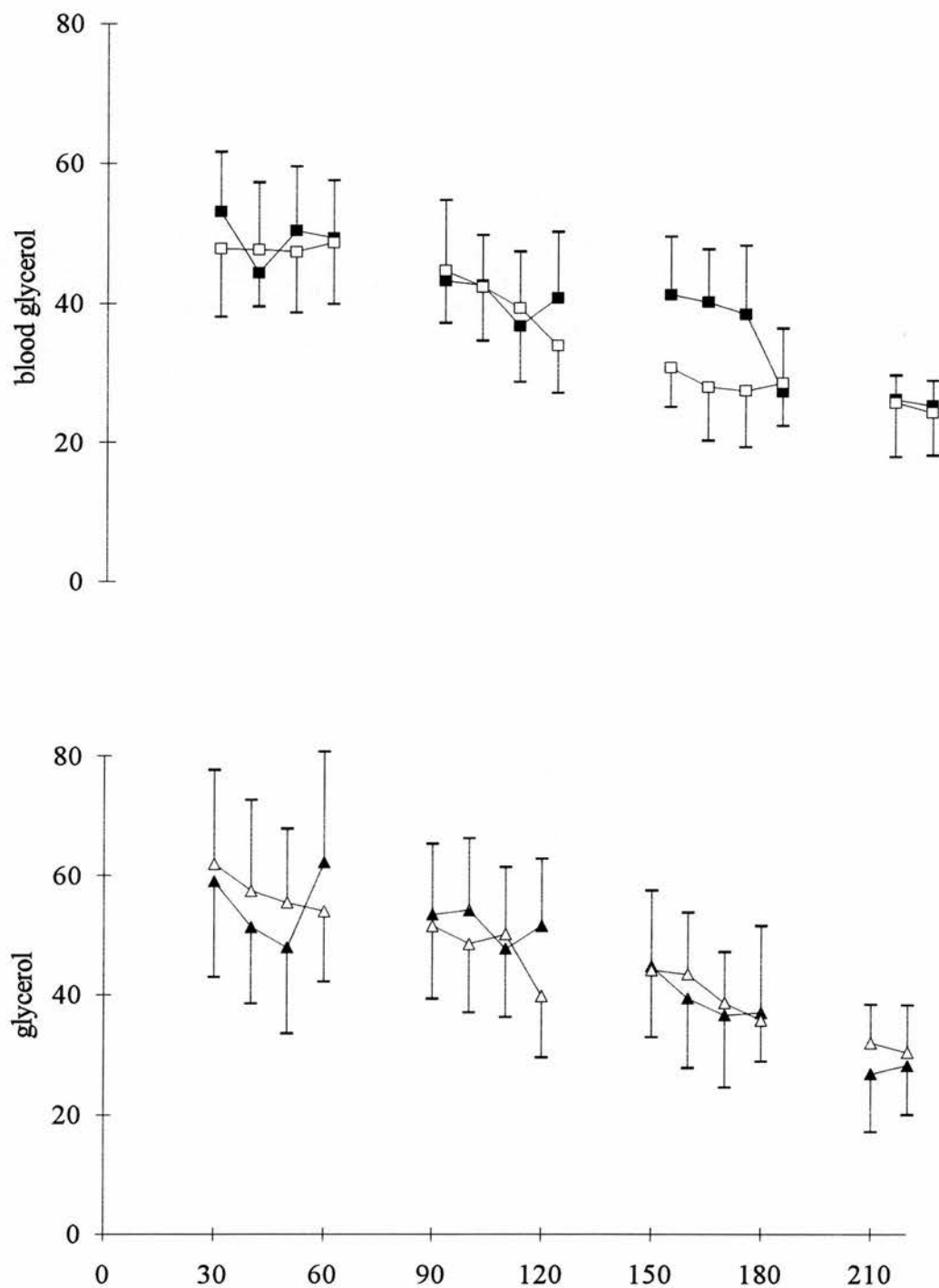


Figure 5.5. Changes in blood glycerol concentration ($\mu\text{mol/L}$; mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (■ before treatment, □ after treatment); bottom - placebo group (▲ before treatment, △ after treatment).

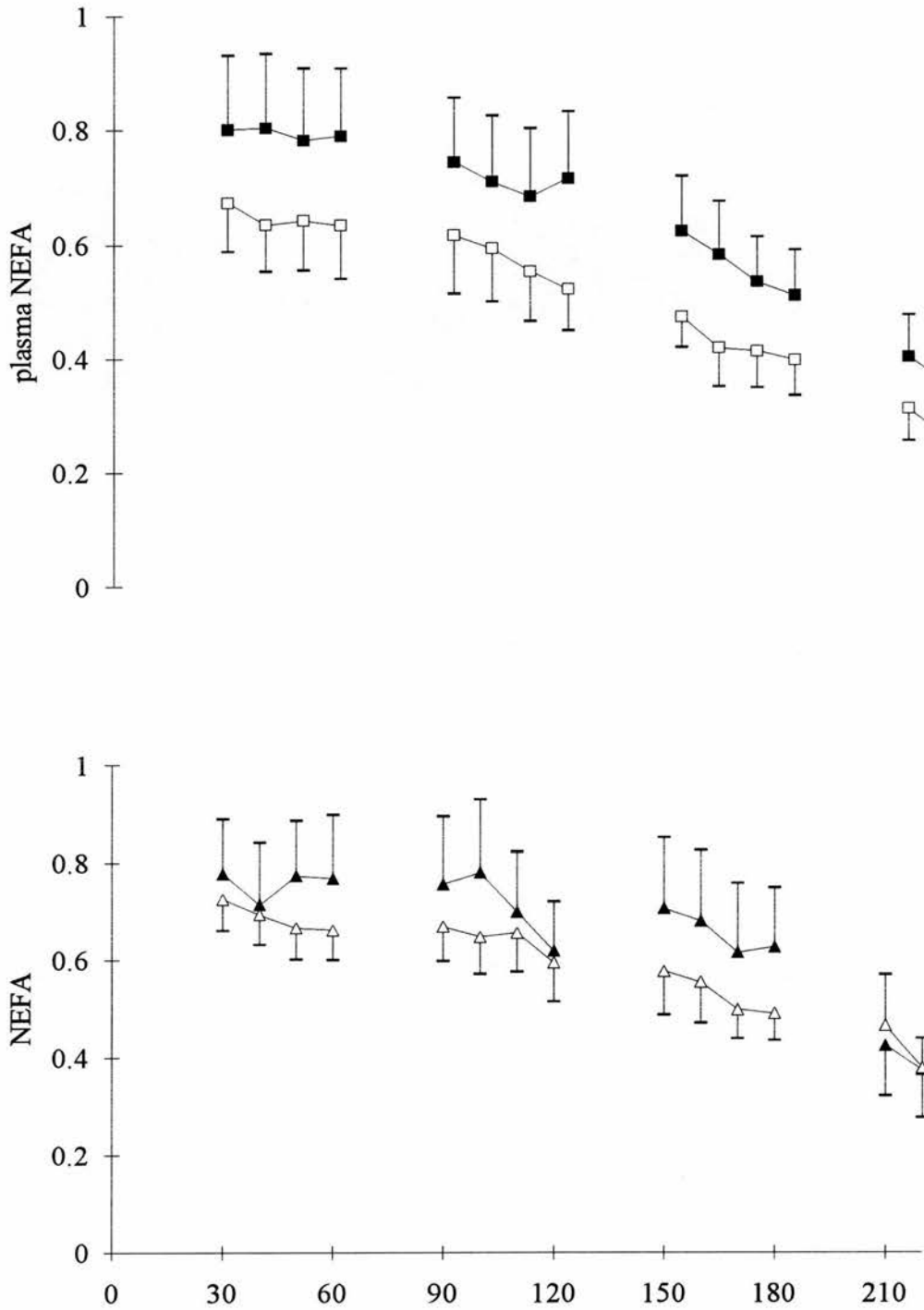


Figure 5.6. Changes in plasma NEFA concentration (mmol/L; mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

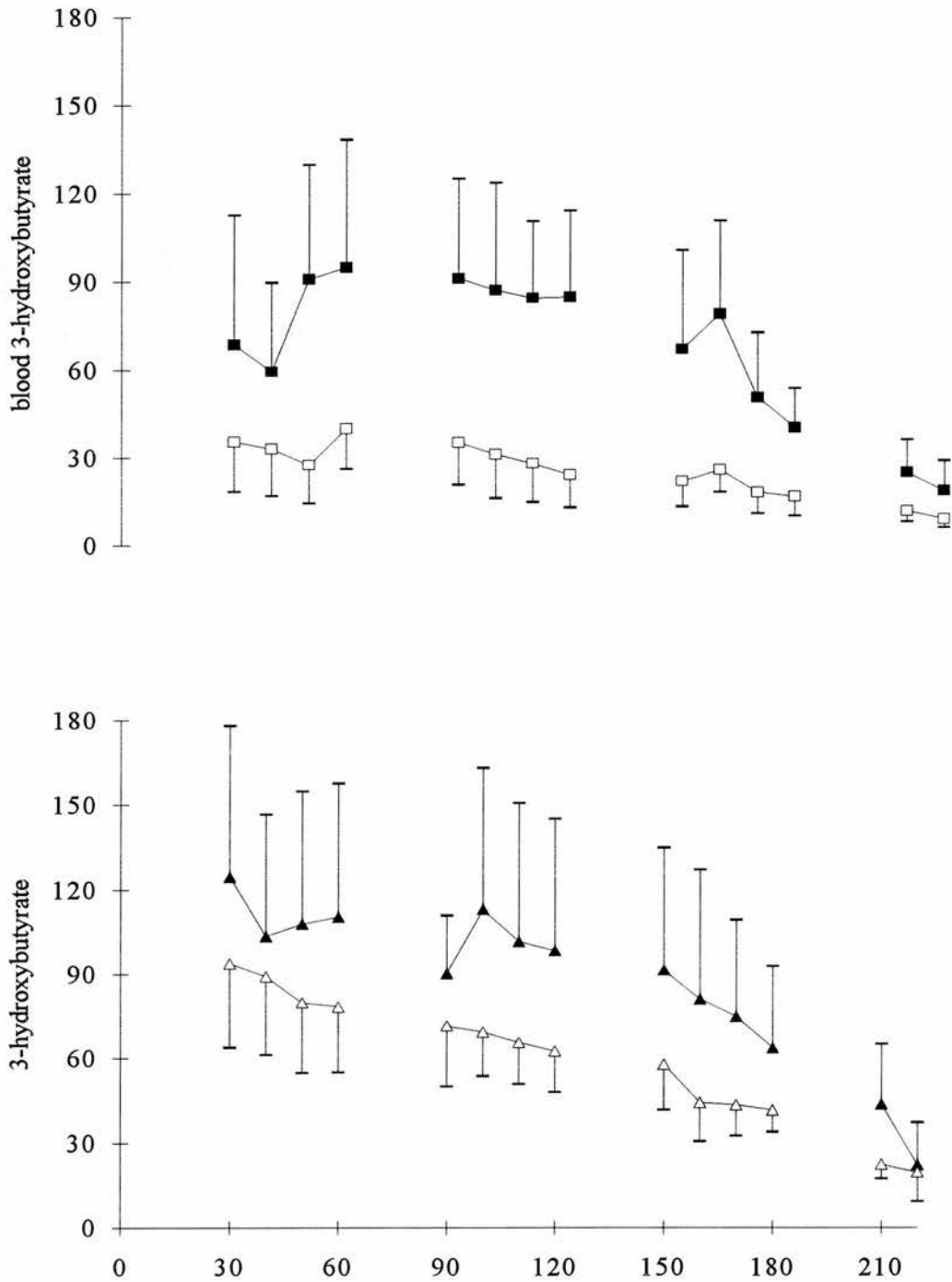


Figure 5.7. Changes in blood 3-hydroxybutyrate concentration ($\mu\text{mol/L}$; geometric mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

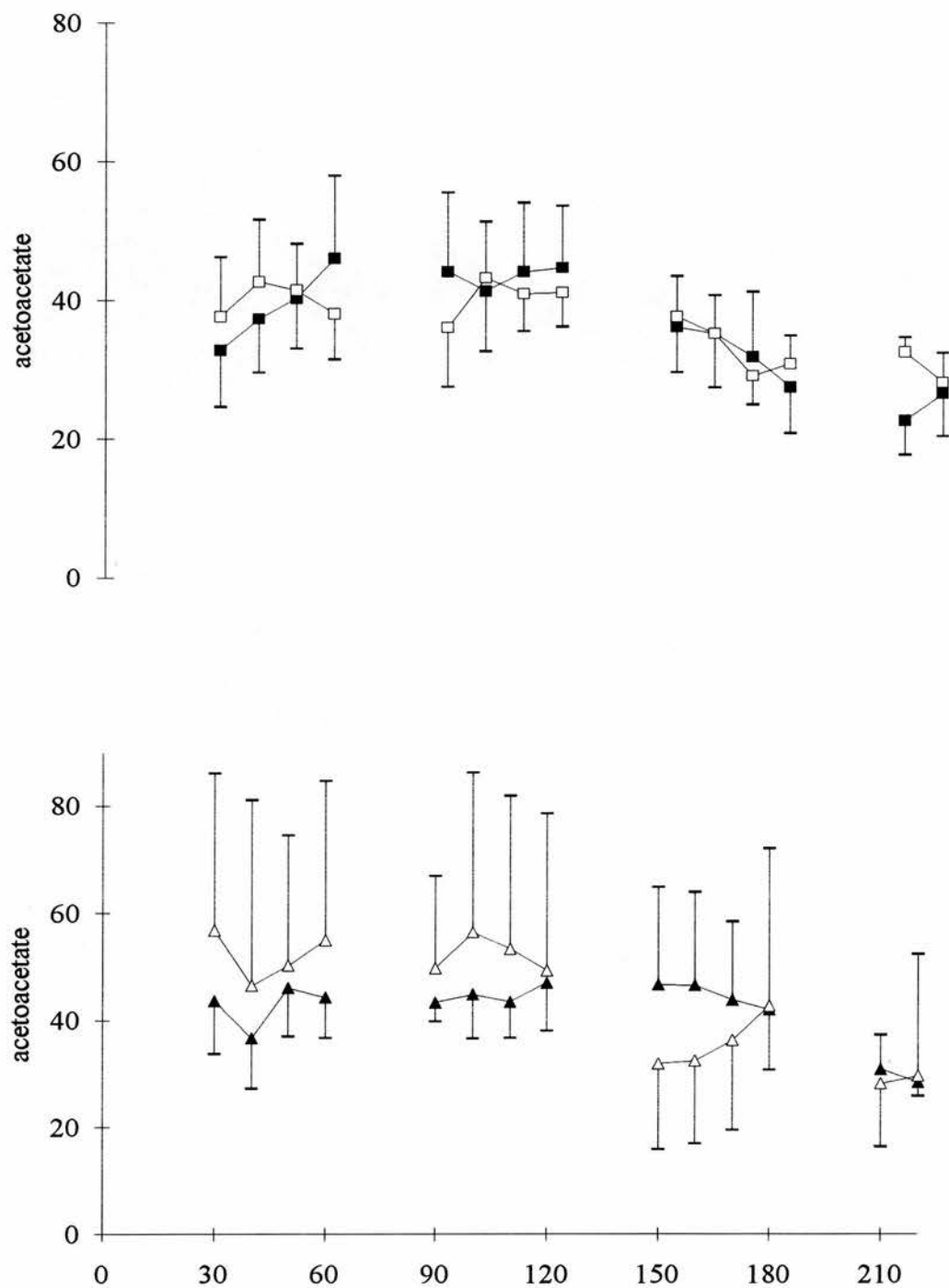


Figure 5.8. Changes in blood acetoacetate concentration ($\mu\text{mol/L}$; geometric mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

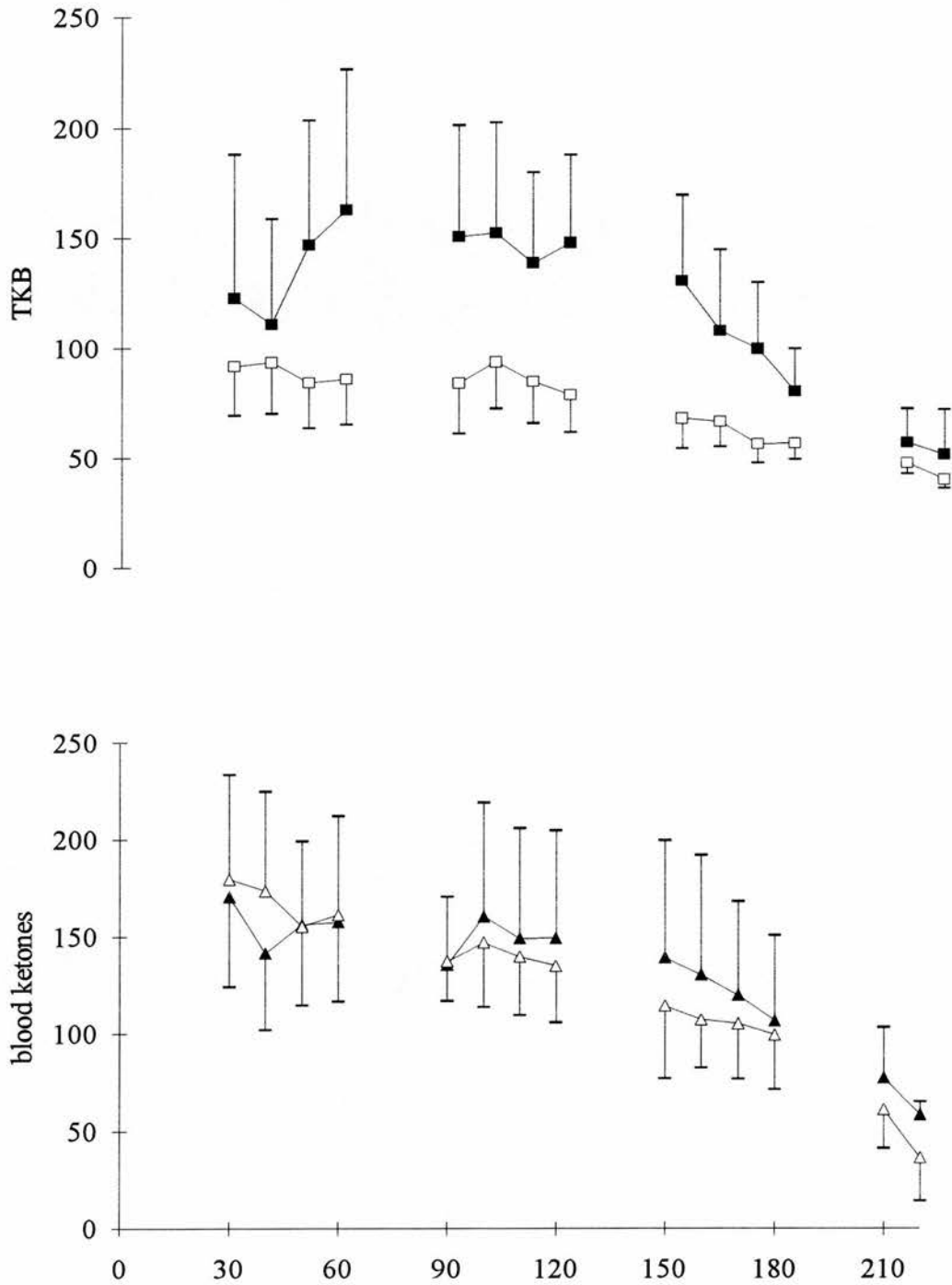


Figure 5.9. Changes in blood total ketone concentration ($\mu\text{mol/L}$; geometric mean \pm SEM) with time (minutes) during low-dose insulin infusion: top - gemfibrozil group (\blacksquare before treatment, \square after treatment); bottom - placebo group (\blacktriangle before treatment, \triangle after treatment).

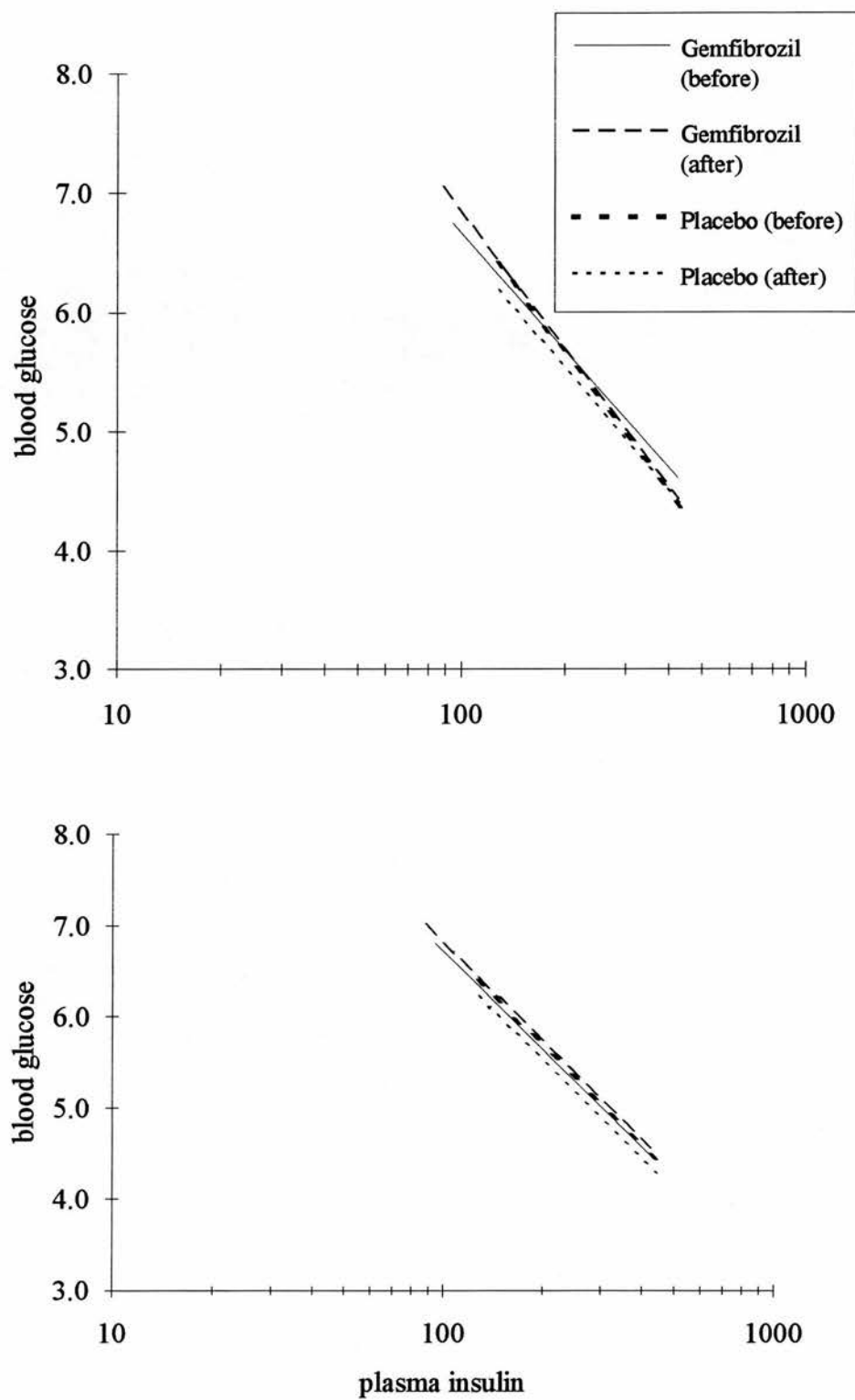


Figure 5.10. Regression slopes for blood glucose (mmol/L) against plasma insulin concentration - calculated for individual regression lines (top) and for common slope (bottom).

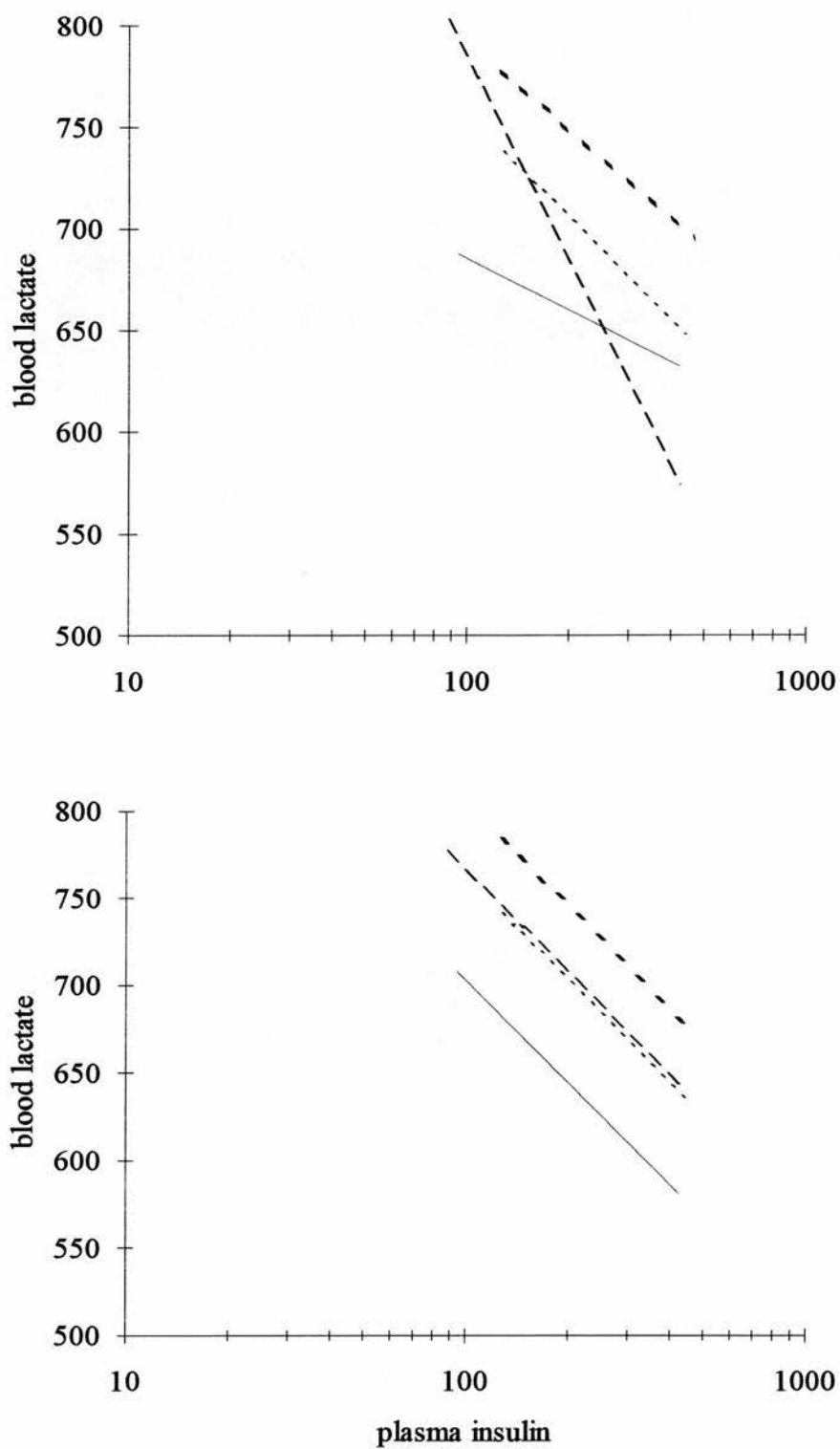


Figure 5.11. Regression slopes for blood lactate ($\mu\text{mol/L}$) against plasma insulin concentration - calculated for individual regression lines (top) and for common slope (bottom). Legend as for fig. 5.10.

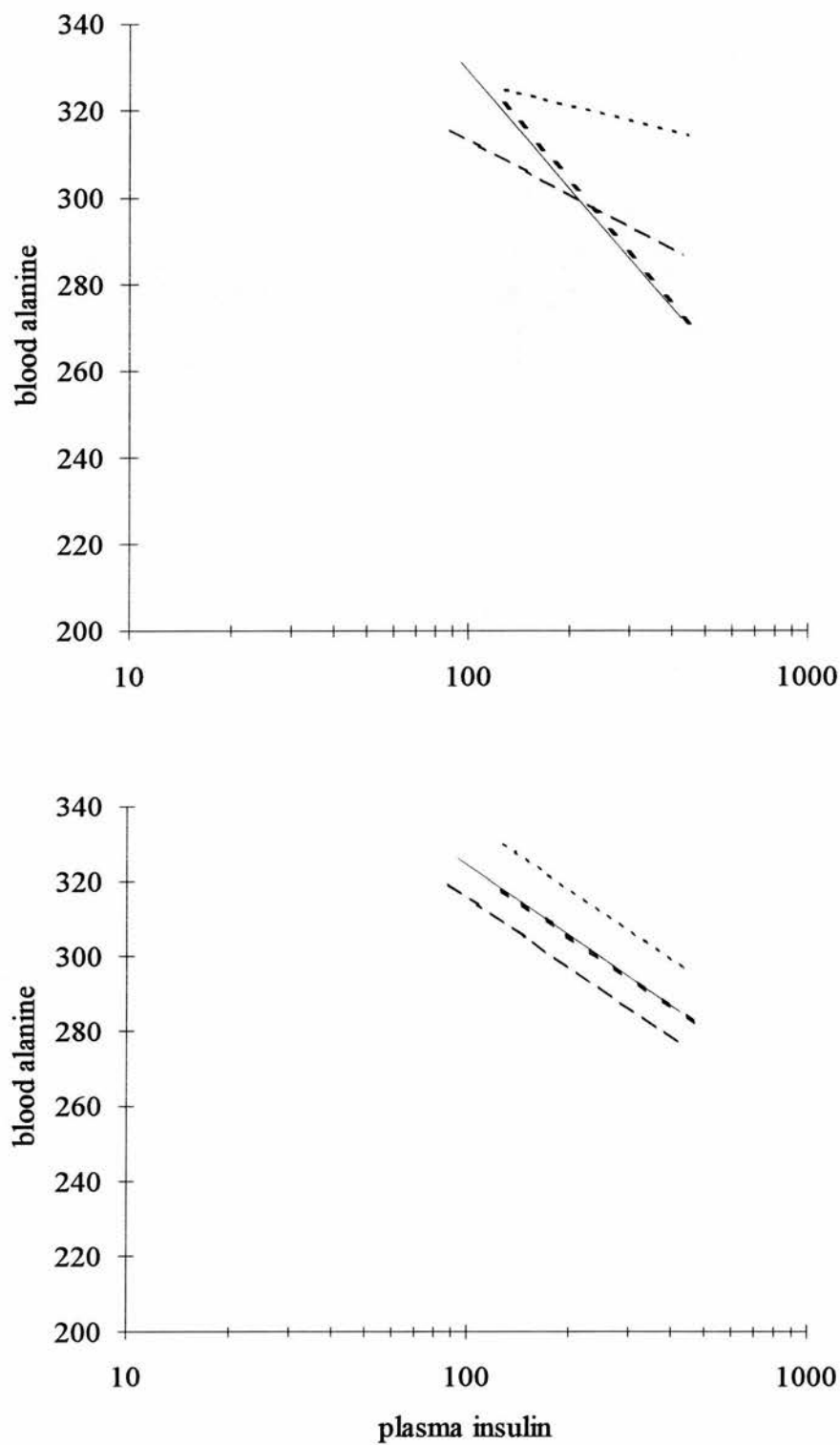


Figure 5.12. Regression slopes for blood alanine ($\mu\text{mol/L}$) against plasma insulin concentration - calculated for individual regression lines (top) and for common slope (bottom). Legend as for fig. 5.10.

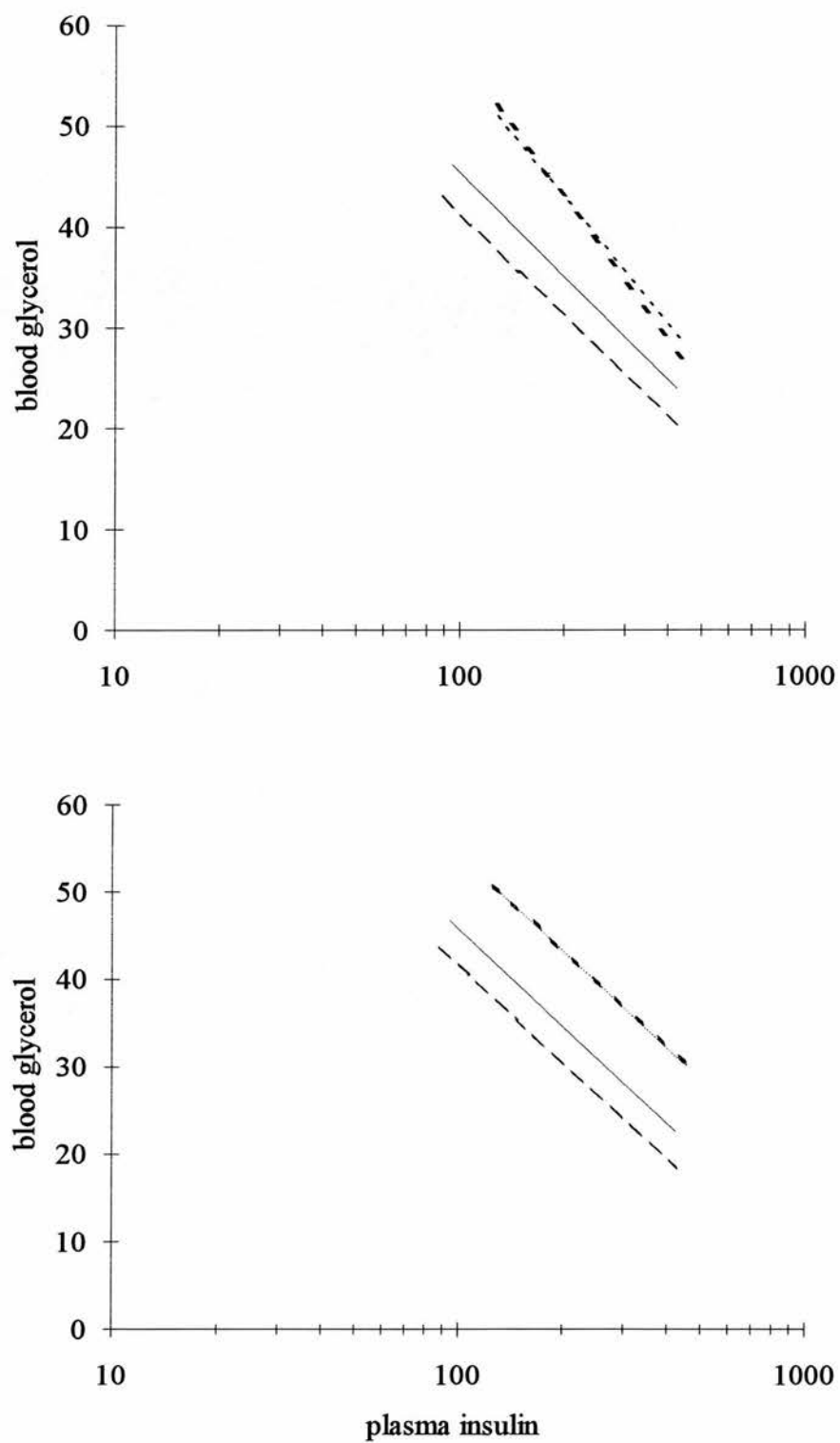


Figure 5.13. Regression slopes for blood glycerol ($\mu\text{mol/L}$) against plasma insulin concentration - calculated for individual regression lines (top) and for common slope (bottom). Legend as for fig. 5.10. (Parallel lines for placebo overlies.)

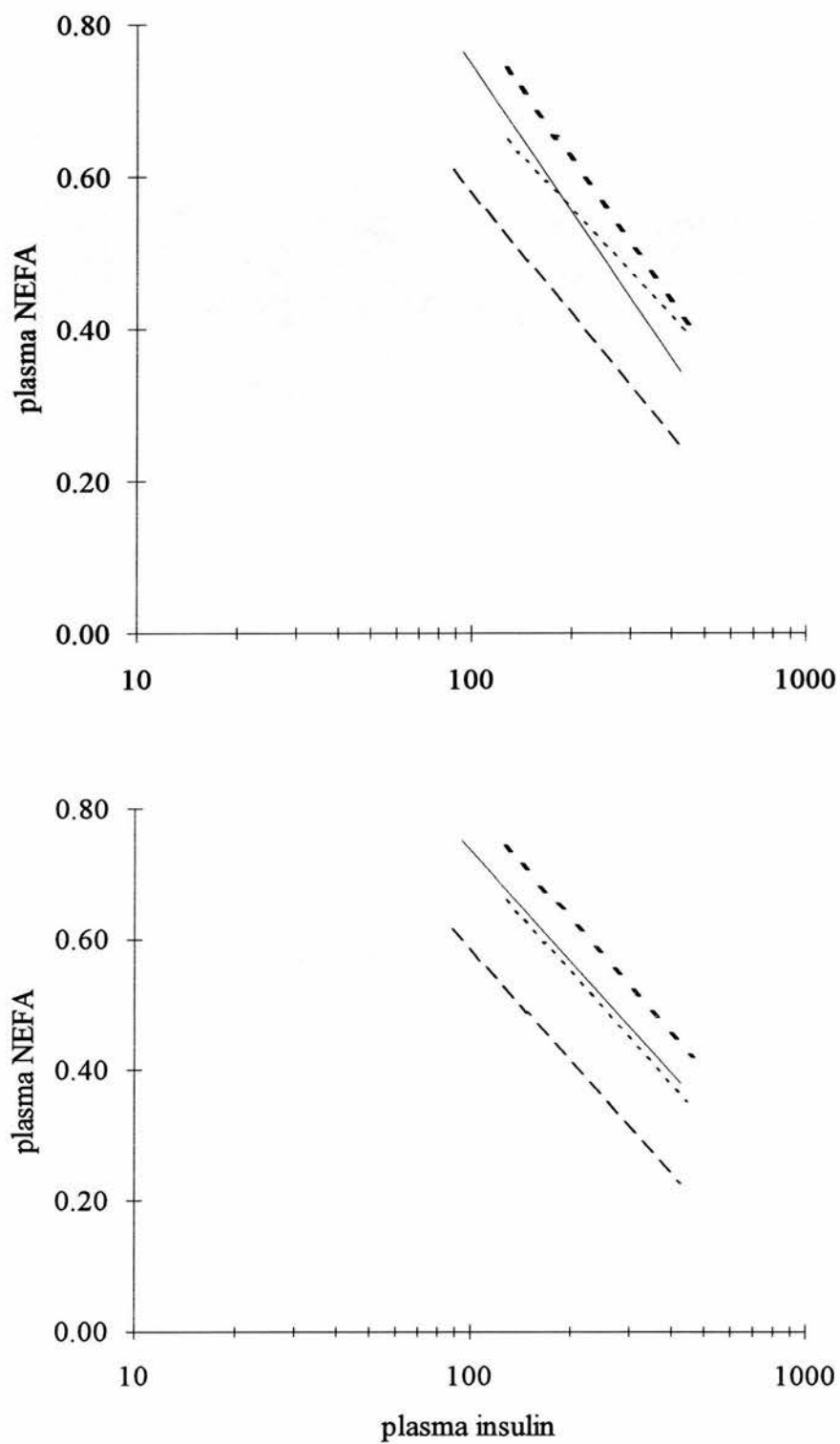


Figure 5.14. Regression slopes for plasma NEFA (mmol/L) against plasma insulin concentration - calculated for individual regression lines (top) and for common slope (bottom). Legend as for fig. 5.10.

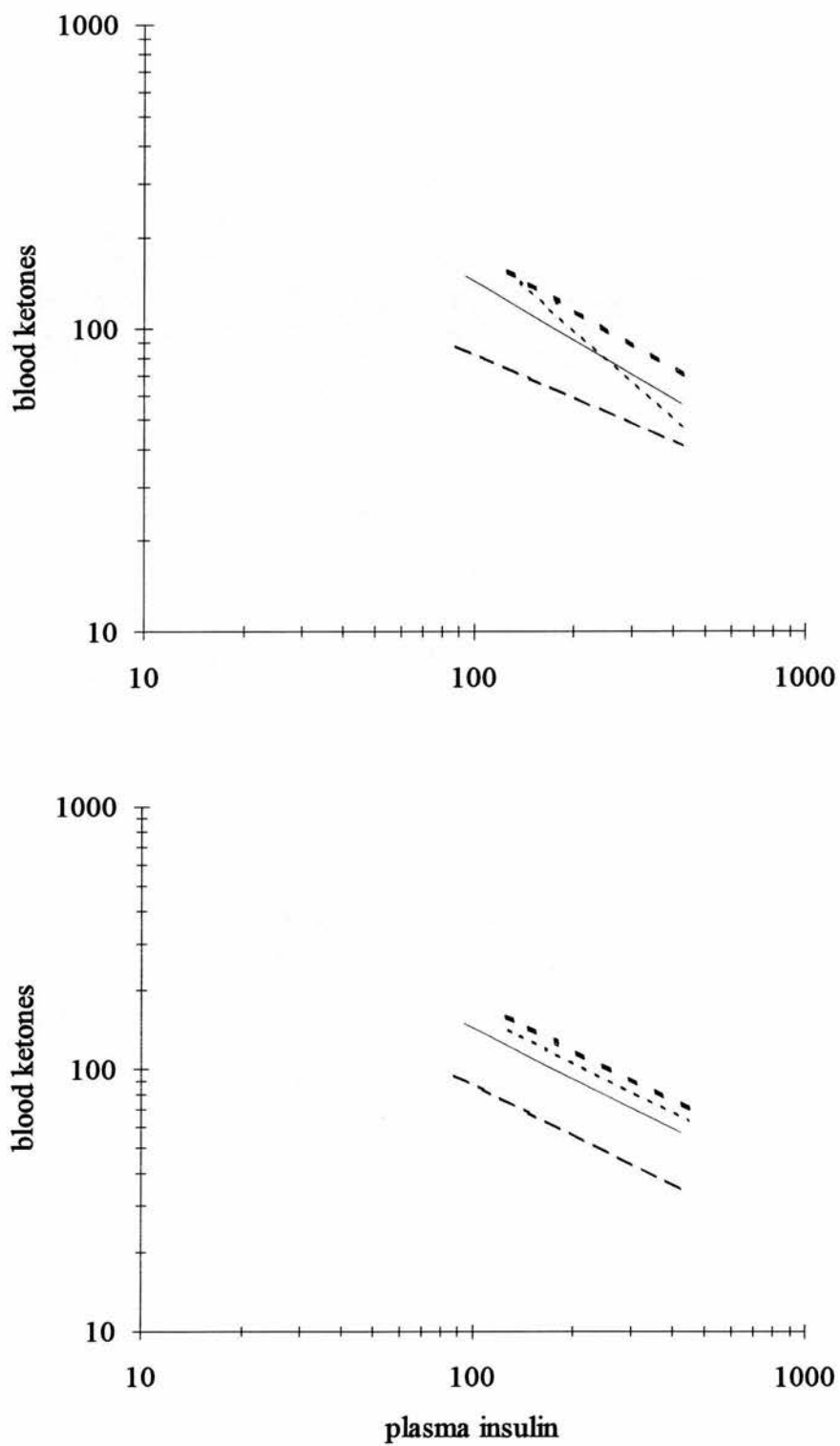


Figure 5.15. Regression slopes for blood total ketones ($\mu\text{mol/L}$) against plasma insulin concentration - calculated for individual regression lines (top) and for common slope (bottom). Legend as for fig. 5.10.

CHAPTER 6

The influence of body fat distribution on insulin resistance in
NIDDM

Introduction

Abdominal obesity, and more particularly visceral obesity, is widely regarded as a risk factor for the development of non-insulin-dependent diabetes and may also be associated with increased cardiovascular risk. It has been proposed that this risk is mediated at least in part by increased insulin resistance although the evidence to support this hypothesis appears to come largely from population studies screened using an oral glucose tolerance test; formal assessments of insulin resistance to assess this have rarely been used.

Assessment of abdominal obesity can most easily be made by measuring the waist-hip ratio. Although the value of this method has been criticised when compared with more specific means of assessing visceral fat (such as magnetic resonance imaging) it is widely accepted as a useful guide to regional adiposity, and is claimed by some to be a better indicator of insulin resistance and risk of diabetes than indices of total body obesity such as body mass index (BMI).

This study attempted to address the question of whether insulin resistance in subjects with non-insulin-dependent diabetes is related to body fat distribution by measuring insulin resistance in two ways, namely the euglycaemic hyperinsulinaemic clamp and the low-dose incremental insulin infusion.

Subjects and Procedures

Subjects were recruited from the Diabetic Clinic at the General and Selly Oak Hospitals, Birmingham between August 1995 and April 1996. Ethical approval was received before the study commenced and all subjects gave signed informed consent to take part after receiving written and verbal details of the study procedure.

Caucasian men aged approximately 35-65 years were considered eligible if they had non-insulin-dependent diabetes of at least two years duration treated with diet alone. Subjects had to be in good general health, on no regular oral medication and have BMI approx. 25-32kg/m². Subjects with known or suspected secondary diabetes were excluded.

Fourteen volunteers were studied; each attended for one clamp study and one insulin infusion. In one subject (subject 4) the clamp procedure had to be terminated because difficulty in maintaining a patent hand vein cannula meant that a steady state blood glucose concentration was not achieved for the required period. For this subject only insulin infusion data are therefore available.

Subjects attended after an overnight fast for both visits; studies were scheduled to start between 8 and 9am.

At visit 1 demographic details were obtained and the following examined: height, weight, waist and hip circumferences, skinfold thicknesses (triceps, biceps, subscapularis, suprailiac and thigh) and resting blood pressure. Waist circumference was determined at the midpoint between the lowest palpable rib border and the superior iliac crest in the mid-axillary line; hip circumference was measured at the level of the greater trochanters. Body density was estimated using the equations of Durnin and Womersley (1974) applied to the skinfold measurements obtained at triceps, biceps, subscapular and suprailiac sites; percent body fat was then calculated using the Siri equation (Siri, 1956).

Fasting blood samples were taken for general biochemical profile, HbA1c, fructosamine, serum total and HDL-cholesterol, triglycerides, thyroid function (T4 and TSH), testosterone, SHBG (sex hormone binding globulin) and cortisol concentrations.

Subjects then proceeded to either a euglycaemic hyperinsulinaemic clamp or low-dose incremental insulin infusion (as described in Chapter 2 - Methods). Clamp studies were more often carried out first but no fixed order was determined. All blood samples were obtained via cannulae sited in warmed dorsal hand veins giving arterialised venous blood. On completion of the procedure subjects were given lunch prior to leaving the hospital. The second procedure was carried out a minimum of 7 days, and where possible not more than 30 days, after the first.

Insulin sensitivity values (M) for the clamp study were obtained from the mean rates of glucose infused during the final thirty minutes of the test (DeFronzo et al, 1979) during which period blood glucose is assumed to be stable at approximately 5mmol/L. Infusing 20% dextrose, M can be calculated as glucose infusion rate per minute (l/min) x 200 (grams glucose/litre infusate); dividing this value by the subject's measured weight (in kg) allows M to be expressed as mg/kg/min. Alternatively, M can be expressed in $\mu\text{mol/kg/min}$ from the molecular weight of glucose (which is 180; thus multiply previous value by 1000/180).

Laboratory analysis

Analyses of plasma insulin and NEFA, and metabolites (in deproteinated whole blood) were made as described previously (Chapter 2); samples were separated and frozen on the day of the study to be stored until analysis. Samples for baseline measures were sent on the day of sampling at visit 1.

Data analysis

Data from the low-dose insulin infusions were analysed in groups. Subjects were divided into two categories - either 'high' or 'low' for the variable under analysis. The

variables of interest studied were waist-hip ratio, body mass index, percent body fat and mean fasting blood glucose. After subjects had been ranked by the variable of interest, the middle two subjects were removed from assessment in order to obtain a clear separation between the high and low groups (since all of the four variables studied are continuous rather than discrete). Each group analysed therefore consisted of six subjects. Analysis of variance applied to the regression lines of metabolite concentration against log mean insulin was then carried out. Data from each subject was thus used for up to four analyses. Scheffe's test was used for significance testing, allowing for multiple comparisons.

In order to demonstrate clear and adequate separation between 'high' and 'low' groups comparison of means for fasting glucose, BMI, waist-hip ratio and percent body fat was made by one-tailed Student's t-test; the one-tailed test was used because the division into 'high' and 'low' groups necessarily indicated that the difference could lie only in one direction.

Correlation coefficients were obtained using Spearman's rank correlation test to examine interrelationships between variables, with particular reference to effects of the four variables above on insulin resistance data obtained from the euglycaemic clamp studies.

Results

General characteristics of the study population are shown in table 6.1.

Table 6.1. General characteristics of the study group (14 subjects)

	<u>mean</u>	<u>range</u>
Age (years)	56	40 - 67
Years since diagnosis of NIDDM		2 - 10
BMI (kg/m ²)	28.7	23.8 - 32.8
Waist-hip ratio	1.01	0.93 - 1.09
% body fat	29.72	24.24 - 38.38
HbA1c (%)*	6.93	4.6 - 10.4
Fasting blood glucose (mmol/L) [†]	7.26	4.85 - 10.65
Fasting triglycerides (mmol/L)	1.82	1.04 - 3.89
Fasting plasma insulin (pmol/L) [†]	78.9	43.5 - 165

* for two subjects HbA1 was measured (prior to change in method); HbA1c derived by subtracting 1.85 from HbA1 value.

[†] mean of fasting values from each visit.

Insulin infusion data

Figures 6.1-6.4 show the metabolite and insulin profiles for each sampling point of the low-dose insulin infusion. From these the infused insulin appears to have little effect on lactate, alanine and pyruvate concentrations. This impression was supported by calculation of the regression lines for these variables, for which the steepness of the regression slope did not differ significantly from zero; i.e. changes in mean plasma insulin concentration did not result in significant changes in metabolite concentration. These data are therefore not further examined here. For all the other metabolites (glucose, NEFA, glycerol and TKB) regression slopes were compared for 'low' and 'high' groups according to fasting blood glucose, waist-hip ratio, BMI, and percent body fat.

Effect of high versus low waist-hip ratio

Table 6.2 (a) summarises the comparisons of the common slopes for each metabolite, comparing the low WHR and high WHR groups (mean WHR 0.961 vs. 1.057; $p < 0.0001$). Only for total ketones was a statistically significant separation between slopes observed, and for all concentrations of insulin, ketone body levels were higher in the low WHR group ($p = 0.019$). A similar observation was apparent when the two individual regression slopes were compared.

Blood glucose concentrations showed a similar trend, with higher concentrations in the low WHR group, but this finding just failed to reach statistical significance ($p = 0.067$).

There were no significant differences between the two regression lines for NEFA or for glycerol. Figure 6.5 (a and b) shows the slopes for 'low' and 'high' WHR groups for regression of each metabolite on insulin.

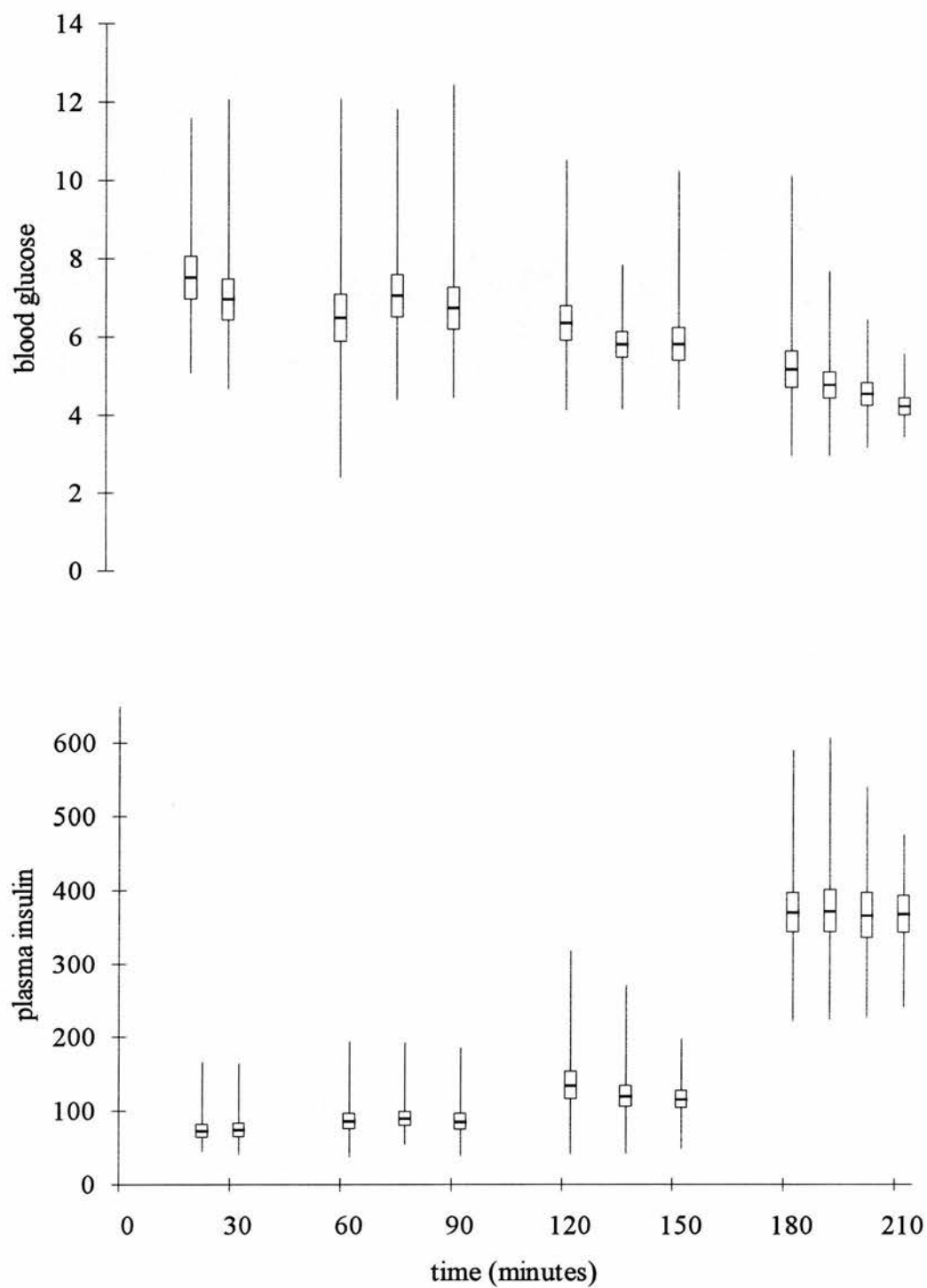


Figure 6.1. Top: mean (—), SEM (box) and range (vertical lines) for blood glucose concentration (mmol/L) at each time point during low-dose insulin infusion. Bottom: plasma insulin (pmol/L); layout as above, (geometric mean).

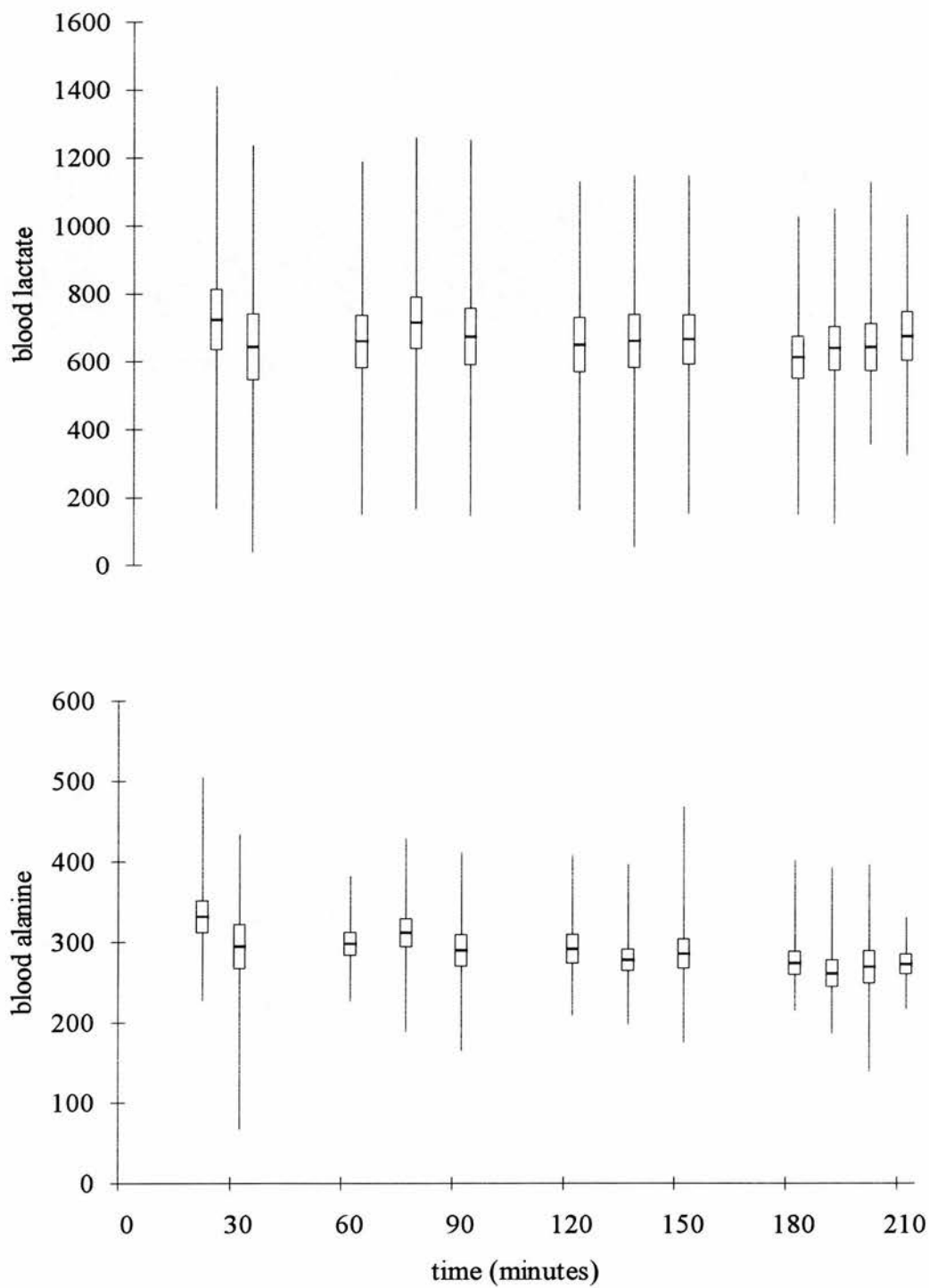


Figure 6.2. Top: mean (—), SEM (box) and range (vertical lines) for blood lactate concentration ($\mu\text{mol/L}$) at each time point during low-dose insulin infusion. Bottom: blood alanine ($\mu\text{mol/L}$); layout as above.

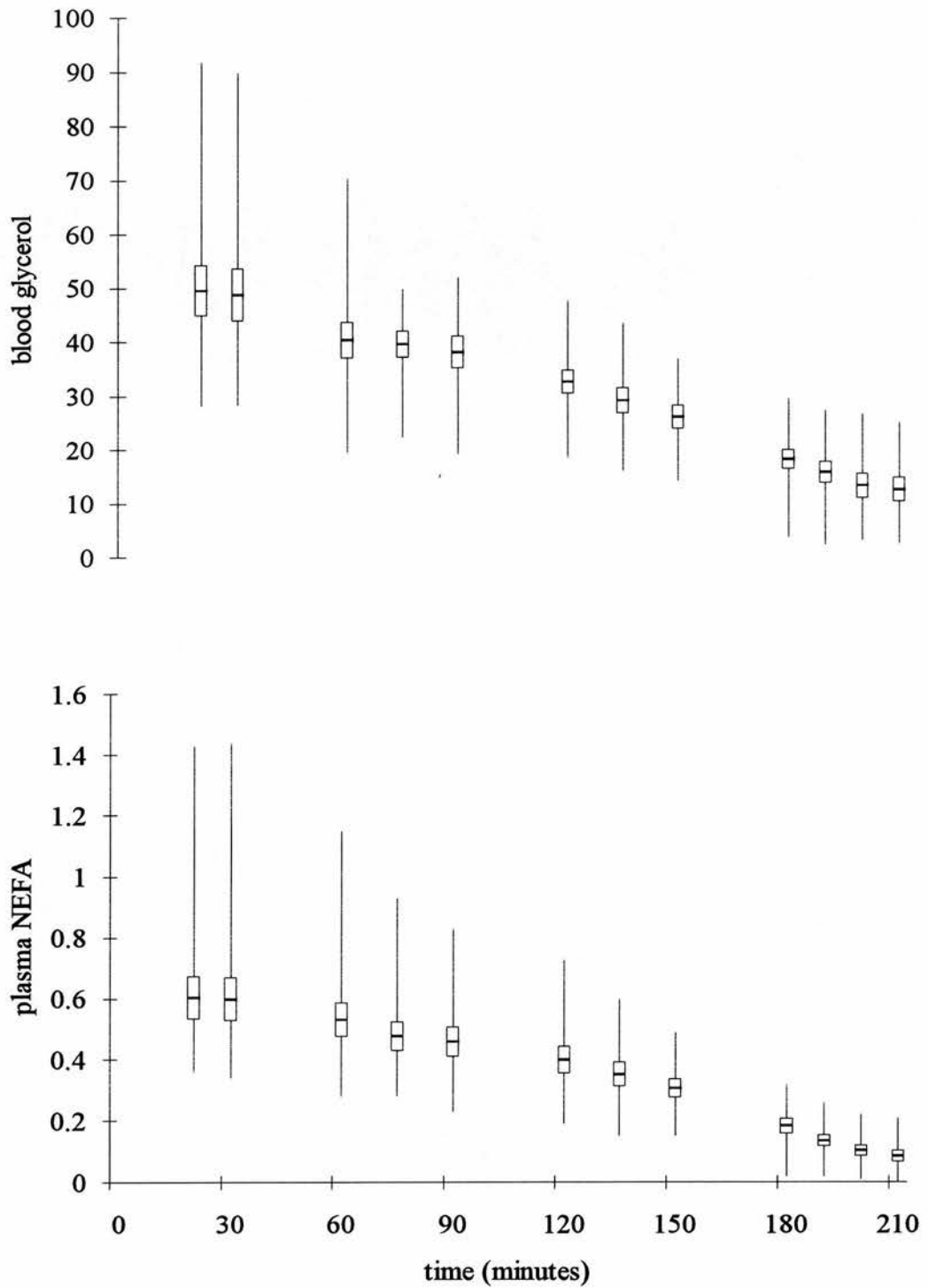


Figure 6.3. Top: mean (—), SEM (box) and range (vertical lines) for blood glycerol concentration ($\mu\text{mol/L}$) at each time point during low-dose insulin infusion. Bottom: plasma NEFA (mmol/L); layout as above.

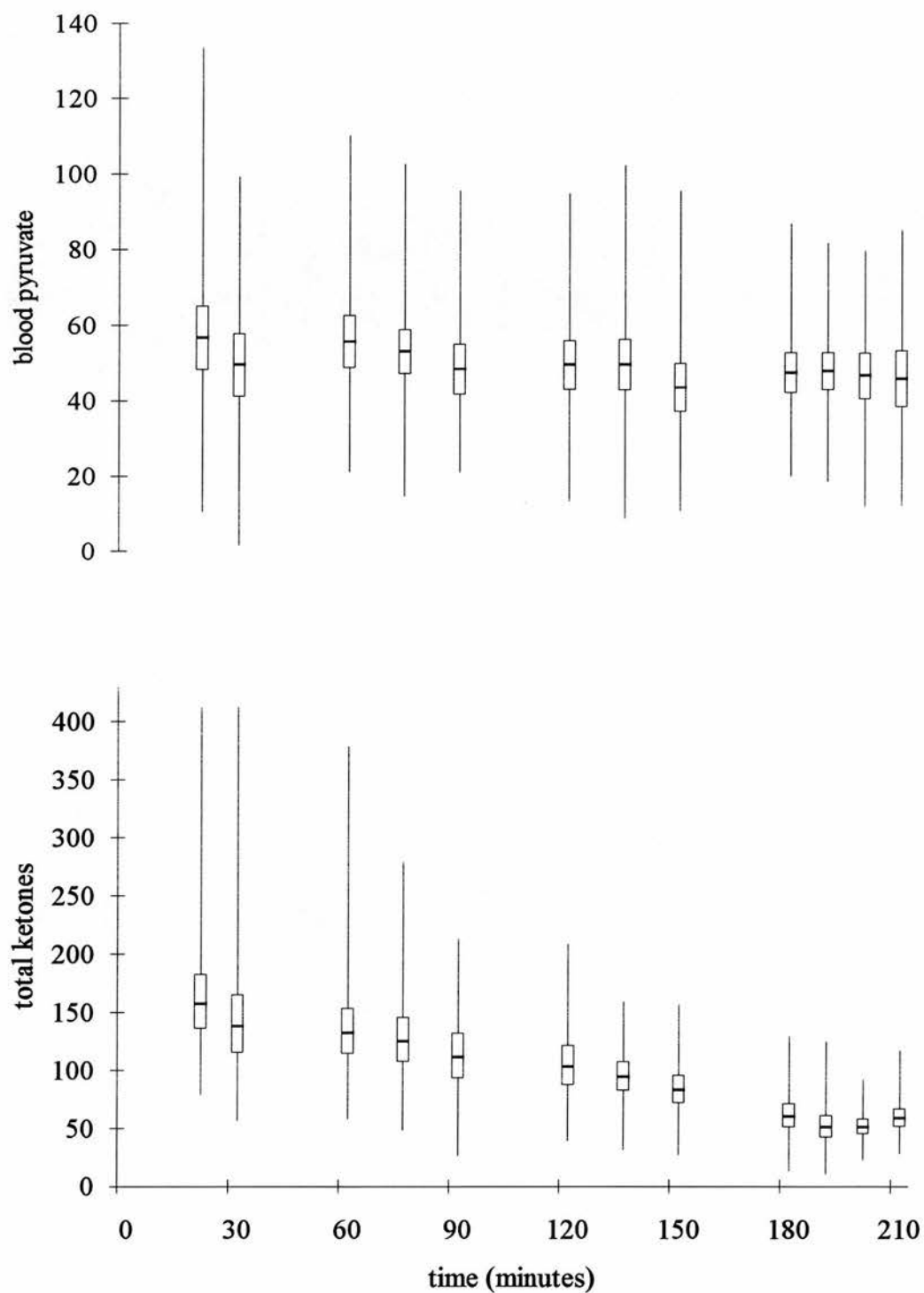


Figure 6.4. Top: mean (—), SEM (box) and range (vertical lines) for blood pyruvate concentration ($\mu\text{mol/L}$) at each time point during low-dose insulin infusion. Bottom: total blood ketones ($\mu\text{mol/L}$); layout as above (geometric mean).

There were no significant differences between 'low' and 'high' groups for mean fasting blood glucose or (geometric) mean fasting plasma insulin concentrations.

Effect of high versus low body mass index

There were no differences observed between the two groups (low BMI vs. high BMI; mean 26.16 vs. 31.26; $p < 0.0001$) for the regression slopes for glucose, nor for ketones.

Clear differences were present between the parallel slopes for glycerol ($p = 0.038$), with low BMI associated with lower blood glycerol concentrations, and there was a trend, not quite reaching statistical significance, for low BMI to be associated with lower NEFA levels ($p = 0.059$).

These data are summarised in table 6.2 (b); regression slopes for the two groups are shown in figure 6.6 (a and b) for each metabolite.

There were no significant differences between 'low' and 'high' groups for mean fasting blood glucose or (geometric) mean fasting plasma insulin concentrations.

Effect of high versus low percent body fat

There was a clear separation between 'low' and 'high' percent body fat (mean 26.19% vs. 33.23%; $p = 0.0007$). However, no differences emerged between regression slopes for any of the four metabolites studied (table 6.2 (c)). (Regression slopes are not shown.)

There were no significant differences between 'low' and 'high' groups for mean fasting blood glucose or (geometric) mean fasting plasma insulin concentrations.

Effect of high versus low fasting blood glucose

Mean fasting glucose concentration was significantly lower in the 'low' glucose than in the 'high' glucose group (5.77 vs. 8.77mmol/L; $p=0.0001$) and this was reflected in lower blood glucose levels at all insulin concentrations ($p=0.002$). However, concentrations of none of the other metabolites differed between the two groups (summarised in table 6.2 (d)). The regression slopes for 'low' versus 'high' FBG are shown in figure 6.7 (a and b). Differences between 'low' and 'high' groups for mean fasting plasma insulin concentration were not statistically significant.

Correlation of influencing variables with insulin sensitivity assessed by euglycaemic clamp

Figure 6.8(a) shows whole blood glucose concentrations during the euglycaemic hyperinsulinaemic clamp, as measured at the bedside using the YSI glucose analyser. The desired blood glucose concentration of 5mmol/L was usually reached within 60 minutes of starting the insulin infusion. The glucose infusion rates are shown in figure 6.8(b); clearly because of the range of fasting blood glucose levels in these subjects the infusion rates varied considerably between individuals. Figure 6.8(a) also shows plasma insulin concentrations reached during the procedure (measured at baseline, approx. 40 and 60 mins, and at 10 minute intervals during the final 20 minutes of the procedure). The tendency for an initial overshoot in insulin concentrations before settling to stable levels after the initial loading infusion accords with that originally described by DeFronzo et al (1979).

Having calculated M values from the clamp studies, and ranked all variables in ascending order, correlation coefficients were obtained for relationships between the clamp-derived index of insulin sensitivity and indices of body fat. M values showed no

statistically significant association with BMI ($r_s=-0.11$; $p=0.71$), waist-to-hip ratio ($r_s=-0.05$; $p=0.87$) or percent fat ($r_s=-0.13$; $p=0.67$), nor with fasting blood glucose ($r_s=-0.44$; $p=0.1$) but M value was significantly related to fasting plasma insulin concentration ($r_s=-0.74$; $p=0.0002$).

Regression slopes for M on each of the body fat indices were calculated, using the actual values rather than ranks. None of these slopes showed a statistically significant difference from zero. Data are shown in table 6.3 with scattergraphs for M against BMI and percentage body fat in figure 6.9, and M versus waist-hip ratio and waist in figure 6.10.

Conclusions

In this group of subjects with established NIDDM no clear pattern emerges to show an influence of any index of body fat on insulin sensitivity with respect to the metabolites studied. Surprisingly, lower waist-hip ratio was associated with greater insulin resistance to glucose and ketone metabolism, although lower body mass index was associated with greater insulin sensitivity for NEFA and glycerol metabolism. Greater numbers of subjects may be needed to confirm these findings, and a type I error cannot entirely be excluded. While other factors not considered here may be important, the diabetic state per se may have an overwhelming effect which obscures any influence of these factors.

Because of the small size of this study it has not proved possible to comment with any confidence (eg. using multiple regression analysis) on the relative contributions of the variables studied. In particular, the effect of fasting blood glucose and the impact of clamping at euglycaemia rather than isoglycaemia may have been important confounders.

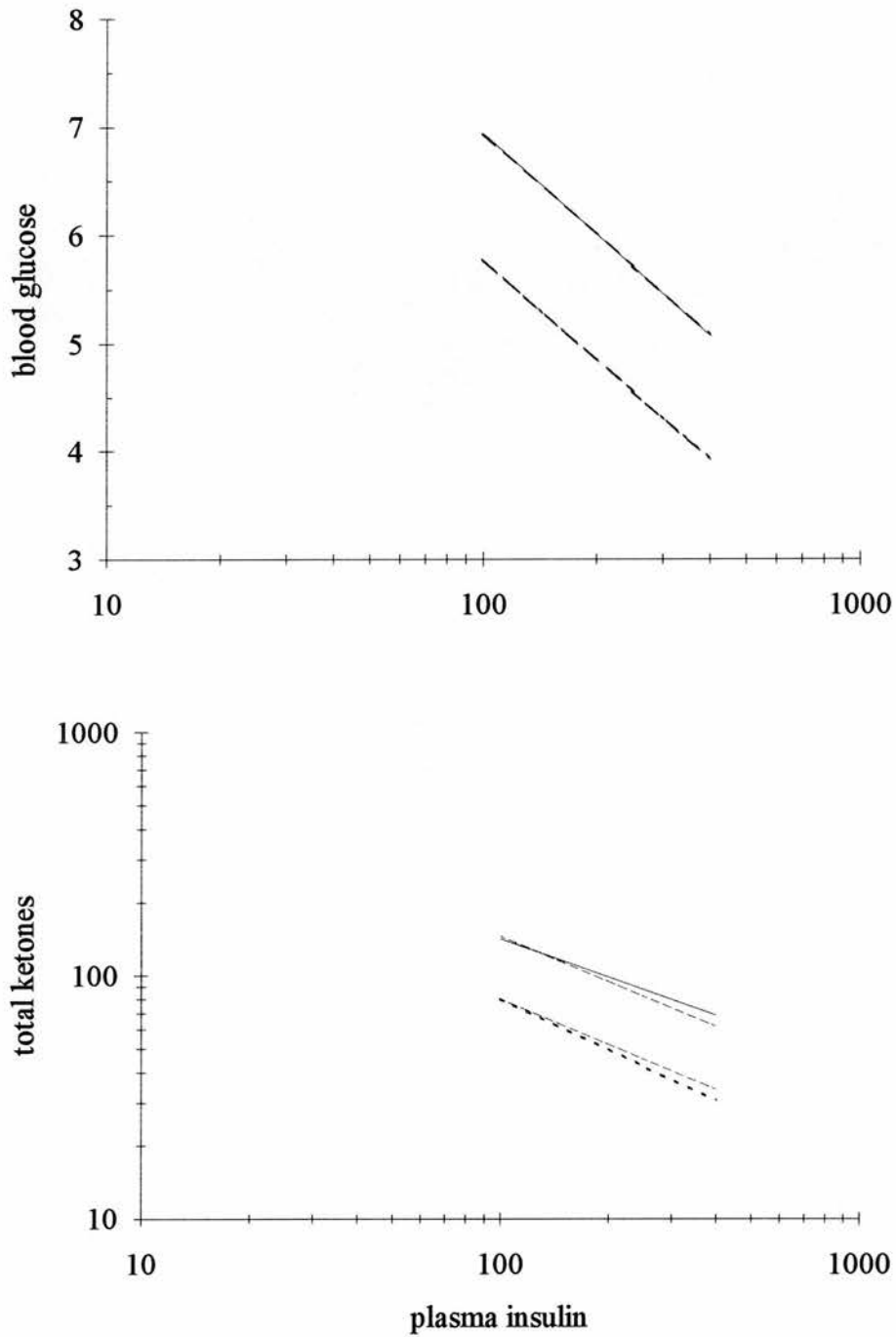


Figure 6.5 (a). Comparison of group regression slopes of metabolite against insulin concentrations calculated for low vs. high waist-hip ratio. Top - glucose (mmol/L); bottom - ketones ($\mu\text{mol/L}$). Legend - low WHR ———; high WHR - - - - (separate slopes); — — — parallel lines for common slope (lines overlie for glucose).

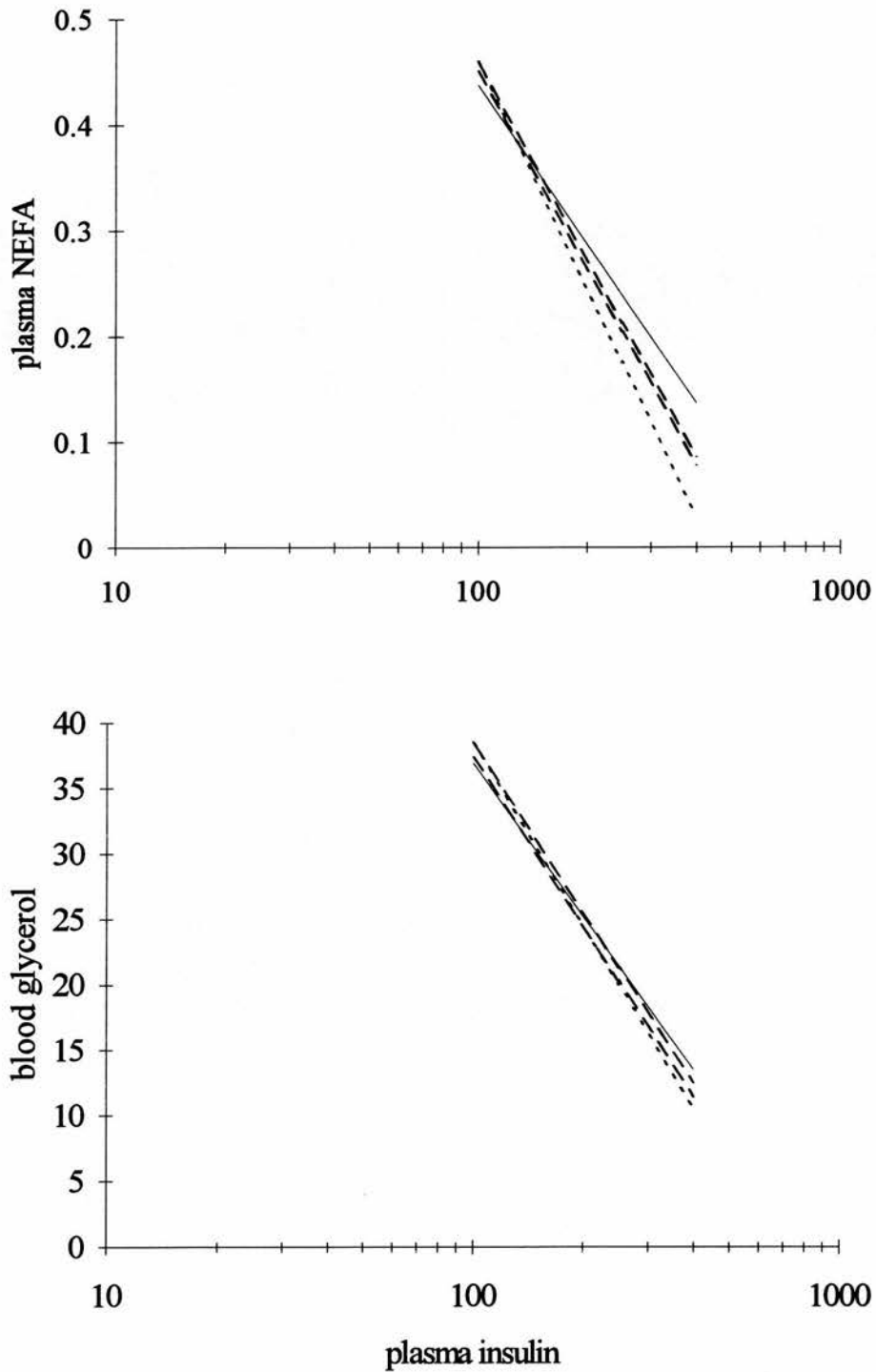


Figure 6.5 (b). Separate regression slopes for low vs. high waist-hip ratio: top - NEFA (mmol/L); bottom - glycerol ($\mu\text{mol/L}$). Legend as for fig. 6.5 (a).

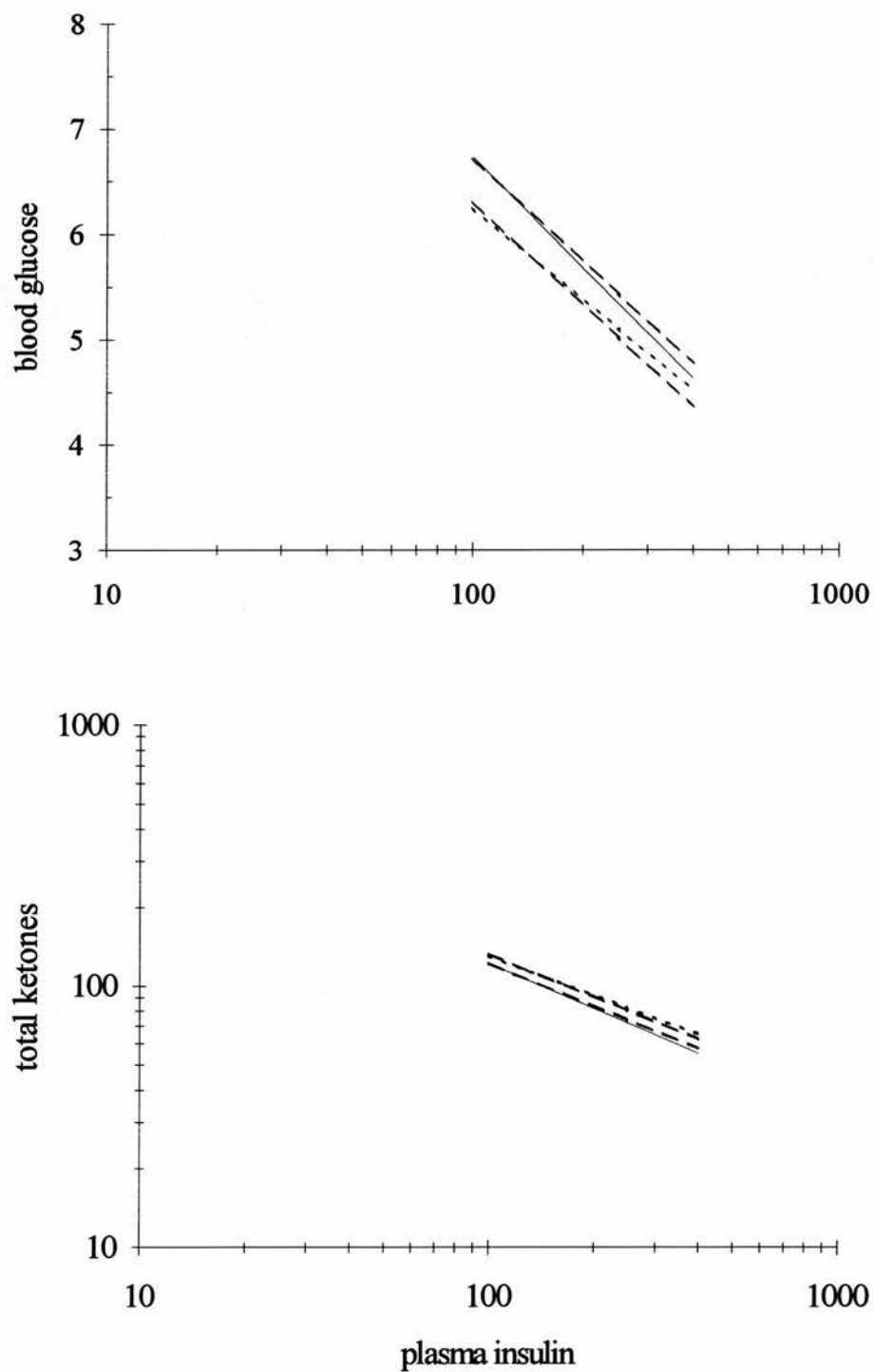


Figure 6.6 (a). Comparison of separate group regression slopes of metabolite against insulin concentrations calculated for low vs. high body mass index. Top - glucose (mmol/L); bottom - ketones ($\mu\text{mol/L}$). Legend as for fig. 6.5.

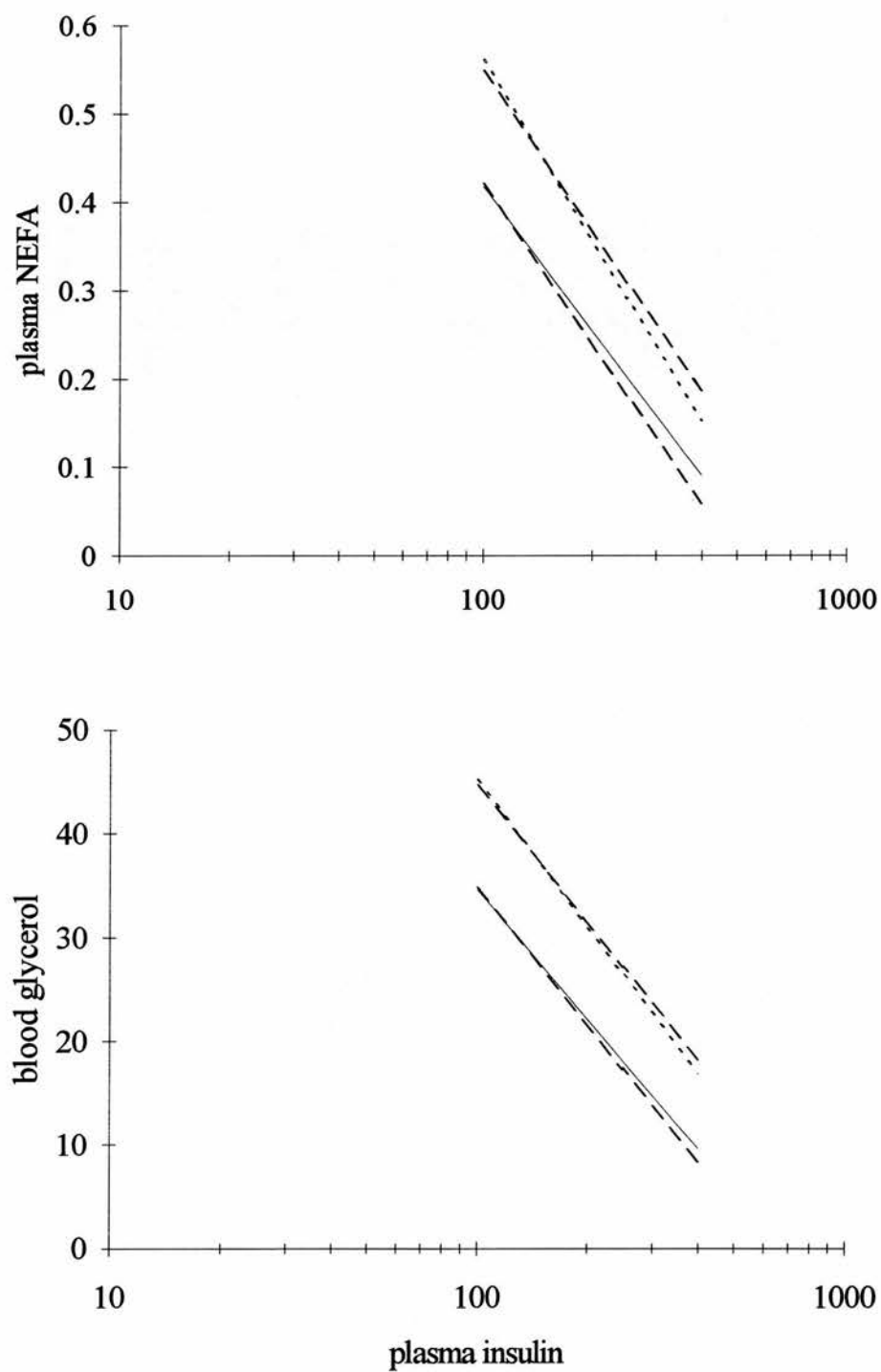


Figure 6.6 (b). Separate regression slopes for low vs. high body mass index: top - NEFA (mmol/L); bottom - glycerol ($\mu\text{mol/L}$). Legend as for fig. 6.5.

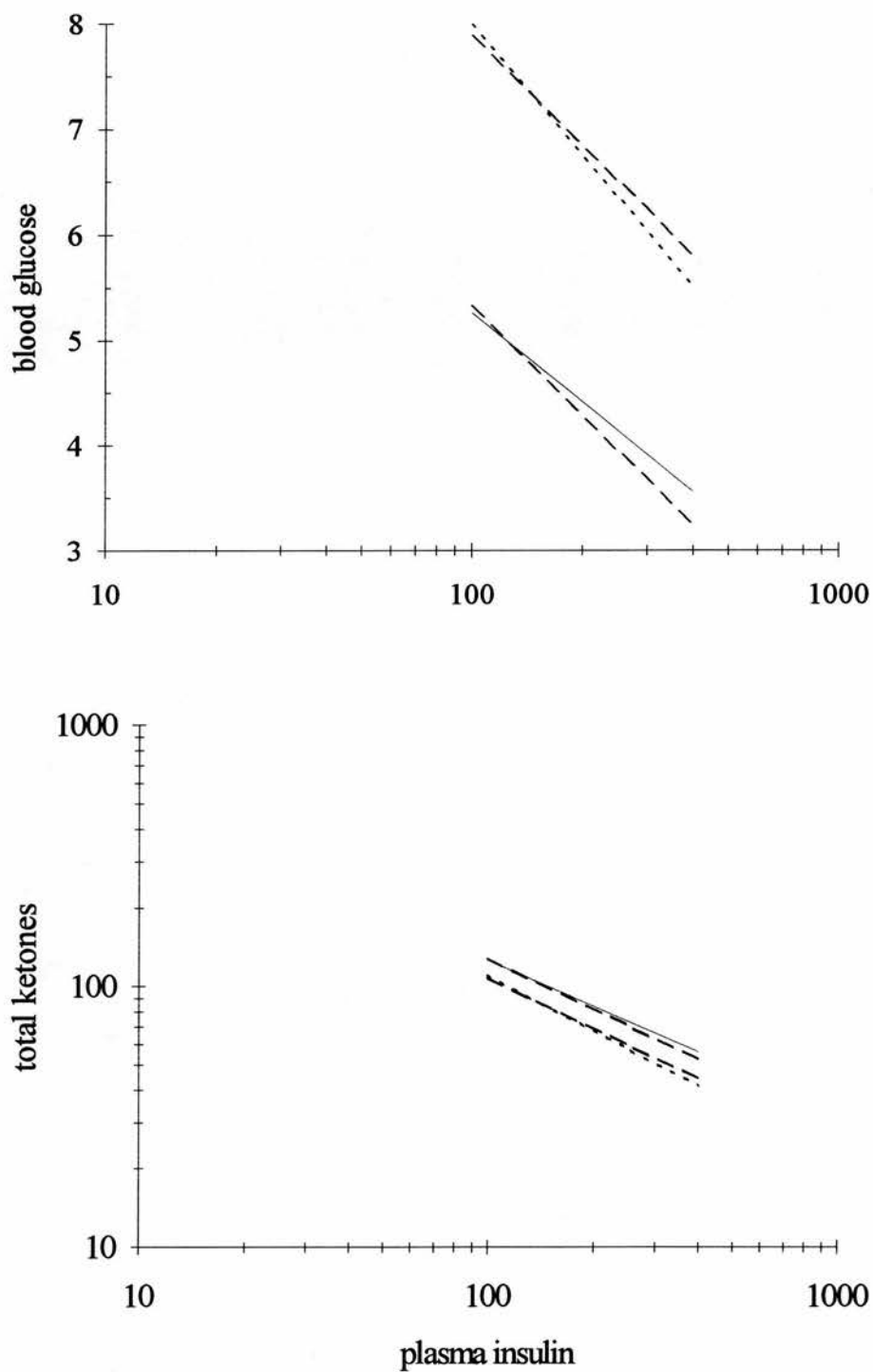


Figure 6.7 (a). Comparison of separate group regression slopes of metabolite against insulin concentrations calculated for low vs. high fasting blood glucose. Top - glucose (mmol/L); bottom - ketones ($\mu\text{mol/L}$). Legend as for fig. 6.5.

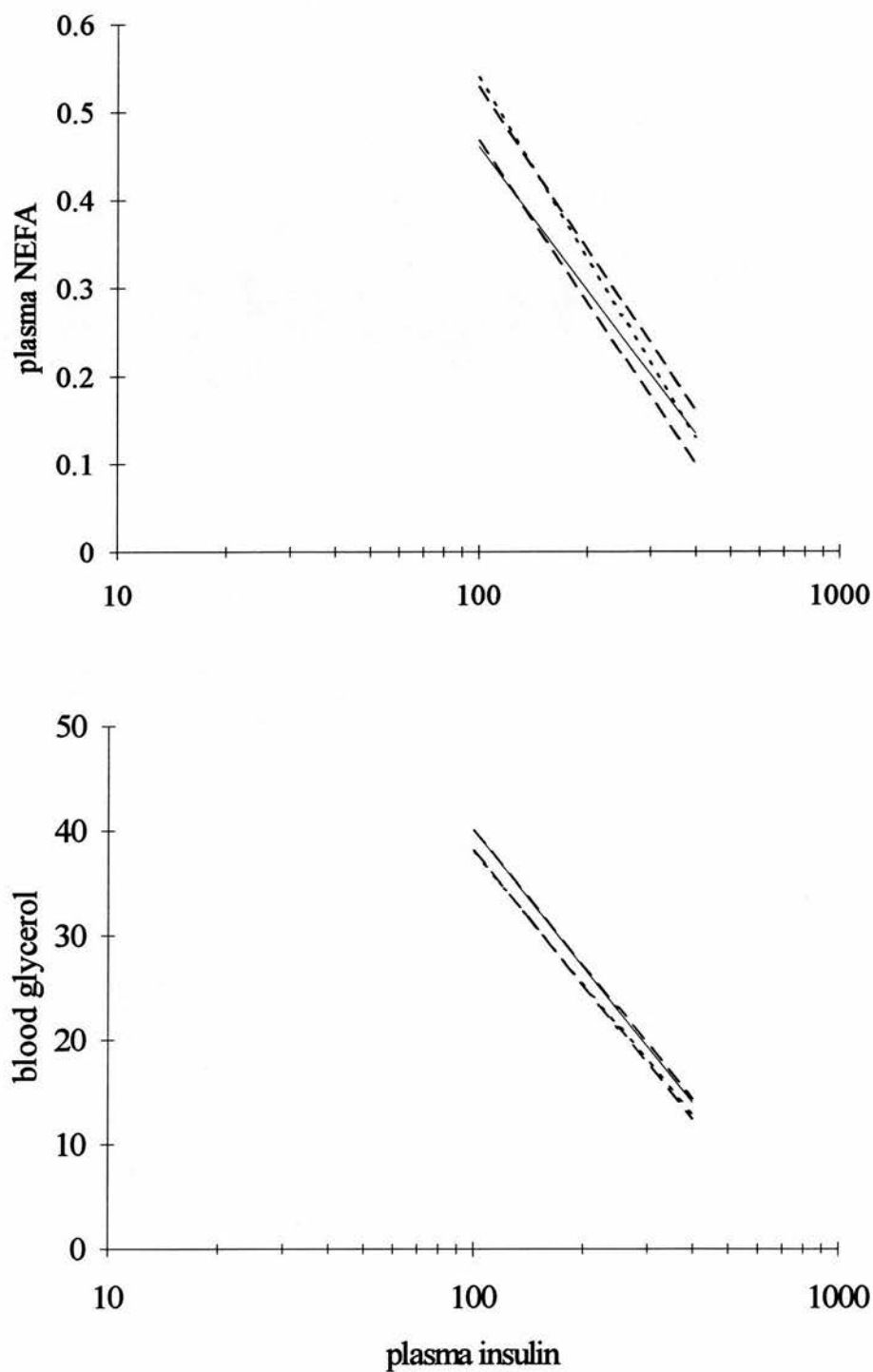


Figure 6.7 (b). Separate regression slopes for low vs. high fasting blood glucose: top - NEFA (mmol/L); bottom - glycerol ($\mu\text{mol/L}$). Legend as for fig. 6.5.

Table 6.2. Effects of variables shown on separation between common regression lines (arithmetic means shown for glucose, NEFA and glycerol; geometric means for ketones and insulin). Corrected mean metabolite concentrations at overall mean insulin concentration are shown; means for insulin are those for 'low' and 'high' groups respectively.

(a) *comparison by WHR*

	corrected mean		<u>separation</u>	<u>p</u>
	<u>low</u>	<u>high</u>		
Glucose	6.74	5.58	1.16	0.067
NEFA	0.42	0.42	0.008	0.63
Glycerol	34.7	35.7	1.06	0.48
*Ketones	133.1	73.5	0.26	0.019
Insulin	128.7	103.4	n/a	

(b) *comparison by BMI*

	corrected mean		<u>separation</u>	<u>p</u>
	<u>low</u>	<u>high</u>		
Glucose	6.38	5.97	0.42	0.28
NEFA	0.36	0.49	0.12	0.059
Glycerol	30.3	40.1	9.89	0.038
*Ketones	107.4	116.6	0.04	0.37
Insulin	109.9	146.1	n/a	

(c) comparison by % fat

	corrected mean		<u>separation</u>	p
	<u>low</u>	<u>high</u>		
Glucose	5.70	6.42	0.72	0.16
NEFA	0.43	0.38	0.05	0.22
Glycerol	33.0	36.3	3.21	0.25
*Ketones	123.2	99.0	0.09	0.13
Insulin	93.8	154.3	n/a	

(d) comparison by fasting blood glucose

	corrected mean		<u>separation</u>	p
	<u>low</u>	<u>high</u>		
Glucose	4.88	7.44	2.56	0.002
NEFA	0.39	0.45	0.06	0.19
Glycerol	34.6	32.6	1.94	0.36
*Ketones	105.9	89.3	0.07	0.21
Insulin	123.5	145.0	n/a	

* antilog

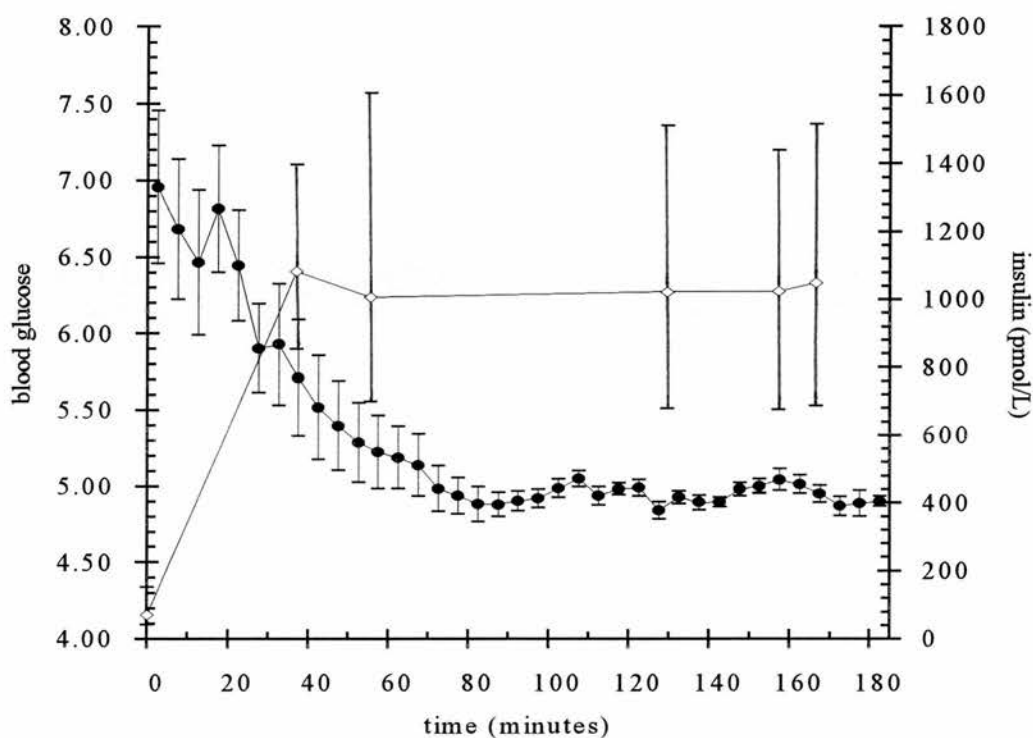


Figure 6.8(a). Mean (\pm SEM) whole blood glucose (\bullet , mmol/L), and geometric mean (\pm range) plasma insulin (\diamond , pmol/L) concentrations achieved against time (minutes) during the euglycaemic hyperinsulinaemic clamp.

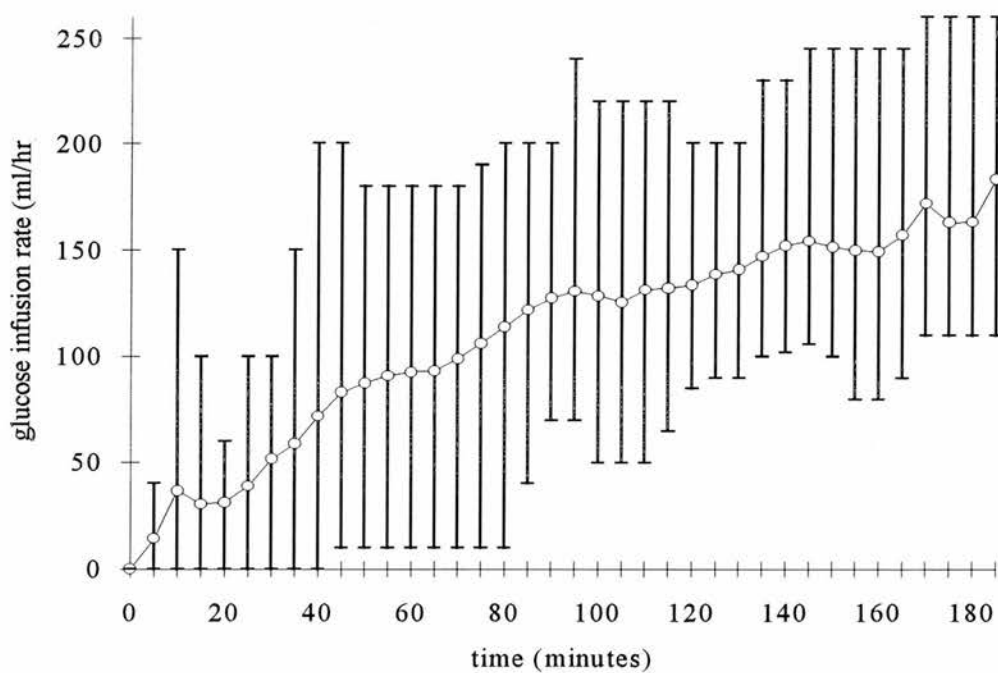


Figure 6.8(b). Mean (and range) glucose infusion rate during the clamp procedure.

Table 6.3. Regression slopes for M values on indices of body fat.

	r^2	a	slope (b)	confidence limits	p
WHR	0.004	7.72	-1.7	-19.8 - 16.4	0.84
BMI	0.013	7.62	-0.06	-0.38 - 0.27	0.71
%fat	0.004	6.63	-0.021	-0.25 - 0.21	0.84
waist	0.0002	6.31	-0.003	-0.13 - 0.12	0.95

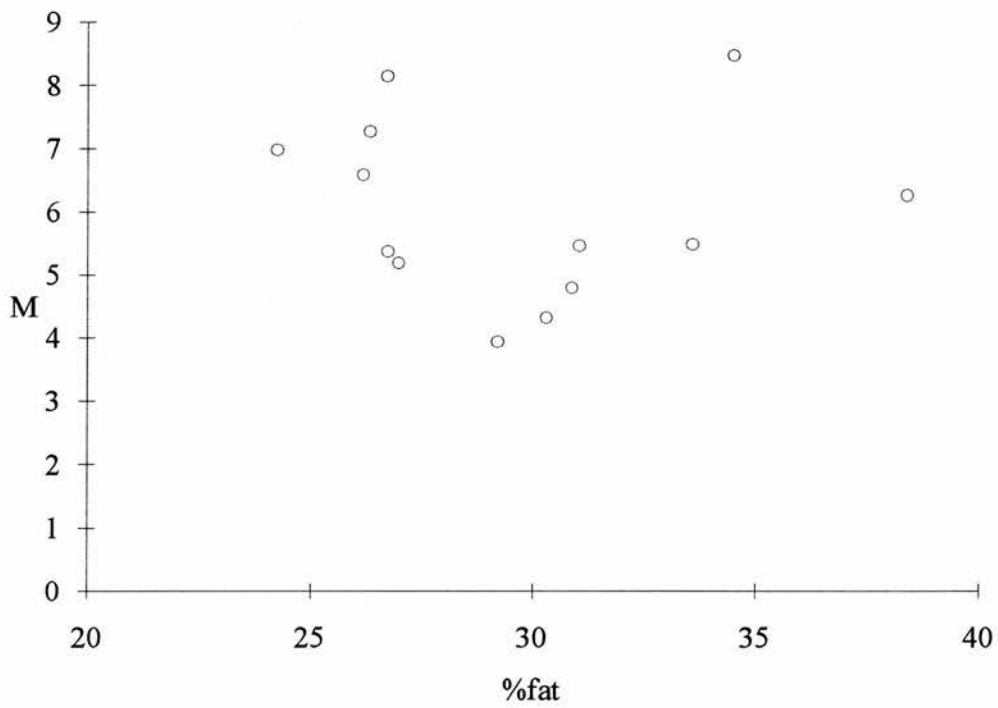
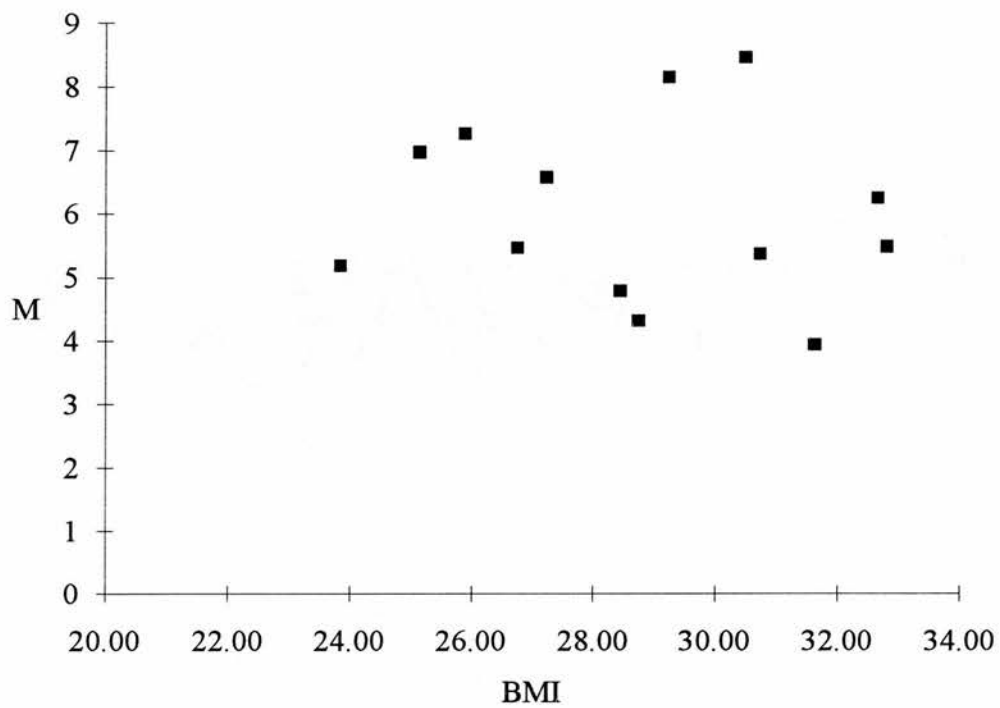


Figure 6.9. Relationships between M (y axis) and BMI (top) and percent body fat (bottom).

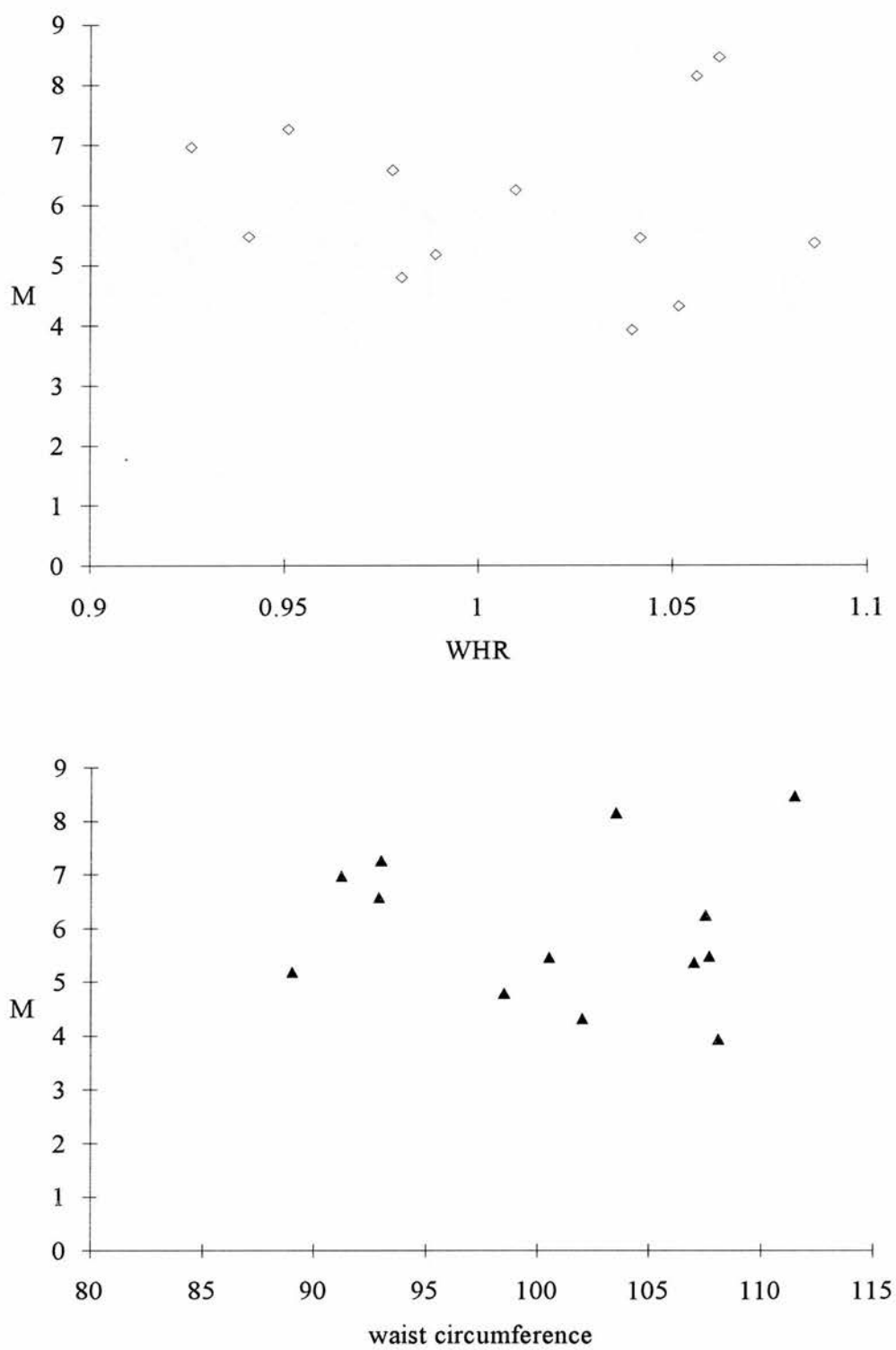


Figure 6.10. Relationships between M (y axis) and waist-hip ratio (top) and waist circumference (bottom).

CHAPTER 7

Use of the low-dose incremental insulin infusion in assessment
of insulin resistance in NIDDM

Introduction

Several methods are currently available for the assessment of insulin resistance, almost all of which address only the role of insulin in glucose metabolism and give little or no attention to its contribution to other aspects of metabolism. The low-dose incremental insulin infusion (Hale et al, 1986) was developed to examine insulin action over the physiological range of insulinaemia, fulfilling the requirement for Kahn's definition (Kahn, 1978) of insulin resistance to assess insulin action over a range of concentrations, and allowing examination of changes in concentration of several metabolites with changes in plasma insulin levels.

This method has not hitherto been subjected to comparison with other techniques for assessing insulin resistance. In this chapter, using data obtained from one of the studies described elsewhere in this manuscript, I propose to address this area.

Subjects and Methods

For comparison with other techniques of measuring insulin resistance, data from the subjects studied in chapter 6 have been used. In this study, as described, subjects underwent a low-dose incremental insulin infusion and a euglycaemic hyperinsulinaemic clamp in random order, separated by 7-35 days. From the fasting blood samples at each visit an estimate of insulin resistance by homeostasis modelling assessment (HOMA-R; Matthews et al, 1985) was also obtained.

Statistical methods

Data from the low-dose insulin infusion procedure are analysed by calculation of the regression line for metabolite on log insulin concentrations, with subsequent analysis

of variance applied to the regression line obtained. This has normally been used for data from groups of subjects with regression lines derived on log mean insulin concentrations. However, calculation of individual regression lines is possible, and I have done this here primarily in order to obtain ranks for subjects. When individual regression lines are calculated several options become available and the choice of which measure to use becomes to some extent arbitrary. The individual regression slopes for glucose on log [insulin] are shown in figures 7.1 and 7.2.

Here regression lines have been calculated for glucose for each individual on his own plasma insulin data. Both separate and common slopes have been used, and three measures of insulin resistance used for comparison with each other and with other methods: firstly, rank order within the group based on the common regression slope (i.e. where the regression line is determined by $y = a + bx$, the common slope derived from a pooled value of b means that this measure is dependent on the individual value for a); secondly and thirdly, from the separate regression lines, estimates of insulin resistance based on the blood glucose concentrations obtained from calculation of the regression equation at insulin concentrations of 100 and 400pmol/L (13.4 and 53.6mU/L), designated here I100 and I400 respectively.

Insulin resistance estimated from the euglycaemic hyperinsulinaemic clamp is calculated as a value M from the quantity of glucose infused during the last 30-60 minutes of the procedure while maintaining a constant measured blood glucose concentration. Higher values of M therefore indicate greater sensitivity to insulin.

Insulin resistance estimated by HOMA-R is calculated from fasting blood glucose and plasma insulin concentrations using the equation insulin resistance value = [glucose (mmol/L)] x [insulin (mU/L)] / 22.5. Higher values indicate greater degrees of insulin resistance.

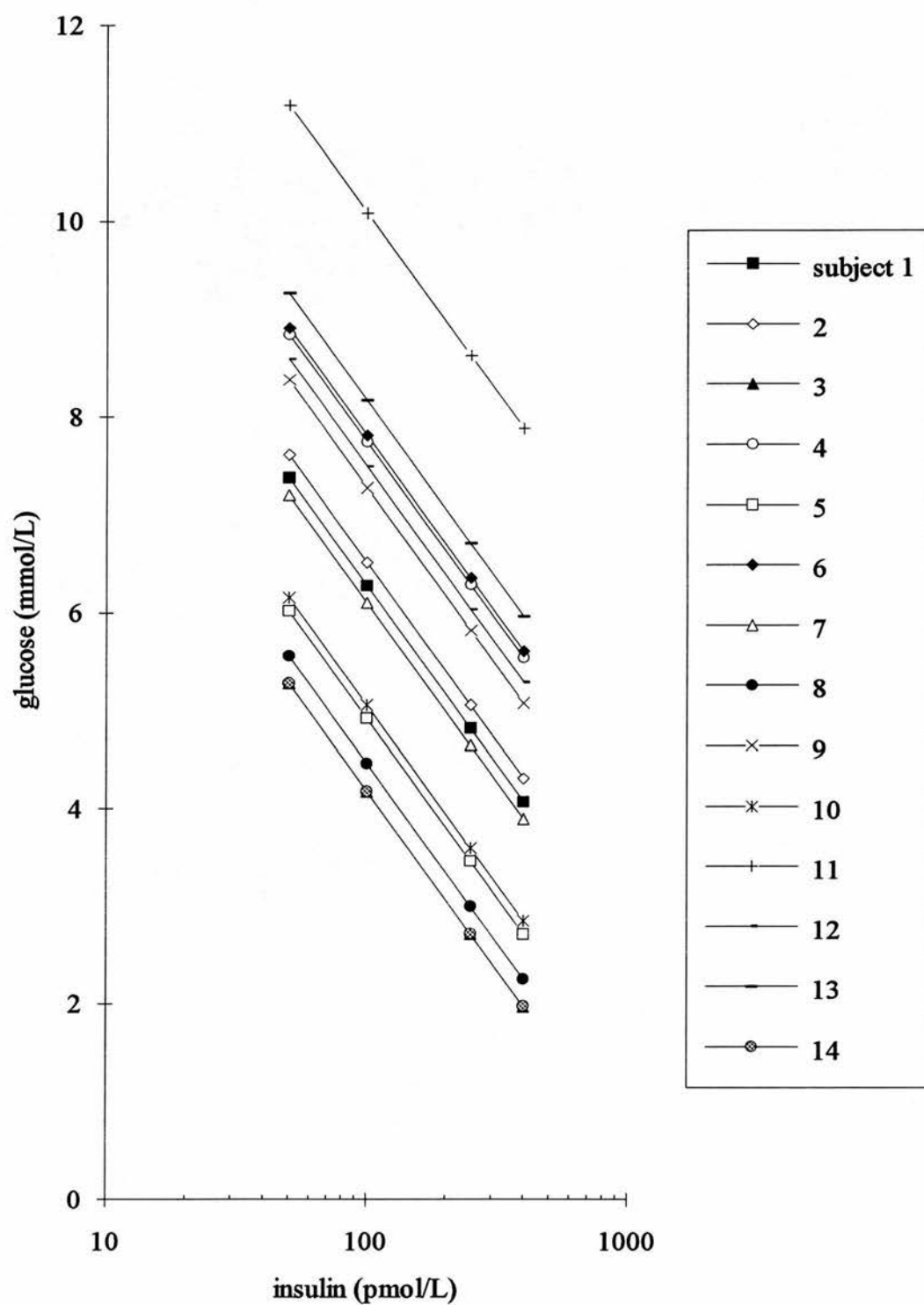


Figure 7.1. Relative positions on common slope for individual insulin sensitivity to glucose metabolism from which data for ranking were obtained.

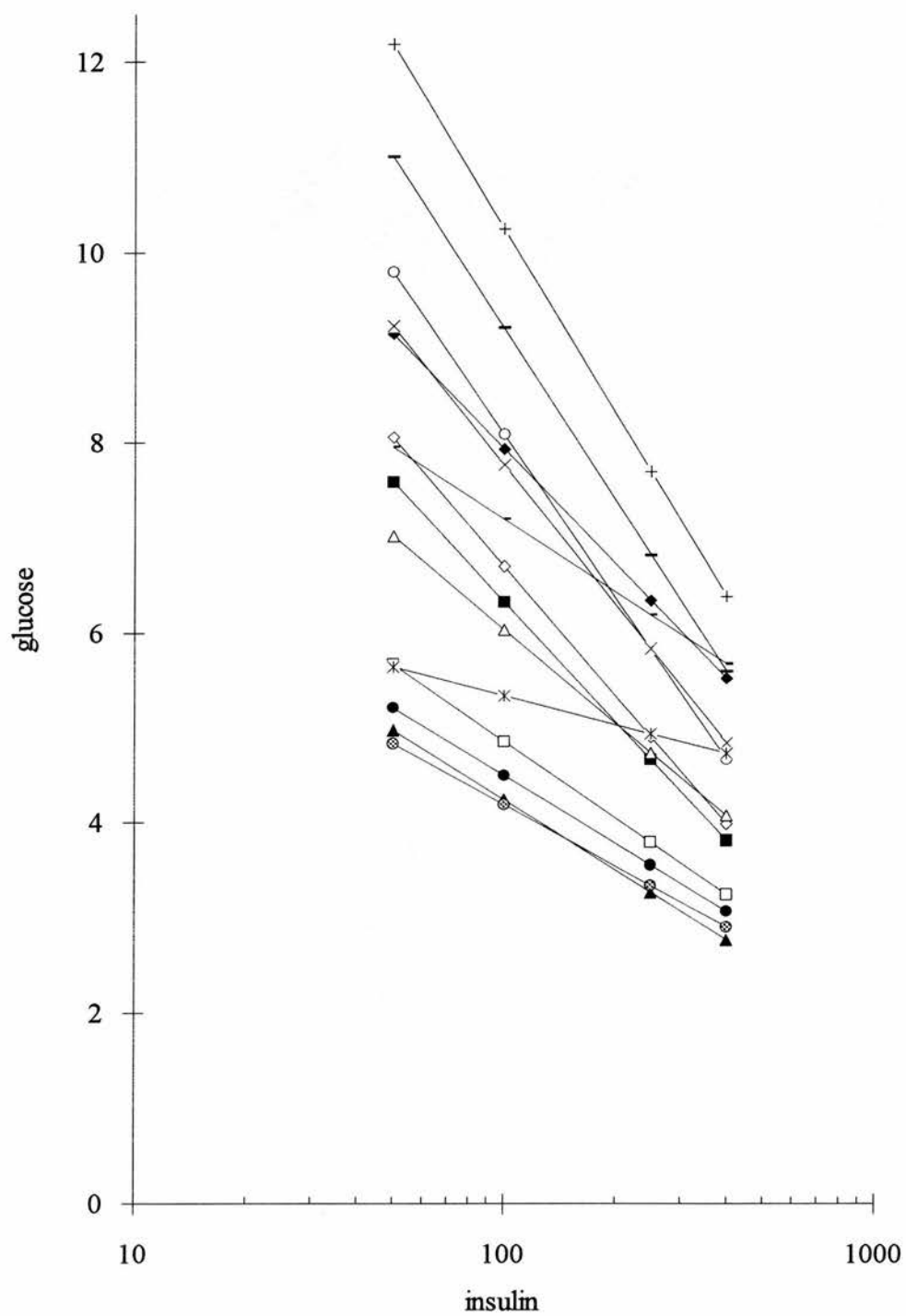


Figure 7.2. Individual slopes for blood glucose on $\log [\text{insulin}]$ from which data for I100 and I400 were obtained. (Legend as for figure 7.1.)

Values obtained by each of these methods were ranked in ascending order and correlation coefficients obtained using Spearman's rank correlation test. The critical p value for statistical significance after correction for multiple comparisons was 0.0025. Using the original data, regression lines were also obtained to examine further the interrelationships between fasting glucose and insulin concentrations and insulin resistance data obtained.

Results

The three estimates of insulin resistance derived from the low-dose insulin infusion were all highly correlated with each other ($r_s > 0.85$; $p < 0.000001$), and all correlated well with insulin resistance estimated from HOMA-R ($r_s > 0.85$; $p < 0.000001$). Although M values from the euglycaemic clamp correlated to a significant extent with HOMA-R values ($r_s = -0.66$; $p = 0.023$), relationships with insulin infusion-derived indices of insulin resistance did not reach statistical significance (r_s for the three comparisons between -0.42 and -0.44 ; $p = 0.10-0.12$).

Fasting plasma insulin concentration did not relate closely to any of the insulin infusion-derived indices, but was closely linked to M values ($r_s = -0.74$; $p = 0.0002$). Since HOMA-R values are obtained by calculation directly from fasting plasma insulin concentrations, a close relationship is to be expected ($r_s = 0.72$; $p = 0.0002$), as was also the case for fasting blood glucose ($r_s = 0.78$; $p = 0.00002$). Fasting blood glucose concentration was also closely related to all three insulin infusion indices ($r_s \geq 0.79$; $p \leq 0.00001$) but not to M values ($r_s = -0.44$; $p = 0.10$).

Regression lines were calculated using the original data obtained, without ranking or transformation, to clarify further the extent of the relationships between these factors.

Table 7.1. Calculated regression lines.

	r^2	<i>slope (b)</i>	<i>a</i>	<i>confidence limits (b)</i>	<i>p</i>
I400 on M	0.21	-0.39	6.70	-0.89 - 0.11	0.113
FBG	0.75	0.6	0.034	0.38 - 0.82	0.00006
FPI	0.031	0.01	4.80	-0.02 - 0.014	0.55
HOMA-R	0.54	0.42	2.91	0.17 - 0.66	0.003
M on FBG	0.17	-0.35	8.51	-0.88 - 0.17	0.17
FPI	0.07	-0.01	6.79	-0.03 - 0.013	0.38
HOMA-R	0.43	-0.44	7.55	-0.77 - -0.1	0.016
I100 on HOMA-R	0.55	0.69	4.18	0.30 - 1.09	0.002

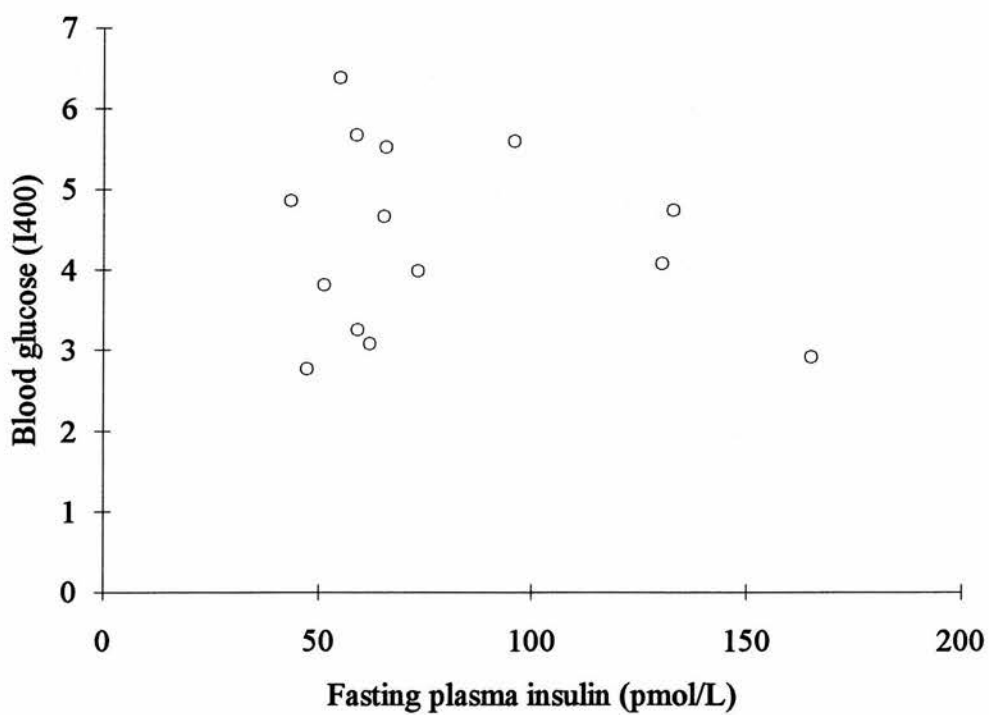
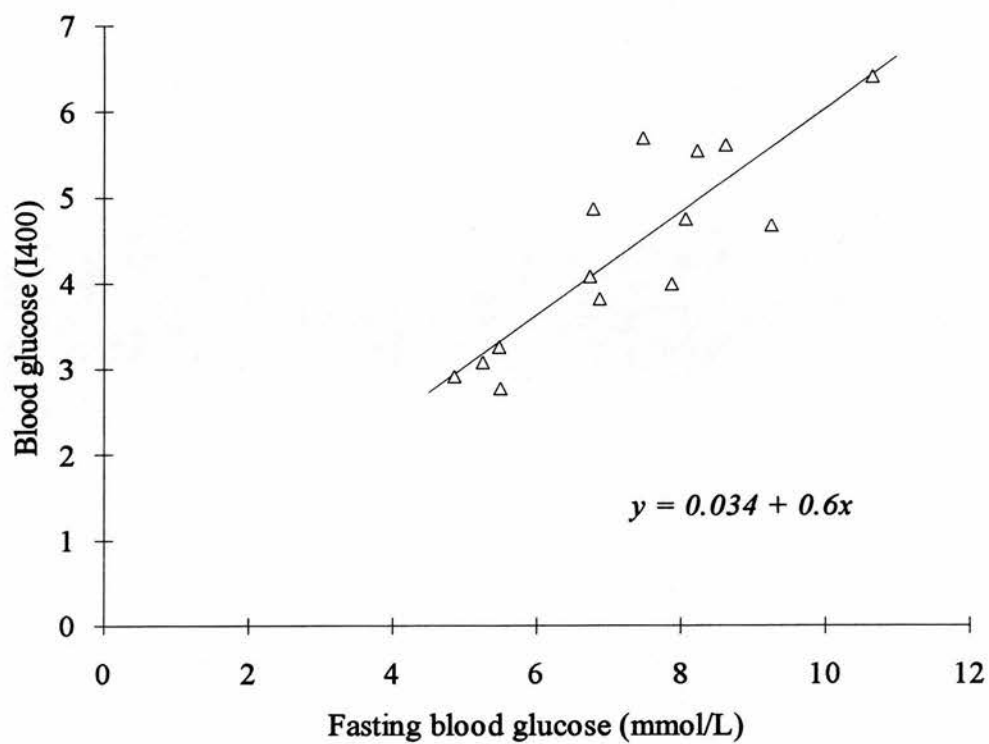


Figure 7.3. Regression slope for I400 on fasting glucose (top) and fasting insulin (bottom) concentrations.

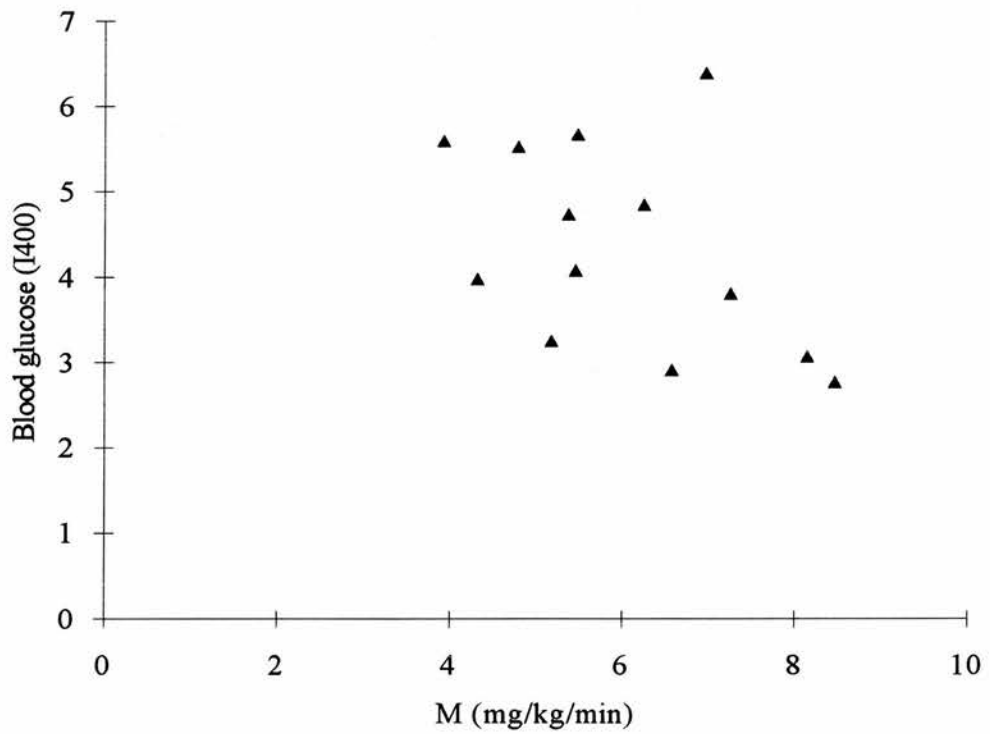
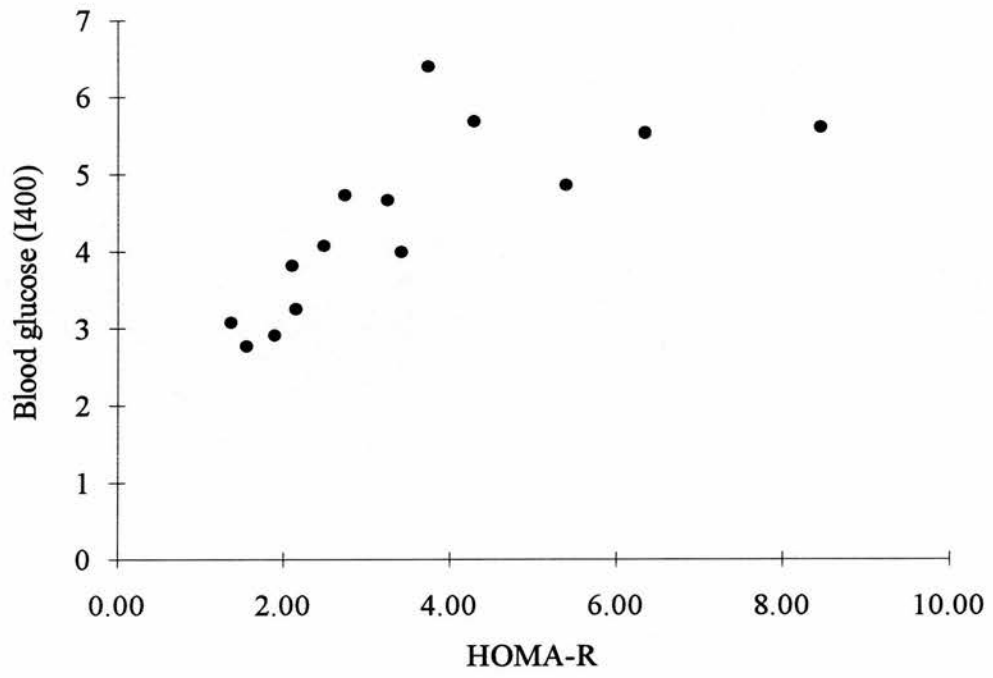


Figure 7.4. Relationships for I400 (y axis) with HOMA-R values (top) and M values (bottom).

Statistically significant associations were obtained for regression of I400 on fasting glucose and HOMA-R, and for M on HOMA-R (see table 7.1 for details). Scattergraphs showing the relationships for I400 with fasting glucose and insulin are shown in figure 7.3, and for I400 with M values and HOMA-R values in figure 7.4.

Conclusions

Assessment of the use of the low-dose insulin infusion technique in this way is necessarily limited and it has not been possible with data from these studies to assess repeatability of the technique.

The three measures of insulin resistance used to assess this technique are all highly correlated, and also correlate well with the HOMA-R values obtained. The relationship with clamp values is less strong, although this is a small dataset and NIDDM subjects may form a special category. It is important to remember that the two methods are assessing different phenomena and to some extent any comparison may be invalid. The strengths of the insulin infusion method lie in its ability to assess effects of insulin on several metabolic variables simultaneously, and at approximately physiological concentrations of insulin. The complexity of the calculations involved make its use more suitable for comparison between groups rather than between individuals.

CHAPTER 8

Conclusions

Introduction

These studies have all been carried out in subjects with NIDDM, all recruited from a general hospital diabetic clinic. This is perhaps an innately heterogeneous group, particularly in terms of fasting blood glucose, and risk factors for both diabetes and coronary heart disease. This very heterogeneity, while in some respects problematic, does nonetheless make the population studied potentially representative of that seen in regular clinical practice.

In this concluding chapter I review each of these studies in turn, to draw conclusions from the evidence presented and relate these to the published literature.

Epidemiology of coronary risk factors in new NIDDM (chapter 3)

Concepts of coronary risk factor clusters are not new (Vague, 1956), although claims for the pre-eminence of one factor over others are more recent (Modan et al, 1985; Reaven, 1988; Kaplan, 1989; Ferrannini et al, 1991; DeFronzo and Ferrannini, 1991). In particular, Reaven's description of 'Syndrome X' stimulated widespread interest in addressing these multiple risk factors, and data have been presented from population studies to support the existence of a syndrome (Zavaroni et al, 1989; Haffner et al, 1992).

While most previous studies of insulin resistance, metabolic syndromes and coronary risk factors have addressed primarily a non-diabetic or perhaps 'potential diabetic' population, once the presence of diabetes is recognised coronary risk increases and it is important to address the needs of this large population. Even in newly-diagnosed diabetes the prevalence of coronary heart disease has been estimated at three times that of the normal population (Uusitupa et al, 1985).

Clustering of metabolic risk factors in terms of Syndrome X or the metabolic syndrome has not previously been studied in a diabetic population. The group chosen, comprising subjects within three months of diagnosis, was intended to examine subjects who would have at least relatively preserved insulin secretory capacity, and in whom confounding influences of diabetes itself and its treatment would not have been superimposed. Thus I believe this to be the most homogeneous diabetic population to study, and perhaps also the 'least diabetic'.

The data from this study show a number of features in broad agreement with those reported elsewhere. A similar prevalence figure for hypertension to that found here (50%) has been reported by the United Kingdom Prospective Diabetes Study (1985), with rates of 40% for men and 53% for women. The pattern of lipid abnormalities, with high rates of hypertriglyceridaemia and low HDL-cholesterol concentrations has also been previously described (Uusitupa et al, 1986; Gibbons, 1986) and it has been suggested that (familial) hypertriglyceridaemia may predict the development of NIDDM (Sane and Taskinen, 1993).

The finding of a normal distribution pattern for the number of features of syndrome X present is at variance with the data from the San Antonio Heart Study (Ferrannini et al, 1991) which reported in a large biethnic (white and Hispanic) population considerable overlap between diabetes or impaired glucose tolerance, obesity, hypertension, hypertriglyceridaemia and hypercholesterolaemia. The authors found a tendency to multiple combinations, and concluded that hyperinsulinaemia was the key unifying factor. The present study gives a certain amount of support to this view in that both hyperinsulinaemia and hypertriglyceridaemia were much more likely to be associated with multiple additional risk factors than with only one other; hence hyperinsulinaemia or hypertriglyceridaemia in conjunction with glucose intolerance (here frank diabetes), seem likely to predict the presence of additional risk factors for

coronary heart disease. In contrast, the other risk factors studied, most notably hypertension and insulin resistance, appeared here not to predict the presence of additional risk factors. The number of additional risk factors in the presence of either hypertension or insulin resistance followed an approximately normal distribution.

A tendency to clustering of metabolic risk factors is shown by the data presented here, although the distribution differs from that described elsewhere (Ferrannini et al, 1991). The distribution was similar for both Caucasian and Asian subjects; the group of black subjects, although too small to analyse formally, showed a tendency to fewer coronary risk factors than the others.

Long-term follow-up data from the San Antonio Heart Study (Haffner et al, 1992) supports the predictive value of hyperinsulinaemia for the development of both hypertriglyceridaemia and low HDL-cholesterol but the relationship with the development of hypertension was weak. Saad et al (1991) have suggested that this association may be present only in some racial groups, and Lind et al (1993) contended that insulin resistance is more closely related (than hyperinsulinaemia) with hypertension. Every et al (1993) found a relationship for higher insulin and C-peptide concentrations with higher diastolic blood pressure among non-diabetic subjects; this association was modified by obesity.

In keeping with previous studies, and notably in support of Reaven's exclusion of it from his original definition of a metabolic syndrome (Reaven, 1988), serum cholesterol concentration had a limited relationship with other coronary risk factors, as did age. It is possible that total/HDL-cholesterol ratios may be a more useful indicator of risk in this context, although I did not address this possibility here. Obesity, however, was related to hyperinsulinaemia and to greater frequency of risk factors. The index of obesity used appeared to be less important; body mass index was at least as good an indicator of risk as was waist-hip ratio, suggesting that obesity

per se rather than fat distribution may be important; this contrasts with the finding of Baynes et al (1991a) who reported that, at least in men with NIDDM, waist-hip ratio was a better predictor of the presence of atherogenic risk factors.

The use of 'number of features of the metabolic syndrome' as the dependent variable in multiple regression analysis here may appear at first sight somewhat specious. However, I would contend that it has allowed exploration of the relative importance of the known contributory factors, notably highlighting insulin and triglycerides, and raising the possibility of a female propensity, albeit modest. The failure of other factors, such as obesity, to take on a significant independent role seems to be accounted for by their associations with existing features of the syndrome (notably insulin concentration in the case of obesity).

The use of factor analysis adds an extra dimension to consideration of the metabolic syndrome and here I have been particularly interested in comparing these data with the findings of Meigs et al (1997). Both studies appear to yield remarkably similar results, with a three-component model based on essentially the same contributory factors. The apparent association of a raised HDL-cholesterol concentration with hypertension and obesity in this study may be anomalous, although it is worth noting that a similar trend was present in Meigs' study. This may warrant further examination.

Clearly this procedure can only be regarded as a descriptive technique to explore interrelationships between features measured or observed. Indeed, when I attempted to develop a model including all the factors used in the multiple regression analysis a highly complex multi-component model resulted with mutually exclusive groups (table 3.7 (b)). Overloading of this procedure appears to run the risk of producing a model which merely describes in complex patterns the population studied.

Probably the most important conclusion from these data, other than the remarkable consistency between these data and those of Meigs et al, is the stark disagreement between these findings and those of others (Ferrannini et al, 1991) over the distribution of risk factors in the respective populations studied. The data presented here, on the definitions used, clearly show an approximately normal distribution of risk factors. It seems reasonable to infer from this that diabetic individuals, like non-diabetics, vary considerably in their coronary risk, and not all will need to be automatically considered at high risk. This may accord better with the 'Common Soil' hypothesis than with concepts based on syndromes of risk factor clustering. This conclusion may render the concept of a metabolic syndrome less useful in a clinical context, but the lack of a clear dichotomy between high-risk and low-risk subjects should only add to clinicians' vigilance in coronary risk prevention.

While these data describe relationships between risk factors, clearly only longitudinal data can fully address the impact of these on outcomes. The proportion of the group studied having previously diagnosed coronary heart disease was small (28 of 231), and the definition of previously diagnosed coronary heart disease weak (self-report of such a diagnosis having been given), allowing only limited interpretation of data comparing subjects with and without known CHD. However, the absence of any significant differences between this and the rest of the study population, other than greater age and obesity (BMI) may raise doubts as to the importance of a metabolic syndrome, although any impact of treatment of heart disease cannot be determined. There are few longitudinal data in the literature, although recently the UKPDS group (Turner et al, 1998) identified five important modifiable risk factors for coronary heart disease in their diabetic population, namely hyperglycaemia, hypertension, low HDL-cholesterol, raised LDL-cholesterol and smoking.

A number of limitations to this study should be recognised. Although every effort was made to apply the duration of (known) diabetes criterion strictly, the often insidious mode of onset of this condition inevitably means that many subjects will have had abnormal glucose tolerance for a considerable time prior to diagnosis. Nonetheless, 84% of the subjects studied were assessed within 2 months of a diagnosis of diabetes being applied. Again where possible all insulin-requiring subjects were removed from analysis. Although subjects were asked to omit diabetic medication, no adjustment has been made for effects of that medication in the analyses shown. Nor has any adjustment been made for any confounding effect of other treatments (eg. effects of antihypertensive treatments on lipid values).

The thresholds defining presence or absence of individual factors is inevitably open to criticism, especially when the variables studied are continuous. I have attempted where possible to use accepted cutoff points, as determined in other epidemiological studies which should aid comparison with other data.

In conclusion I have for the first studied a population of newly-diagnosed type 2 diabetic subjects for features of Reaven's metabolic syndrome and examined interrelationships between the known features and additional features considered of a priori importance. I have identified important associations between the coronary risk factors defined, but have seen no separation into 'few risk factor' and 'multiple risk factor' categories as reported in other populations; instead a normal distribution of risk factors is observed. Analysis of these data has identified a 3-component model for risk factor clustering, almost identical to that reported from the Framingham Offspring Study. Long-term follow up of this population will help to establish whether such a model is indeed predictive of coronary disease.

Use of insulin secretagogues (chapter 4)

A number of potential new oral hypoglycaemic drugs are currently under evaluation. Despite their usefulness, the sulphonylureas have important disadvantages, notably a propensity for weight gain and risk of hypoglycaemia as their action on insulin secretion is not glucose-dependent. The biguanides too have important limitations, as does acarbose. The meglitinide family has attracted particular interest (Malaisse, 1995a); the main property of this group appears to be stimulation of insulin secretion, but other characteristics have not previously been determined. A4166, a short-acting agent of this group, was studied here primarily to confirm its effects on insulin secretion in diabetic man, and also to examine its effects on glucose and other intermediary metabolites.

Insulin secretion in response to an intravenous glucose bolus in normal subjects is biphasic (Porte and Pupo, 1969) with an early acute insulin release measurable during the first 10 minutes after injection of glucose. This acute or first phase is characteristically lost in NIDDM (Pfeifer et al, 1981); indeed a paradoxical decrease in insulin secretion may occur (Metz et al, 1979), as observed here in the studies with placebo. It has been suggested that first phase insulin response is lost with fasting plasma glucose concentrations above 6.5 mmol/L (115 mg/dl; Brunzell et al, 1976) and the defect may therefore be expected to have become established before diabetes is diagnosed in most cases. The second phase response to glucose, being related primarily to the duration of the hyperglycaemic stimulus, is usually better preserved and tends not to deteriorate until markedly higher fasting blood glucose levels are reached (Pfeifer et al, 1981). Insulin secretory responses to other stimuli are usually preserved in NIDDM, although most of these are enhanced by the presence of adequate circulating glucose concentrations (Pfeifer et al, 1980).

The data presented here clearly show the ability of A4166 to stimulate insulin secretion and restore first phase insulin response to glucose in non-insulin-dependent diabetic subjects. Studies of sulphonylurea agents have reported enhancement or partial restoration of the first phase insulin response to intravenous glucose. Turtle (1970) described in three patients an increased early insulin response (measured at 10 minutes) to 50g IV glucose given 3 hours after 250mg tolazamide given orally. Chiasson et al (1991), using an intravenous glucose tolerance test with minimal modelling, found increased acute and total insulin responses to glucose both in healthy volunteers and NIDDM subjects 90 minutes after administration of gliclazide (80mg).

Tolbutamide, which may be expected to be the most closely comparable sulphonylurea agent to A4166 in terms of rate of onset and duration of action, has been relatively little studied in this way. Siegal et al (1972) reported significantly enhanced insulin secretion, and also enhanced glucose clearance rates in healthy volunteers, after submaximal (but not maximal) β cell stimulation by intravenous glucose given 2 hours after 1g oral tolbutamide. Gadgil et al (1983) were able to demonstrate, again in healthy volunteers, lower plasma glucose concentrations following intravenous glucose loading 30 minutes after oral tolbutamide (1g), although insulin concentrations were not measured. In their study this effect was not observed when the IVGTT was performed 4 hours after tolbutamide dosing, and again was only present when β cell stimulation by glucose was submaximal.

Effects of A4166 on metabolite concentrations have not previously been examined. We observed, prior to diet, a rise in lactate concentrations and a fall in NEFA, glycerol and ketone body concentrations. It seems likely that most of these effects are secondary to enhanced insulin secretion in the presence of the drug, although direct effects of A4166 or improvements in insulin sensitivity cannot be ruled out. The lack of a statistically significant effect with A4166 after diet may also, or alternatively, be a

result of the diet treatment itself. No data exist to indicate whether any effects of A4166 on metabolite concentrations may be demonstrable in subjects with established diabetes. Effects of diet on metabolite concentrations have been previously described (Sheppard et al, 1983) with falls in concentrations of lactate, pyruvate, alanine and glycerol observed after 6 weeks of dietary treatment in newly-diagnosed diabetic subjects. These changes were associated with a fall in blood glucose concentrations and with reduction in subjects' body weights. It is notable, albeit disappointing, that the attempts in the present study at vigorous dietary management had no significant impact on either fasting or overall blood glucose concentrations and subjects' body weights did not change. Although this was not a primary objective of this study, the trend towards lower blood glucose concentrations with A4166 suggests that a larger study may be better able to demonstrate a glucose-lowering effect.

Bakkali-Nadi et al (1994) have reported from isolated rat islet studies that the insulinotropic effects of the meglitinide analogues are glucose-dependent, which would appear to be at variance with the findings here, as indicated by the rise in plasma insulin concentration observed following A4166 administration even before onset of glucose injection (figure 1). However, in our study fasting blood glucose concentration in all cases was at least 6 mmol/L and it seems likely that this concentration provides an adequate co-stimulus for insulin release.

Effects on glucose clearance are also of interest, since any drug to be used in NIDDM may have considerable advantages if it can be shown to beneficially affect insulin sensitivity. The findings here must be regarded as somewhat equivocal, since glucose clearance was only enhanced in the studies following diet treatment. Whether this is a reflection of enhanced insulinaemia overcoming insulin resistance is not clear; if this is so the absence of an effect prior to diet cannot be explained. The variability seen in absorption of the drug is also likely to be important. That some individuals

appear to be non-absorbers and hence non-responders to A4166 is clearly shown here although reasons for this are not apparent. This was an unexpected finding of this study and requires further elucidation in future if A4166 is to come into clinical use.

The size of this study has adequately demonstrated the ability of A4166 given prior to IVGTT to enhance significantly endogenous insulin secretion in NIDDM subjects whose secretory responses are otherwise grossly impaired. Further work is necessary to elucidate the mechanism of action of A4166 in enhancing insulin secretion and whether the metabolic improvements suggested by this study are specific to A4166 or rather a consequence of higher circulating insulin concentrations.

Effects on insulin resistance of reducing hypertriglyceridaemia (chapter 5)

Associations between insulin resistance and hypertriglyceridaemia have been previously described (Olefsky et al, 1974; Bernstein et al, 1978; Steiner et al, 1980). With the increased interest in this relationship further stimulated by concepts of clustering of metabolic coronary risk factors, an attempt to assess whether insulin resistance might be modifiable by reducing serum triglyceride concentrations seemed appropriate. Previous studies have yielded conflicting data for any effect of bezafibrate on glycaemic control (Jones et al, 1990; Karhääpää et al, 1992) with no effect on insulin resistance (Karhääpää et al, 1992). With gemfibrozil no effect (Eisalo et al, 1982; Lintott et al, 1992) or a deleterious effect (Marks and Howard, 1982) has previously been shown on glycaemic control; no effect has been reported on insulin resistance (Shen et al 1991; Vuorinen-Markkola et al, 1993), although Shen et al (1991) reported some improvement in insulin sensitivity in a less well-controlled diabetic subgroup of their study.

The mechanism of action of the fibrate family has only recently been elucidated. It is now clear that fibrates exert most if not all of their effects via stimulation of peroxisome proliferator activated receptors (PPAR) of the mainly hepatic α subgroup (Staels et al, 1997). This results in a number of effects on lipid metabolism. Hepatic synthesis of apolipoprotein A-II, a major constituent of HDL, is increased (Vu-Dac et al, 1995) and apolipoprotein C-III synthesis reduced. This latter effect correlates closely with fibrate-induced falls in triglyceride concentrations; gemfibrozil can be shown to reduce plasma apo C-III concentrations as well as reducing hepatic apo C-III mRNA synthesis (Haubenwallner et al, 1995). In vitro fibrates inhibit certain key enzymes in fatty acid synthesis; gemfibrozil is particularly potent in this respect (Sanchez et al, 1992). In cell culture VLDL-receptors are upregulated by fibrates, an effect shared with insulin (Wittmaack et al, 1995).

In this study a significant reduction in triglyceride concentrations has been demonstrated with gemfibrozil; this effect has been well documented in both diabetic (Marks and Howard, 1982; Garg and Grundy, 1989; Shen et al, 1991; Lintott et al, 1992; Vinik et al, 1993; Lahdenperä et al, 1993) and non-diabetic populations (Frick et al, 1987; Tsai et al, 1992). An association between impaired NEFA suppression and hypertriglyceridaemia in NIDDM has also been described previously (Yki-Järvinen and Taskinen, 1988; Baynes et al, 1991b).

The triglyceride reduction shown here was associated with a statistically significant reduction in mean concentrations of NEFA and ketone bodies during insulin infusion. The downward and leftward shift of the insulin dose-response relationship could initially be interpreted as showing that insulin sensitivity has improved (i.e. that metabolite concentrations are lower after gemfibrozil treatment at each concentration of insulin achieved). However, the absence of concomitant changes in glycerol concentrations which would then be expected makes this hypothesis less likely.

Gemfibrozil appeared to have no effect on glycerol concentrations nor on the relationship between insulin and glycerol. Since circulating glycerol levels reflect predominantly lipolysis, a process particularly sensitive to inhibition by insulin, the absence of any observed change in this relationship suggests that gemfibrozil does not influence the sensitivity of lipolysis to insulin.

Circulating NEFA concentrations reflect both lipolysis and also hepatic synthesis. In the context of what is now known about the mode of action of fibrates it seems most likely that the changes observed here are the result of a direct effect on lipid metabolism - particularly hepatic fatty acid synthesis - by the drug. This finding contrasts with the findings of Vuorinen-Markkola et al (1993) who reported no effect on glucose or NEFA metabolism despite a similar reduction in triglyceride concentrations with gemfibrozil. Nonetheless, the findings from the present study are in accord with the emerging evidence for the role of PPARs in insulin sensitivity of lipid metabolism.

No effect on insulin stimulated glucose clearance has been shown, nor was there any change in overall glycaemia as measured by fasting blood glucose, HbA1c and fructosamine concentrations. These findings are broadly in keeping with those previously reported (Shen et al 1991; Vuorinen-Markkola et al, 1993).

Important limitations to this study centre around its size, as a result of problems in recruitment. Effects of treatment with other oral hypoglycaemic and antihypertensive drugs are unlikely as these remained stable throughout the study. (Only sulphonylureas were used by the subjects in this study.) Although a type II error is possible because of its small size, the data presented, and the evidence from other studies, suggest that the overall lack of effect on glucose metabolism is likely to be a true finding. Gemfibrozil may have important advantages in NIDDM if these effects on fatty acid metabolism can be confirmed.

Body fat distribution and insulin resistance in NIDDM (chapter 6)

Vague's description of android (upper body) obesity and its association with other metabolic derangements (Vague, 1956) has been supported by more recent observations on coronary risk and insulin resistance among non-diabetic subjects. Whether body fat and its distribution remain relevant once diabetes is established is less clear.

In this study a group of men with established NIDDM on no medical treatment was studied; a deliberate attempt was made to ensure homogeneity in as many aspects as possible, allowing a limited range for BMI (24-33) and age (40-66) to minimise confounding factors.

No consistent effect of any of the indices of obesity or body fat distribution used (BMI, waist-hip ratio or percentage body fat) was shown on the assessments of insulin resistance. There appears to be no clear explanation for the apparently reduced insulin sensitivity to glucose and ketone metabolism shown by the 'low' waist-hip ratio group; previous experience with this methodology suggests that the group size was adequate to show a true effect, and the finding is supported by the trends in the other variables including insulin. In contrast the greater insulin sensitivity for NEFA and glycerol metabolism in the 'low' BMI group suggests more efficient triglyceride metabolism among less obese individuals. However, these findings are of questionable clinical significance set against the absence of an overall effect on insulin sensitivity shown by any index of body fat.

The powerful effect of basal glycaemia suggests that the diabetic state per se may well have an overwhelming effect on insulin sensitivity, at least to glucose metabolism, and this accords with concepts of glucose toxicity (Yki-Järvinen, 1992) suggesting that hyperglycaemia itself may inhibit normal metabolic response to insulin.

This is in keeping with studies previously reported addressing effects of body fat on insulin sensitivity in NIDDM (Hollenbeck et al, 1984; Firth et al, 1987; Ludvik et al, 1995), concluding that the contribution of obesity was dwarfed by that of diabetes itself. Even among non-diabetic offspring of individuals with NIDDM the family history may be an important contributor to insulin sensitivity to glucose metabolism (Osei et al, 1991). While this effect of glycaemia was prominent with the low-dose insulin infusion it should be noted that it was not seen with the euglycaemic hyperinsulinaemic clamp.

A further possible explanation for the apparent inconsistency in these findings is the possibility that different factors may influence different aspects of insulin resistance, and there is no a priori reason to suppose that all modifiers of insulin resistance will influence it in the same direction. Thus, obesity (measured by BMI) may only be relevant for insulin sensitivity to fat metabolism in this population, and fasting glucose only for glucose metabolism. Similarly, reducing triglyceride concentrations would appear from the data presented above only to influence insulin sensitivity to fat and not to glucose metabolism. There are few data in the literature concerning relationships between sensitivities to different aspects of insulin action, and further study is warranted.

This was a small study and it may be that the range of values for waist-hip ratio and percent body fat studied were too narrow, and those for glucose too wide. Interpretation of the findings is limited by the inability (small study size) to compare the relative contributions of factors studied (by multiple regression). There are conflicting reports on whether BMI is important in non-obese subjects (Bogardus et al, 1985; Campbell and Gerich, 1990); here again better control of ranges may have allowed differences to be observed. Nonetheless it seems likely that hyperglycaemia is a more important determinant than body fat of insulin resistance, and only where basal

glycaemia is more homogeneous (i.e. among non-diabetic subjects or those with impaired glucose tolerance) will effects of body fat emerge.

Role of the low-dose incremental insulin infusion (chapter 7)

The use of both low-dose insulin infusions and euglycaemic hyperinsulinaemic clamps in the study of body fat and insulin resistance allowed comparison between the two techniques, as well as comparison with fasting insulin concentrations and the HOMA-R method. The validity of many measures of insulin resistance, perhaps most notably the FSIGT (minimal model), become questionable in diabetic subjects.

Before making direct comparison between the two methods it is important to note that they are designed to assess different aspects of insulin resistance. The euglycaemic clamp is designed to assess insulin-mediated glucose clearance at a fixed normal blood glucose concentration, using supraphysiological concentrations of insulin. This is clearly artificial, and problems can immediately be seen to arise with the question as to whether blood glucose in diabetic subjects should be set at normoglycaemia (i.e. 5mmol/L as in this study) or at euglycaemia for each individual (i.e. at fasting glucose level; in this study 4.8-10mmol/L). The use of normoglycaemic concentrations here may have introduced confounding metabolic perturbations conceivably rendering the procedure invalid. However, the use of individual euglycaemic concentrations would inevitably have made comparison between subjects more difficult.

The low-dose incremental insulin infusion is designed to assess effects on several metabolic variables of a range of insulin concentrations broadly within the physiological range (Hale et al, 1986). It has been used to study subjects with normal and impaired glucose tolerance, and with IDDM and NIDDM; with this method the

fasting blood glucose of the individual is less important. Comparison of the two techniques therefore has important limitations which include the need to derive arbitrary point values from the insulin infusion to create comparable units for matching with M values.

It is therefore not surprising to find only limited correlations between the indices of insulin resistance derived from insulin infusion and the clamp-derived M values. That both are well correlated with HOMA-R indicates a useful role for each of the three methods in appropriate circumstances. The low-dose insulin infusion has usually been used to compare groups and it is probably invidious to use individual values as applied here; the slopes obtained from the regression equations for individuals will all have wide confidence intervals (although these have not been formally calculated here). Its strengths appear to lie in giving more reliable assessments of insulin resistance in diabetic subjects than do many of the alternative methods, and in its ability to yield dose-response relationships not only for glucose but for other metabolites simultaneously.

The close relationship observed in the present study between fasting blood glucose and individual insulin sensitivity seems likely to reflect the impact of chronic hyperglycaemia itself in diabetic subjects on insulin sensitivity, i.e. glucose toxicity (Yki-Järvinen, 1992).

It has not been possible here to make any assessment of repeatability for the insulin infusion method, and this remains for future study. Other methods, notably HOMA (Matthews et al, 1985) and CIGMA (Hosker et al, 1985), and the IVGTT with minimal modelling (Steil et al, 1994) have been subjected to repeatability assessments and may prove less reliable than might be expected, with reported coefficients of variation exceeding 20%. The short insulin tolerance test appears to be the most reliable of those tested in this way with quoted intra-individual coefficients of

variation of 5-15% (Akinmokun et al, 1992; Hirst et al, 1993). All of these methods may be better suited to comparison of groups than of individuals.

Concluding remarks

Despite the myriad of confounding factors present, some clear findings emerge. The metabolic syndrome appears not to be homogeneous, even in a diabetic population, and is probably composed of 'subsyndromes', each contributing to coronary risk. Obesity, measured by either body mass index or waist-hip ratio, belongs in its grouping of risk factors, although other important contributors such as cholesterol appear to exert an effect independently.

Once diabetes is established, obesity becomes of much lesser significance in relation to insulin resistance. It remains to be established whether improvements in glycaemic control without changes in body fat would improve insulin sensitivity. Improving hypertriglyceridaemia with gemfibrozil probably reduces fatty acid concentrations directly rather than by any improvement in insulin sensitivity; this effect may nonetheless be valuable.

Restoration of a more normal insulin response to glycaemic stimulus by the insulin secretagogue A4166 may improve glucose clearance but overall has little effect on intermediary metabolism other than that resulting from increased insulin concentrations.

The various techniques used here to assess insulin action can all provide valuable information, although comparison of results obtained with different methods must be cautious. Appropriate use of each technique requires careful consideration of the question to be addressed, and importantly of the population studied.

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