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Publication Date:
1988

DOI:
https://doi.org/10.26190/unsworks/7782

The Autonomic Nervous System and Glucose Metabolism with Reference to Noninsulin Dependent Diabetes Mellitus

by

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A thesis submitted for the degree of Doctor of Medicine in the Faculty of Medicine of the University of New South Wales

October, 1988

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Acknowledgements

The work described in this thesis was performed at the Garvan Institute of Medical Research, St Vincent's Hospital, Sydney under the excellent supervision of Dr Donald Chisholm. I would like to thank Don for his unfailing and invaluable help, guidance and constructive criticism during the experimental work and the thesis preparation. I would also like to acknowledge the enthusiastic support of Dr Len Storlien whose intellectual contribution to the project was considerable. I wish to thank Drs George Smythe and Harry Grunstein whose pioneering work into the role of the central nervous system in glucose metabolism provided much of the theoretical background which encouraged the development of this project. I would also like to thank Dr Ted Kraegen for his invaluable advice and insights throughout this work. I would like to express my gratitude to Dr Les Lazarus, Director of the Garvan Institute, for enabling me to carry out this work at the Institute.

I wish to acknowledge the following people for their contributions to certain aspects of this work: Arthur Jenkins, in particular for his guidance with the radioactive tracer studies and the other metabolic assays, but also for his helpful and insightful comments; Stuart Furler who collaborated in the cephalic phase studies and developed the program for the estimation of portal insulin levels and the clamp algorithm; and Dr Mark Borkman who aided in the recruitment of subjects and with several of the studies.

I would like to thank Sisters Mary O'Brien, Gail Plummer and Judy Sowden for their excellent assistance in carrying out these studies. I would like to thank Debbie Barnett, Sue Mitchell, Peter Clarke, Jane McFadden, Walter Tok, Radik Zeleny and the other members of the Garvan Institute hormone assay service for their assistance with the substrate and hormone assays.

I would particularly like to pay tribute to the many willing volunteers who participated in these studies. These people cheerfully placed themselves in the hands of this researcher, usually at considerable inconvenience to themselves, in the belief that their actions would ultimately help others. Most were required to return for repeated studies (one gentleman participated in eight) and most were willing to volunteer for more. I will always feel deeply indebted to these remarkable people.

Several bodies provided financial assistance for this research including the NH & MRC, CSL-NOVO and the Garvan Research Foundation. I would particularly like to thank the Australian division of CSL-NOVO for their interest in this work as well as their financial support.

Finally, I would like to thank my wife, Ann McDonald, for her faith and tremendous support through trying times and acknowledge my two children, Kirsten and Robert, without whom this thesis might have been completed considerably earlier.

Publications arising from this thesis

Manuscripts

Bruce DG, Storlien LH, Furler SM, Kraegen EW, Chisholm DJ. Cephalic phase metabolic responses in normal weight humans. Metabolism 1987; 36:721-725.

Storlien LH, Bruce DG. Mind over metabolism: the cephalic phase. Biol Psychol, 1988; in press.

Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW. The physiological importance of the deficiency in early prandial insulin secretion in noninsulin dependent diabetes mellitus. Diabetes 1988; 37:736-744.

Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW. Prandial insulin delivery to subjects with noninsulin dependent diabetes mellitus: studies with intranasal insulin administration. Submitted for publication.

Abstracts

Bruce DG, Storlien LH, Furler SM, Chisholm DJ. Cephalic phase metabolic responses in normal weight humans. Proc Endo Soc Aust 1986; 29:78.

Bruce DG, Storlien LH, Kraegen EW, Chisholm DJ. The physiological importance of early insulin release during meals in non-insulin dependent diabetes. Proc Aust Diab Soc 1987; Abstract no 9.

Bruce DG, Chisholm DJ, Storlien LH, Smythe GA, Kraegen EW. Noradrenaline causes excessive glycaemic and pressor responses in noninsulin dependent diabetes mellitus. Proc Endo Soc Aust 1987; Abstract no 139.

Bruce DG, Chisholm DJ, Storlien LH, Smythe GA, Kraegen EW. Acute psychological stress does not cause hyperglycaemia in noninsulin dependent diabetes mellitus despite an increased sensitivity to sympathomimetic agents. Proc Aust Diab Soc 1987; Abstract no 13.

Abbreviations used in this thesis

ACTH	adrenocorticotrophin
CHO	carbohydrate
Ci	curies
CNS	central nervous system
CRF	corticotrophin releasing factor
DHPG	dihydroxyphenylethylene glycol
FFA	free fatty acids
GH	growth hormone
GIP	gastric inhibitory polypeptide
IDDM	insulin dependent diabetes mellitus
LHA	lateral hypothalamic area
μ	micro
MHPG	methoxyhydroxyphenylethylene glycol
min	minute
NIDDM	noninsulin dependent diabetes mellitus
R_a	rate of hepatic glucose production
R_d	rate of glucose disposal
SD	standard deviation
SEM	standard error of the mean
U	units
VMH	ventromedial hypothalamus

Contents

Summary

The role of the autonomic nervous system in normal and abnormal metabolic regulation is still unclear in many respects. In noninsulin dependent diabetes mellitus (NIDDM), the deficient early prandial insulin response may be due to a defect in neural stimulation of insulin secretion and may contribute to prandial hyperglycaemia. Additionally, fasting hyperglycaemia in NIDDM may be due to abnormal regulation of hepatic glucose production by the sympathetic nervous system.

The aims of this thesis were to determine whether (i) neurally stimulated insulin secretion is demonstrable in humans and has a role in glucoregulation, (ii) prandial hyperglycaemia in NIDDM is related to the deficient early prandial insulin response, (iii) there is evidence of abnormal sympathetic nervous regulation of hepatic glucose production in NIDDM.

The cephalic phase of insulin secretion was stimulated in normal subjects using a combination of food-related stimuli. This was associated with a small fall in basal glycaemia and inhibition of the glycaemic response to a glucose infusion. In subjects with NIDDM, physiological correction of the deficient early insulin response substantially improved prandial hyperglycaemia, reduced hyperinsulinaemia, partially corrected glucagon and free fatty acid secretion and increased meal-related thermogenesis. The results of a pilot study suggested that the reduction in prandial hyperglycaemia was due to improved suppression of endogenous hepatic glucose production. These studies indicate the critical importance of early insulin secretion in prandial glucoregulation, implicate its deficiency in the pathogenesis of hyperglycaemia and suggest alternative therapeutic strategies for insulin delivery in NIDDM.

Activation of the sympathetic nervous system by tyramine increased hepatic glucose production and glycaemia in nondiabetic and diabetic subjects. Noradrenaline infusion resulted in abnormally increased pressor and glycaemic responses in subjects with NIDDM, however there was no hypersensitivity to psychological stress. There were significant correlations between basal hyperglycaemia and basal sympathetic tone in the diabetic subjects. These findings suggest that NIDDM may be characterised by abnormal sensitivity to basal sympathetic tone and to high circulating noradrenaline levels.

The results of this thesis indicate that abnormal autonomic regulation of prandial insulin secretion and basal hepatic glucose production could contribute to hyperglycaemia in NIDDM. These conclusions indicate new directions for research into the pathogenesis of NIDDM and suggest possible new therapeutic modalities.

CHAPTER ONE

General Introduction

1.1 Introduction

Since the original finding of Claude Bernard (1849) that dogs became glycosuric after puncture of the floor of the fourth ventricle, an abundant body of knowledge has accumulated confirming that the central nervous system has an important regulatory role in metabolism. It is now universally accepted that the central nervous system is able to modulate pancreatic beta cell function (Woods and Porte, 1974; Miller, 1981; Steffens and Strubbe, 1983; Rohner-Jeanrenaud et al., 1983; Ahren et al., 1986) and there is convincing evidence that glucose production by the liver is also subject to control by the nervous system (Shimazu, 1981; Lautt, 1980). A complex neural system exists which is able to detect changes in nutrient supply and hormone levels within the brain and viscera (Powley and Laughton, 1981; Oomura, 1984). However relatively little is known of the physiological importance of these neural mechanisms in directing or modulating normal metabolism. There is even less known about a possible role for the nervous system in causing or contributing to the metabolic disturbances of diabetes mellitus.

This chapter will summarise current knowledge regarding neural control of metabolism focussing primarily on normal glucose and insulin regulation and then consider available research that implicates neural mechanisms in causing or promoting the metabolic abnormalities of diabetes mellitus. The studies in this thesis are concerned only with noninsulin dependent diabetes mellitus (NIDDM). However, many of the concepts discussed could be relevant to insulin dependent diabetes mellitus (IDDM).

1.1.1 Peripheral nervous system and insulin secretion

The islets of Langerhans receive a rich autonomic innervation via the pancreatic nerves which enter the gland with the pancreatic arteries (Woods and Porte, 1974). These include parasympathetic fibres from the vagal system, sympathetic fibres from the splanchnic/coeliac system and visceral afferent nerves. Parasympathetic axons from the vagus synapse with parasympathetic postganglionic neurones near or within the islet. Efferent fibres have been seen in close proximity to all four main islet cell types without any apparent specialisation of synaptic contact (Esterhuizen et al., 1968). The presence of gap junctions between nerves and endocrine cells and between endocrine cells, suggest that the secretory islet cells are electrically coupled and that activation of an individual cell is able to be rapidly dispersed through the entire islet (Meda et al., 1980). There are also afferent or sensory fibres within the pancreatic nerves whose function is uncertain.

The nerves to the islet have been traced back to the CNS with most of the work having been done on the vagal system. Preganglionic cholinergic nerves which track to the islet originate in the dorsal motor nucleus of the vagus and the nucleus ambiguus and polysynaptic pathways interconnect with several hypothalamic nuclei (Akmayev and Rabkin, 1978; Laughton and Powley, 1979; Saper et al., 1979).

Several neurotransmitters have been identified within the islets. These include the parasympathetic neurotransmitter acetylcholine, the sympathetic neurotransmitter noradrenaline and several peptidergic neurotransmitters (Smith and Madson, 1981). Pharmacological studies have demonstrated that the cholinergic neurotransmitters stimulate insulin secretion (Malaise et al.,

1967; Iversen, 1973) whereas sympathetic neurotransmitters usually inhibit insulin secretion.

The effect of sympathetic stimulation is complex. ß-adrenergic receptor agonists cause increased insulin secretion whereas alpha-adrenergic receptor agonists inhibit insulin secretion and the net effect of any sympathomimetic compound depends on its relative affinity for these receptors. At physiological concentration, adrenaline and noradrenaline cause inhibition of insulin secretion (Beard et al., 1982; Porte and Williams, 1966). However, the effect of adrenergic agents on insulin secretion is also dependent on other factors such as prevailing levels of blood glucose (Beard et al., 1982) and the sensitivity of adrenergic receptors. Adrenergic receptor sensitivity can be altered, for example by hypoxia (Baum and Porte, 1980). Recent pharmacological studies have shown that alpha$_1$-adrenergic receptors are responsible for the inhibition of insulin secretion (Skoglund et al., 1986) and that ß$_2$-adrenergic receptors are mainly responsible for stimulating insulin secretion (Kaneto et al., 1975).

Stimulation of the parasympathetic nervous system increases insulin secretion. Electrical stimulation of the vagus nerves (Bergman et al., 1973), the dorsal motor nucleus of the vagus in the brainstem (Ionescu et al., 1983) and the pancreatic nerve after appropriate sympathetic blockade (Frohman et al., 1967; Porte et al., 1973), all result in increased insulin secretion.

In contrast to stimulation studies, vagotomy or parasympathetic blockade have much less of an effect on insulin secretion. Vagotomy does not alter basal insulin levels in several species including man (Russel et al., 1974; Lund et al., 1975). The insulin response to oral or intravenous glucose in vagotomised man has been reported to be reduced (Russel et al., 1974) or unchanged (Lund

et al., 1975). On the other hand, pretreatment with atropine decreased the insulin response to a glucose challenge in monkeys and calves (Daniel and Henderson, 1975; Bloom and Edwards, 1980). The available evidence therefore suggests that the vagal innervation of pancreatic islets has a role in stimulating insulin secretion following glucose administration but has less importance in maintaining basal insulinaemia (Miller, 1981).

Stimulation of the sympathetic nerves inhibits insulin secretion by an alpha-receptor mediated mechanism (Porte et al., 1973; Miller, 1975; Bloom and Edwards; 1975). Basal sympathetic nervous tone appears to have tonic alpha-adrenergic inhibitory activity as insulin levels decline following sympathetic ganglionic or ß-receptor blockade (Goodner et al., 1973) and alpha-receptor blockade results in an increased insulin response to a glucose challenge (Robertson et al., 1973).

In addition to the classical neurotransmitters, many peptides (including substance P, somatostatin, vasoactive intestinal polypeptide, bombesins, cholecystokinin, insulin, galanin and several enkephalins) are present in the vagus and splanchnic nerves and some of them are secreted into the blood following electrical stimulation (Lundberg et al., 1980; Miller, 1981; Uvnas-Wallensten, 1981; Ahren et al., 1986). The function of these peptidergic neurotransmitters is unclear although most of the neuropeptides identified in pancreatic nerves can influence islet hormone secretion under certain experimental conditions (Ahren et al., 1986).

Thus in summary, sympathetic nervous activity causes reflex inhibition of insulin secretion and vagal nerve activity stimulates insulin secretion. Current evidence suggests that the main function of the parasympathetic innervation is

to enhance meal related insulin secretion. The role of the sympathetic innervation appears to be mainly inhibitory with basal sympathetic activity having a tonic inhibitory action.

1.1.2 Central nervous system and insulin secretion

Numerous studies have shown that the autonomic nerves which control insulin secretion are themselves modulated by hypothalamic regulatory centres. The ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic area (LHA) are particularly important in this regard although recent evidence suggests the importance of other less well studied areas including the periventricular nucleus and the dorsomedial hypothalamic nucleus (Tokunaga et al., 1986; Bernardis, 1985; Weingarten et al., 1985).

The VMH and the LHA have been considered to be the hypothalamic centres which control the sympathetic and parasympathetic arms of the autonomic nervous system respectively (Ban, 1975). The concept of localised centres may not be correct as the hypothalamus is an area of convergence of complex circuits and is part of the limbic-hypothalamic-reticular system (Luiten et al., 1987). The distinction is probably important as the hypothalamus may not be the highest integrative site of the autonomic nervous system.

Destructive lesions of the VMH cause hyperphagia, obesity and hyperinsulinaemia. The hyperinsulinaemia does not occur secondary to obesity or hyperphagia as it is seen within minutes of lesioning the VMH and is abolished by vagotomy (Inoue and Bray, 1980; Berthoud and Jeanrenaud, 1979). In addition to the importance of vagal nerve activity, reduced sympathetic tone has been shown to contribute to the hyperinsulinaemia (Inoue and Bray, 1979; Nishizawa and Bray, 1978). Electrical studies have recorded

both reduced sympathetic activity and increased vagal activity in pancreatic nerves after VMH lesions (Yoshimatsu et al., 1984).

Lesions of the LHA have in general opposite effects leading to hypophagia and weight loss. Such lesions do not appear to influence insulin secretion substantially although reduced insulin levels have been reported (Steffens et al., 1972). Electrical recordings from the vagus and splanchnic nerves after LHA lesions record reduced vagal activity and variable effects in the splanchnic sympathetic fibres (Yoshimatsu et al., 1984).

Stimulation of the VMH electrically causes an inhibition of insulin secretion (Frohman and Bernardis, 1978) whereas stimulation of the LHA has variable effects on insulin secretion although appears to cause increased insulin levels in the presence of glucose (Steffens et al., 1972; Berthoud et al., 1980).

Thus there is substantial evidence that the hypothalamus can modulate insulin secretion and that this modulation is mediated via the autonomic nervous system. There is considerable anatomical evidence for this and there are strong connections between the hypothalamus and the autonomic preganglionic neurons of the medulla (Powley and Laughton, 1981) and projections from the hypothalamus have been traced to the pancreatic islets (Ter Horst at al., 1984; Luiten et al., 1984).

Separation of the hypothalamus from the brainstem autonomic nuclei leaves autonomic reflexes intact. This decerebrate animal model demonstrates the importance of hypothalamic modulation as although insulin secretion continues to be partially regulated, supernormal basal and prandial insulin levels are seen, suggesting that there is a disinhibition of control (Flynn et al., 1986).

1.1.3 Central nervous system and hepatic glucose production

There is convincing evidence that glucose metabolism within the liver is under direct control by the autonomic nervous system and that this regulation is modulated by the same hypothalamic areas important in insulin regulation.

The liver receives innervation from both branches of the autonomic nervous system and the parenchymal cells of the liver of most species including man receive both sympathetic and parasympathetic fibres (Forssman and Ito, 1977; Nobin et al., 1977).

The extensive studies of Shimazu and coworkers demonstrated that glycogen metabolism in the liver is influenced by activity within the hypothalamus. Electrical stimulation of the VMH caused a rapid rise in blood glucose and a depletion of liver glycogen, whereas stimulation of the LHA resulted in liver glycogen accumulation (Shimazu et al., 1966). Subsequent studies indicated that the effect of hypothalamic stimulation was to alter the state of activation of certain key enzymes involved in glycogen metabolism. Stimulation of the LHA resulted in activation of glycogen synthetase from its inactive form and at the same time caused inactivation of glycogen phosphorylase, resulting in net stimulation of glycogen synthesis. VMH stimulation on the other hand caused activation of glycogen phosphorylase and stimulated glycogenolysis (Shimazu et al., 1978). Chemical stimulation studies using microinjection of a variety of neurotransmitters into the hypothalamus demonstrated that noradrenergic stimulation of the VMH and cholinergic stimulation of the LHA produced similar results to the electrical studies (Matsushita and Shimazu, 1979; 1980; Shimazu et al., 1976).

The effect of VMH stimulation was reproduced by stimulating the splanchnic sympathetic nerves. Simultaneous stimulation of the vagus nerves blocked this response (Shimazu and Fukuda, 1965). The effects of splanchnic stimulation persisted in adrenalectomised or pancreatectomised animals demonstrating that the effect was due to direct stimulation of the hepatocytes rather than indirectly by altering pancreatic or adrenal hormone secretion. Stimulation of the hepatic splanchnic nerves has been shown to cause hyperglycaemia in several other species including man (Edwards, 1972; Nobin et al., 1977; Lautt and Wong, 1978).

In contrast to the effects of stimulating the splanchnic sympathetic nerves, stimulation of the hepatic vagus caused increased glycogen synthetase activity and incorporation of glucose into glycogen in the liver. This effect was eliminated by simultaneous stimulation of the splanchnic nerves and was independent of pancreatic hormone effects (Shimazu and Fujimoto, 1971; Shimazu, 1967; 1971). Cholinergic stimulation of glycogen synthesis has also been demonstrated in vitro (Ottolenghi et al., 1971; Akpan et al., 1974).

Gluconeogenesis also appears to be subject to hypothalamic regulation as demonstrated by electrical stimulation studies. Stimulation of the VMH resulted in altered activity of key gluconeogenic enzymes (activated phosphoenol pyruvate carboxykinase and suppressed pyruvate kinase), whereas LHA stimulation caused reciprocal changes. These changes occurred at a slow rate compared with the effects on glycogen metabolism and were judged to be of lesser importance at least in terms of rapid control (Shimazu and Ogasawara, 1975).

Recent studies of brain neurotransmitter activity have extended the above findings and suggest that blood glucose may be under direct hypothalamic control within the physiological range. The concentrations of central neurotransmitters and their major neuronal metabolites were determined (using sensitive methodology) to assess neurotransmitter turnover and provide an index of neuronal activity within discrete areas of the brain (Smythe et al., 1982; 1983). Such methods uncovered strong positive correlations between medial basal hypothalamic noradrenergic activity (assessed by the ratio of noradrenaline and its major metabolite, dihydroxyphenylethylene glycol) and blood glucose levels under a variety of conditions including stressed and nonstressed states (Smythe et al., 1984). These relationships were found to be independent of adrenal or pancreatic hormones and were considered to reflect direct hypothalamic control of liver glucose output.

These workers examined brain neurotransmitter activity during 2-deoxyglucose administration which causes central neuroglycopaenia and results in a rise in peripheral blood glucose levels (due to increased hepatic glucose output) and a counterregulatory hormonal response with the release of glucagon and catecholamines. Neuroglycopaenia, induced by 2-deoxyglucose administration, was found to be accompanied by an increase in hypothalamic noradrenergic activity. Furthermore, the administration of the sympathetic blocking drug guanethidine prevented the systemic hyperglycaemic response. Further studies in adrenomedullectomised animals indicated that the hyperglycaemic effect of 2-deoxyglucose was mediated by direct sympathetic nervous stimulation of liver glucose production (Storlien et al., 1985). These studies confirmed the work of Brodows et al (1973, 1975) who concluded from their studies in adrenalectomised and spinal-injured humans, that the hepatic

sympathetic innervation contributes to the hyperglycaemic response following 2-deoxyglucose administration.

1.1.4 Afferent nervous system and metabolism

The afferent neural systems concerned with central nervous control of metabolism have been less well defined than the efferent connections. However there is evidence for an extensive sensory system both within the brain and the splanchnic organs, which can sense blood (or tissue) glucose and insulin levels and thus determine the state of nutrient supply. The existence of a peripheral sensory system which may play a role in metabolism is emphasised by the fact that 75-90% of the fibres in the abdominal vagus and 50% of all splanchnic fibres are afferent (Sawchenko and Friedman, 1979).

The observation that insulin infusion into the brain of rats resulted in a fall in systemic blood glucose levels first suggested that an insulin sensitive area in the brain exists which can regulate metabolic activity (Szabo and Szabo, 1972). The hypothalamus is itself sensitive to insulin as microinjection of insulin into the VMH results in a fall in blood glucose (Storlien et al., 1975; Iguchi et al., 1981). This response has since been shown to be abolished by vagotomy or atropine administration and may be mediated by the vagus (Szabo et al., 1983). Insulin injection into the VMH also causes a reduction in the firing rate in sympathetic nerves which supply brown adipose tissue (Sakaguchi and Bray, 1987). Therefore the detection of insulin by the hypothalamus can result in an alteration in the activity of both arms of the autonomic nervous system.

The hypothesis that the hypothalamus is sensitive to insulin and may be part of an afferent sensory system was strengthened when it was found that insulin

could bind to CNS structures and that insulin could cross rapidly into the brain at sites where the blood-brain barrier is incomplete (i.e. near the circumventricular organs, where specific binding sites for insulin are concentrated, Van Houten et al., 1979; 1981). There is anatomical evidence for the existence of neural connections between these areas and the medial basal hypothalamus and therefore communication between blood borne insulin and hypothalamic structures may be possible (Ricardo and Koh, 1978; Broadwell and Brightman, 1976). Other groups have confirmed the presence of insulin receptors in the brain with the highest concentrations being in the olfactory bulbs and the hypothalamus (Havrankova et al., 1978; Pacold and Blackard, 1979).

Studies of central neurotransmitter turnover have shown that peripheral insulin administration rapidly alters medial basal hypothalamic neurotransmitter activity independent of peripheral blood glucose levels or brain glucose uptake (Smythe et al., 1985). Insulin rapidly suppressed hypothalamic noradrenergic activity (previously shown to be positively correlated with blood glucose, Smythe et al., 1984) and caused a reciprocal increase in serotoninergic activity. These studies indicated that changes in peripheral insulin concentrations could be detected within the brain and resulted in altered hypothalamic neuronal activity and a reduction in blood glucose levels (Smythe et al.,1984).

There is also evidence pointing to the functional significance of glucose sensitive neurones within the brain (Oomura et al., 1984). Single cell electrophysiological studies have demonstrated that glucoreceptor neurones within the VMH increase their firing rate following the iontophoretic application of glucose. This response was enhanced when both glucose and insulin were applied. Neurones from the LHA behaved differently in that glucose application

reduced the rate of firing whereas insulin application increased the rate of firing of the glucose sensitive cells (Oomura et al., 1974).

The possibility that glucoreceptors could be present in the liver and other visceral organs was originally suggested by Russek (1963). Niijima recorded neural discharges from branches of the hepatic and pancreatic nerves and found that the administration of intraportal glucose reduced the firing rate of hepatic nerve afferents and increased the firing rate of pancreatic efferents (Niijima, 1969; 1980). Subsequent studies showed that intravenous glucose administration also modulated efferent activity in the hepatic branch of the vagus nerve (Niijima, 1985). There may be a close relationship between central and peripheral glucoreceptor cells in that intraportal glucose injection inhibited hypothalamic LHA activity in a manner similar to direct glucose application to the LHA (Shimizu et al., 1983). In other electrophysiological studies, intravenous glucose injection was followed by a simultaneous increase in pancreatic vagal firing and a decrease in pancreatic sympathetic firing (Yoshimatsu et al., 1984). Lee and Miller (1985) have demonstrated a functional role for these putative hepatic glucoreceptors in that portal glucose administration to rats caused a stimulation of insulin secretion which was mediated via the hepatic (afferent) vagus nerve as no insulin secretion occurred when the nerve was sectioned. They also found that the hepatic vagal afferents play a tonic inhibitory role in insulin regulation as section of these nerves caused an elevation of basal insulin levels.

In summary, recent evidence suggests that insulin itself acts within the CNS and can evoke neural reflexes which can alter metabolic activity and reduce blood glucose levels. This makes teleological sense as under physiological conditions an animal only experiences a rise in insulin levels consequent on

meal ingestion and concomitantly with a rise in blood glucose levels. Glucoreceptors within the brain and the viscera also play a role in the regulation of insulin secretion and hepatic glucose production. When blood or tissue glucose levels are rising, afferent neural activity modifies hypothalamic responses in an integrated fashion and results in increased insulin secretion and directly or indirectly causes a reduction in liver glucose production. Thus there is evidence that a complex sensory system exists which can monitor insulin, glucose and possibly other substrate and hormone levels and these signals may alter efferent neural activity in order to maintain nutrient homeostasis and nutrient supply.

1.1.5 Overview of hypothalamic regulation of CHO metabolism

There is now good functional and anatomical evidence that central regulation of metabolism involves a hierarchy of control with hypothalamic structures modulating both the parasympathetic and sympathetic limbs of the autonomic system. The autonomic nervous system has some ability to respond when isolated from the hypothalamus but the higher centres are necessary for precise regulation (Flynn et al., 1986).

The two arms of the autonomic system appear to be controlled in a coordinated tightly coupled fashion. Anatomically, the important central relays project simultaneously to both branches of the autonomic system (Powley and Laughton, 1981). Manipulations of the medial and lateral areas of the hypothalamus produce reciprocally organised responses in the two autonomic branches. Thus, electrical activity within the vagal and splanchnic efferent nerves are changed reciprocally following manipulations of the VMH or LHA (Kita et al., 1980; Yoshimatsu et al., 1984). Stimulation of the medial hypothalamus typically causes a series of catabolic responses which result in

hyperglycaemia, due to increased hepatic glycogenolysis and a suppression of insulin secretion. On the other hand, stimulation of the lateral areas of the hypothalamus results in essentially anabolic responses including increased basal and glucose stimulated insulin secretion, reduced hepatic glycogenolysis and increased hepatic glycogen synthesis.

The hypothalamus is also involved in the modulation of a number of other important aspects of metabolism. Thus VMH-sympathetic stimulation typically results in increased free fatty acid, glucagon and adrenaline levels (Texeira et al., 1975; Frohman and Bernardis, 1971), causes increased thermogenesis (Minokoshi et al., 1986) and inhibits feeding. These responses are clearly catabolic in nature. On the other hand, VMH lesions typically result in reduced glucagon levels (Inoue et al., 1977), reduced sympathetic nervous activity (Vander Tuig et al., 1982) and reduced mobilisation of free fatty acids (Nishizawa and Bray, 1977).

It is not known to what extent direct neural activity at the target organs or indirect actions due to altered secretion of metabolically active hormones are responsible for regulating various metabolic processes. Shimazu hypothesised that direct neural effects are rapid and likely to be of greater importance during times of stress or for initiating changes whereas the hormonal actions are important for consolidating or prolonging effects (Shimazu, 1981). There is evidence that hormonal mechanisms and neural systems may act synergistically in some circumstances (Beckh et al., 1982). Finally, a redundancy of systems often exists such that one system assumes importance when a primary regulatory system fails. Such redundancy has been demonstrated during insulin hypoglycaemia in humans where adrenergic

mechanisms assume critical importance in maintaining blood glucose levels when glucagon deficiency is present (Tse et al., 1983; Rizza et al., 1979).

1.2 Physiological role for the CNS in carbohydrate metabolism

There is relatively little data indicating the importance of these neural mechanisms in regulating carbohydrate metabolism. However neural control may be important in several key areas.

1.2.1 Basal metabolism

Basal insulin levels are maintained by a balance of a number of inhibitory and stimulatory factors including prevailing levels of glucose, amino acids, catecholamines, cortisol and gastrointestinal peptides (Ward et al., 1984). There is considerable evidence that basal insulin secretion is tonically inhibited by sympathetic (alpha-adrenergic) tone (Robertson et al., 1973; Berthoud et al., 1980). Basal sympathetic tone to the beta cells is modulated by the VMH (Flynn et al., 1986) and influenced by portal glucose levels which are detected by putative hepatic glucoreceptors (Lee and Miller, 1985).

The factors controlling basal liver glucose output have not been extensively studied. Basal insulin and glucagon secretion are important (Liljenquist et al., 1977) but there is evidence that direct sympathetic ß-adrenergic and alpha-adrenergic tone have a role in maintaining basal hepatic glucose production in man (Rizza et al., 1980; Rosen et al.,1983).

1.2.2 Prandial metabolism

Following the ingestion of a meal or glucose load, glucose homeostasis is maintained by a number of metabolic alterations. These include an increase in peripheral glucose uptake particularly into skeletal muscle, a reduction of endogenous liver glucose production and an increase in hepatic glucose

storage although this last may be quantitatively less important than previously thought (Radziuk et al., 1978; Katz and McGarry, 1984). Other alterations include falls in glucagon, free fatty acid and adrenaline levels (Trunet et al., 1984) and increases in noradrenaline levels (Young et al., 1980). Insulin secretion is clearly important in promoting some of these metabolic changes.

The rise in insulin levels following a mixed meal is due to a number of stimulatory factors including neural activation, stimulation of the beta cells by absorbed glucose and amino acids, and insulinotropic gut hormones which potentiate glucose stimulated insulin secretion. The proportion of insulin secretion after oral glucose ingestion which is not due to direct glycaemic stimulation of the beta cells has been variously estimated to account for approximately 20-70% of the total in man (Nauck et al., 1986; Shuster et al., 1988) indicating that the effects of neural stimulation and gut hormones are considerable. Another study in rats suggested that the neural component of insulin secretion comprised at least 26% of the total (Berthoud, 1984).

Cephalic phase insulin release has been extensively studied in a number of animal species including man (Berthoud et al., 1981; Powley and Berthoud, 1985). Cephalic responses are neural reflexes which are stimulated by sensory stimuli arising from the oropharynx and can occur rapidly after the onset of meal ingestion (Powley and Berthoud, 1985). Cephalic phase insulin secretion is abolished by vagotomy (Louis-Sylvestre, 1976) and probably also by VMH lesions (Berthoud et al., 1980; Storlien, 1985). In one study, VMH lesioning augmented rather than abolished cephalic insulin responses (Louis-Sylvestre, 1976). In this latter study, the rats were studied some time after the lesioning and thus the increased responses may have been related to beta cell hyperplasia and/or hyperphagia. It is clear however, that cephalic phase

insulin secretion is mediated via the hypothalamus and the vagus. These studies demonstrate that there is an increase in vagal stimulation of the beta cells during the ingestion of meals.

Cephalic phase insulin secretion may have an important homeostatic role in prandial glucose metabolism. When cephalic responses were abolished in rats, either by bypassing the oropharynx (intragastric feeding) or by curing diabetic rats of their diabetes with transplanted beta cells (resulting in denervated beta cells), the early rise in insulin levels did not occur and impaired glucose tolerance resulted (Louis-Sylvestre, 1978; Trimble et al., 1980). Replacement of the deficient early phase with intravenous insulin corrected these abnormalities (Berthoud et al., 1981). This phenomenon has a clinical counterpart in that insulin dependent diabetic subjects have considerably worse prandial hyperglycaemia if their preprandial insulin treatment is slightly delayed (Kraegen et al., 1981; Dimitriadis and Gerich, 1981).

Thus it seems likely that the initial phase of insulin secretion during meals is mediated at least partly by neural mechanisms and that the neural reflex includes the VMH and the vagus nerve. The early phase of prandial insulin secretion appears to have considerable importance in prandial glucoregulation. The early rise in insulin levels may act to initiate a series of metabolic events including suppression of hepatic glucose production, in order to prepare the animal for the influx of nutrients consequent on ingesting the meal (Storlien, 1985). The implications are that the hypothalamus and the autonomic nervous system have an important role in modulating normal prandial insulin secretion and that abnormalities of this system could have pathogenetic consequences which could cause hyperglycaemia.

Alterations in sympathetic nervous activity occur during meal ingestion and increases in noradrenaline levels during meals have been considered to represent increased sympathetic activation (LeBlanc et al., 1984; Steffens et al, 1986). Furthermore, chronic fasting and overnutrition have been shown to be associated with reduced and increased noradrenaline turnover respectively in both rats and humans (Landsberg and Young, 1978; O'Dea et al., 1982). Several possible functions for increased sympathetic activity during meal ingestion have been suggested. Sympathetic activation may be responsible for meal-induced thermogenesis (LeBlanc et al., 1984) and may attenuate the vagally mediated rise in insulin secretion (Steffens et al., 1986). There are also shifts in blood flow during food ingestion which may be regulated by the sympathetic nervous system and could give rise to the observed changes in plasma noradrenaline levels. The relationship between the sympathetic nervous system and food ingestion is complex as an increase in insulin levels itself causes an increase in sympathetic activation (Rowe et al., 1981).

An increase in sympathetic nervous system activity during meals appears to be at variance with the hypothesis that hepatic glucose production may be promoted by sympathetic activation as hepatic glucose production falls during food ingestion (Radziuk, 1978). Similarly an increase in sympathetically mediated inhibition of the pancreatic beta cells would seem unlikely to occur during food ingestion. However, the techniques usually used for evaluating sympathetic activity are imprecise and are unable to detect sympathetic activation in discrete organs (Esler, 1982). Sympathetic nervous system outflow to different organs is non-uniform and local organ-specific increases in sympathetic activation can occur (Esler et al., 1984). Thus, an increase in sympathetic nervous system activity at, for instance, the site of meal-induced

thermogenesis could coexist with a reduction in sympathetic activation elsewhere e.g. at the liver or beta cells. The studies of Yoshimatsu et al (1984) support this view as they have reported a reduction in sympathetic neural firing in the pancreatic nerve following glucose administration.

1.2.3 Exercise

During physical exercise there is a rapid increase in peripheral uptake of glucose by skeletal muscle and at the same time there is an equally rapid increase in hepatic glucose production. The initial increase in glucose production arises initially from glycogenolysis but subsequently gluconeogenesis becomes of increasing importance. The control of hepatic glucose production in this situation is extremely precise and changes in peripheral blood glucose levels do not usually occur during acute exercise.

The rapidity and precision of the system suggested that a neural control mechanism rather than a hormonal mechanism regulates this response (Richter et al., 1981; Vranic and Berger, 1979). Hoelzer et al (1986) have demonstrated the importance of sympathoadrenal activation in promoting hepatic glucose output in normal exercising humans. Normal glucoregulation occurred during somatostatin infusion with physiological replacement of glucagon and insulin (to prevent changes in these hormones during exercise), whereas the addition of combined alpha- and ß-adrenergic blockade resulted in a fall in hepatic glucose output and plasma glucose levels. They replicated this study in adrenalectomised men and found that glucoregulation was normal during somatostatin infusion and insulin and glucagon replacement. They therefore concluded that direct sympathetic control of hepatic glucose production appeared to be critical in the control of hepatic glucose output during exercise.

Recent evidence demonstrates that hepatic glucose output is subject to negative feedback control by plasma glucose during exercise in humans. This feedback relationship is extremely precise and sensitive and therefore peripheral or central glucoreceptors may have a role in the sympathetic neural control of hepatic glucose output during exercise (Jenkins et al., 1985; 1986).

In summary, there is considerable evidence that the hypothalamus and the autonomic nervous system have an important role in the physiological regulation of blood glucose. The hypothalamus and the peripheral autonomic nervous system have become increasingly the focus of attention by researchers interested in human metabolic diseases, particularly diabetes and obesity (Woods and Porte, 1974; Lautt, 1980; Feldberg et al., 1985; Bray et al., 1981; Jeanrenaud et al., 1985). Abnormalities in central or peripheral autonomic control systems may cause or contribute to the pathophysiology of these metabolic disorders.

1.3. Pathogenesis of noninsulin dependent diabetes mellitus

The cause of NIDDM is unknown but both inherited and acquired factors are important. A strong genetic component has been demonstrated by twin and family studies. A high concordance rate of NIDDM is found in monozygotic twins (Barnett et al., 1981) and a strong familial tendency for the condition is seen in some patients (Koberling et al., 1985). Lifestyle factors are also important and this is most vividly illustrated by the increase in the incidence of NIDDM in communities which have changed their lifestyles from a rural agrarian one to an urban Western style of living (Zimmett, 1982). The main lifestyle factors considered to be important include obesity, low levels of physical exercise and the consumption of diets high in saturated fats and relatively low in fibre and complex carbohydrates.

NIDDM is characterised by a number of complex and apparently interrelated metabolic defects. The major abnormalities include qualitative and quantitative abnormalities of insulin secretion (Ward et al., 1985), peripheral insulin resistance due to a combined receptor and post-receptor defect in cellular insulin action (Olefsky et al., 1982) and elevated rates of hepatic glucose production (Bowen and Moorehouse, 1973; Best et al., 1982). Whether or not some one or more of the major metabolic abnormalities is the primary cause of NIDDM is unknown.

A considerable debate has continued for a number of years over whether the primary problem with NIDDM lay with inadequate insulin production or with insulin resistance. However most recent studies have found that the major metabolic abnormalities usually coexist in subjects with NIDDM (Olefsky, 1985; Efendic et al., 1984). Furthermore, neither insulin resistance nor impaired

insulin secretion alone necessarily cause hyperglycaemia. For instance, obese humans can be severely insulin resistant without ever developing NIDDM, presumably due to their ability to compensate by increasing insulin secretion (Luft et al., 1967; 1968). As for insulin secretion, subjects with NIDDM probably retain between 60 and 100% of normal beta cell volume (Westermark et al., 1978; Rahier et al., 1983) and it has been estimated that humans may not develop diabetes even after removal of up to 80% of their pancreas (Turner et al., 1979). Thus there appears to be a substantial reserve in both insulin-mediated glucose uptake and insulin secretory capacity and it may be that a combination of insulin resistance and impaired insulin action is necessary to cause the metabolic abnormalities of NIDDM (Weir, 1982; Olefsky, 1985).

Recently, several groups have studied nondiabetic individuals considered to be at high risk of subsequently developing NIDDM in an attempt to determine which metabolic defect occurs first and is therefore likely to be the primary cause of NIDDM. Normoglycaemic women with a history of recent gestational diabetes (Ward et al., 1985) and a substantial number of nondiabetic first degree relatives of diabetic patients (O'Rahilly et al., 1986) were found to have subclinical abnormalities of both insulin secretion and action. In addition, it has been shown that some nondiabetic children of subjects with NIDDM have slightly elevated levels of blood glucose (Leslie et al., 1986) and increased glucose turnover (Osei and Holland, 1987). Thus a number of subclinical defects can be detected in high risk populations many years before the development of overt diabetes. It is not known which of these defects, if any, are the primary cause of NIDDM.

Each of the three major defects in NIDDM has been shown to be partially reversible with dietary therapy (Stanik et al., 1980; Henry et al., 1986),

sulphonylurea therapy (Kolterman et al., 1984; Simonson et al;., 1984) and insulin therapy (Turner et al., 1976; Garvey et al., 1985). This has given rise to the hypothesis that hyperglycaemia itself may, in part, be responsible for the defects in insulin action and secretion (Unger and Grundy, 1985). The concept that hyperglycaemia is somehow 'toxic' in some tissues (the 'glucotoxicity' hypothesis) has recently been verified experimentally. Hyperglycaemia independently contributes to the defect in insulin secretion in some animal models of diabetes mellitus (Bonner-Weir et al., 1983; Grill and Rundfeldt, 1986). Rossetti et al (1987) demonstrated that both reduced insulin sensitivity and abnormal insulin secretion could be normalised in diabetic rats (partial pancreatectomy model) following treatment of hyperglycaemia by phlorizin, an agent which reduces hyperglycaemia by increasing urinary glucose losses without altering insulin secretion or action.

Chronic hyperglycaemia may therefore 'desensitise' the beta cells and impairs their ability to respond to a glucose stimulus. This desensitisation could be common to other cells in the body. Hyperglycaemia can down-regulate the glucose transport system in non-insulin dependent tissues such as brain (Matthei et al. 1986) and insulin resistance in the rat is associated with a reduced number of cellular glucose transporters (Karnieli et al., 1981). Therefore, a generalised impairment in glucose transport could account for peripheral and hepatic insulin resistance as well as impaired beta cell responses to glucose.

In subjects with established NIDDM, hyperglycaemia causes a further impairment of insulin secretion and insulin action. Therefore in subjects destined to develop NIDDM, a small increase in blood glucose levels could cause pre-existing subclinical abnormalities in insulin action and secretion to

become functionally significant. These more severe defects in insulin action and secretion could then cause more severe hyperglycaemia to develop. A vicious cycle of worsening hyperglycaemia on the one hand and worsening abnormalities of insulin secretion and action on the other, could ultimately lead to frank diabetes.

1.3.1 Hepatic glucose production in NIDDM

Recent studies have indicated that elevated rates of hepatic glucose output play an important role in determining the severity of the diabetic state. In hyperglycaemic patients with NIDDM, hepatic glucose output is usually greater than normal (Bowen and Moorehouse, 1973; Best et al., 1982; Bogardus et al., 1984) and there is a strong correlation between fasting blood glucose and the basal rate of glucose production by the liver (Kolterman et al., 1984; Bogardus et al., 1984). In addition, the improvement in fasting glucose following weight loss or hypocaloric diets correlates with a reduction in hepatic glucose production (Henry et al., 1985). In contrast, little or no correlation was found between fasting blood glucose levels and measures of either insulin secretion or action in NIDDM (Bogardus et al., 1984).

Prandial hyperglycaemia in NIDDM is also related to hepatic glucose production. Following the ingestion of a mixed meal or oral glucose, the absorbed glucose is taken up predominantly by skeletal muscle, and liver glucose output is suppressed (Radziuk et al., 1978). In a recent study utilising a double radioisotope approach, prandial hyperglycaemia in a group of NIDDMs was found to be due to the combination of impaired suppression of hepatic glucose production and reduced peripheral glucose disposal (Firth et al., 1986). Thus, it appears that excessive glucose output from the liver may be

important in contributing to prandial as well as basal hyperglycaemia in NIDDM.

Total glucose uptake can be divided into two components, non-insulin mediated glucose uptake (includes glucose uptake into non-insulin sensitive tissues e.g. brain, and the amount of glucose uptake measured during hypoinsulinaemia) and insulin-mediated glucose uptake. Non-insulin mediated glucose uptake has been estimated in both nondiabetic subjects and subjects with NIDDM to account for approximately 70% of the total in the basal state (Baron et al., 1985). This means that insulin-mediated glucose disposal is relatively unimportant basally and impaired insulin action at the periphery is unlikely to cause or contribute substantially to basal hyperglycaemia. This suggests that overproduction of glucose by the liver is the most direct cause of fasting hyperglycaemia in NIDDM (Olefsky, 1985).

The cause of excess liver glucose output in NIDDM is poorly understood. Possible causes include reduced insulin secretion, hepatic insulin resistance (Revers et al., 1984), reduced suppression of hepatic glucose production by glucose itself (Liljenquist et al., 1979), increased availability of gluconeogenic substrates (Ferrannini et al., 1983), abnormal secretion of a number of metabolically active hormones or increased sensitivity of the liver to one or more of these stimulatory factors. Glucagon (Unger and Orci, 1981), growth hormone (Hansen, 1973) and possibly also cortisol (Cameron et al., 1984) are abnormally regulated in NIDDM. All are capable of promoting hepatic glucose production in man and several groups have postulated that hyperglucagonaemia (Baron et al., 1987; Moltz et al., 1984) or increased growth hormone secretion (Martin and Jeanrenaud, 1985) could be important determinants of this. Abnormal adrenaline secretion from the adrenal medulla

has also been suggested as a cause of hyperglycaemia in NIDDM (Feldberg et al., 1985).

Given the importance of the autonomic nervous system in controlling hepatic glucose output in normal animals (Shimazu, 1981; Smythe et al., 1984), it is feasible that an abnormality in this system could be responsible for the excessive glucose production in NIDDM (Lautt, 1980). There is some evidence to suggest that the sympathetic nervous system is overactive in NIDDM and an excess sympathetic drive to the liver could cause increased hepatic glucose production. Several workers have reported elevated circulating catecholamine levels (Robertson et al., 1976; Halter and Porte, 1977) and increased levels of urinary noradrenaline or noradrenaline metabolites have been reported in NIDDM (Grunstein, 1985). Studies of alpha-adrenergic blockade and insulin secretion suggest that there may be increased alpha-adrenergic activity at the beta cells in NIDDM (Robertson et al., 1976; Broadstone et al., 1987). Sympathetic blockade induced by the drug guanethidine improved glucose tolerance in three subjects with NIDDM (Gupta, 1969) and reversed the impaired glucose tolerance of patients with thyrotoxicosis (Woeber et al., 1966). A recently described alpha$_2$-adrenergic blocking drug has been found to be an effective hypoglycaemic agent in NIDDM although whether its hypoglycaemic action is due to adrenergic receptor blockade has not been elucidated (Kashiwagi et al., 1986; Kawazu et al., 1987). Finally, the regulation of hepatic glucose output during moderate exercise in NIDDM is deranged (Jenkins et al., 1988) and in exercising nondiabetic humans the sympathetic nervous system is a major regulator of hepatic glucose production (Hoelzer et al., 1986).

1.3.2 Prandial insulin secretion in NIDDM

In contrast to the basal state where noninsulin-mediated glucose uptake is of major importance, insulin levels rise and insulin-mediated glucose uptake becomes quantitatively more important following a meal or glucose ingestion (Olefsky, 1985; Kingston et al., 1986). Insulin secretion following oral ingestion of carbohydrate is stimulated by a complex interplay of neural and hormonal factors and by absorbed nutrients (Ward et al., 1984). Studies of cephalic phase insulin secretion suggest that neural stimulation occurs rapidly and is responsible for the initial insulin response. Nutrient stimulated (glucose and amino acids predominantly) insulin secretion then follows and is greatly amplified by insulinotropic gut hormone secretion (Creutzfeldt, 1979). The amount of insulin released following an oral glucose load which is due to neural and gut hormone factors, has been variously estimated to account for 20-60% of the whole in humans (Nauck et al., 1986; Shuster et al., 1988). This non glucose-mediated insulin secretion has considerable importance and explains why oral glucose loads are better tolerated than intravenous loads (Elrick et al., 1964; Perley and Kipnis, 1967).

In established NIDDM, there is invariably a deficiency in early insulin secretion and insulin levels are subnormal during the first 30-60 minutes after the meal, whereas later insulin levels are usually normal or higher than normal (Yalow and Berson, 1960; Perley and Kipnis, 1967; Kosaka et al., 1977). This later hyperinsulinaemia may occur secondary to the prandial hyperglycaemia seen in NIDDM. Beta cell function is influenced by the degree of hyperglycaemia and severely hyperglycaemic individuals often have reduced insulin secretion throughout the prandial period. Insulin secretion can be substantially improved following therapeutic correction of hyperglycaemia, however the defective early

phase does not improve suggesting that it may not occur as a secondary phenomenon (Garvey et al., 1985).

Similar defects in prandial insulin secretion have been reported in subjects who were considered to be at high risk of developing NIDDM (Colwell and Lein, 1967) and in one longitudinal study in subjects subsequently developed diabetes (Kosaka et al., 1977). These data indicate that the delay in early prandial insulin secretion occurs early during the development of NIDDM and could even occur as a primary abnormality.

The cause of the early deficiency of insulin secretion is unknown but is quantitatively mainly due to a reduction in non-glucose mediated insulin secretion (Nauck et al., 1986). Several groups have considered that a deficiency in insulinotropic gut hormone secretion is the likely cause. Gastrointestinal polypeptide is the most potent of the known gut hormones in terms of its insulinotropic effects. However, its secretion is often excessive in NIDDM and therefore does not explain the deficiency in insulin secretion (Crockett et al., 1976; Ross et al., 1977). This augmented secretion may occur as an attempted compensatory mechanism for the deficient insulin secretion (Osei et al., 1986). It has been suggested that an as yet unidentified "incretin" may be responsible for the prandial augmentation of insulin secretion (Creutzfeldt, 1985). However, none of the currently identified insulinotropic gut hormones are able to stimulate insulin secretion in the absence of glucose. Their main physiologic action appears to be to potentiate glucose stimulated insulin secretion (Brown et al., 1975; Andersen et al., 1978). Thus a gut hormone abnormality may not explain the early deficiency in prandial insulin secretion in NIDDM.

Glucose stimulated insulin secretion is also abnormal in NIDDM and in particular there is a defect in the first phase of the normal biphasic insulin response to intravenous glucose (Brunzell et al., 1976). It is unknown how this pattern of insulin response to parenteral glucose relates to abnormal prandial insulin secretion. However, the studies of Nauck et al (1986) indicated that abnormalities of glucose stimulated insulin secretion are not the major cause of the deficiency in early prandial insulin secretion in NIDDM. These researchers used glucose infusions to simulate the peripheral glucose response to glucose ingestion in subjects with NIDDM and compared insulin and C peptide responses with the prandial responses. They concluded that the early deficiency in insulin secretion in subjects with NIDDM was due predominantly to 'incretin' factors, i.e. either gut hormone or neurally mediated insulin secretion.

An abnormality of the neural component of insulin secretion is therefore a potential candidate for delayed insulin secretion in NIDDM. Support for this hypothesis comes from cephalic phase studies which demonstrate that neurally based insulin secretion occurs early after the start of meal ingestion (Berthoud et al., 1981). Furthermore, as detailed previously, it has been shown in rats that where cephalic responses have been eliminated or bypassed, early insulin release is greatly reduced, higher than normal insulin levels subsequently occur and prandial hyperglycaemia results (Louis-Sylvestre, 1978; Trimble et al., 1980; Berthoud et al., 1982). Thus the absence of cephalic phase insulin secretion in these animals causes a pattern of prandial metabolic abnormalities similar to that seen in NIDDM.

A number of studies in humans also indicate that the early phase of insulin secretion is important in carbohydrate metabolism. A delay in preprandial

insulin delivery to subjects with IDDM results in a marked worsening of prandial hyperglycaemia (Kraegen et al., 1981; Dimitriadis and Gerich, 1981) and the presence of insulin antibodies, which slows the absorption of preprandially injected insulin, also caused prandial hyperglycaemia (Van Haeften et al., 1987).

Thus there is sufficient data to postulate that the abnormal prandial insulin response in NIDDM could be due to an abnormality of the autonomic control of pancreatic beta cells. Reduced insulin secretion could result from reduced vagal activity and/or increased sympathetic tone to the beta cells during meal ingestion. Furthermore, this early deficiency in prandial insulin secretion may contribute to the metabolic disturbance of NIDDM by causing prandial hyperglycaemia. As hyperglycaemia itself causes functional beta cell abnormalities and insulin resistance, prandial hyperglycaemia could contribute to these metabolic abnormalities and lead to a worsening of hyperglycaemia.

1.3.3 Stress and diabetes

Severe physical stress associated with surgery, burns or myocardial infarctions can cause hyperglycaemia in nondiabetic and diabetic individuals (Porte and Woods, 1983). The mediators of this effect include direct sympathetic nervous activation and the release of stress hormones which include adrenaline, cortisol, glucagon and growth hormone. This results in suppression of insulin release, stimulation of hepatic glucose production and reduced insulin action (Rizza et al., 1980; 1981). There is evidence that adrenaline, glucagon and cortisol act synergistically and therefore the effects of these stress hormones are quite potent (Shamoon et al., 1980). The role of the autonomic innervation during stress is unclear but it is likely that sympathetic activation has similar actions and may also promote hyperglycaemia.

NIDDM is characterised by a number of endocrine and neuroendocrine abnormalities which are similar to those seen in stress states. Basal glucagon levels are elevated and there is abnormal prandial regulation of glucagon secretion (Muller et al., 1979). There is also some evidence for increased growth hormone levels (Hansen, 1977), catecholamine levels (Robertson et al., 1976) and abnormal regulation of cortisol secretion (Cameron et al., 1984). It has therefore been proposed that NIDDM may be analogous to chronic stress and that neuroendocrine activation could be responsible for some of the metabolic abnormalities of NIDDM (Porte and Woods, 1983). A glucoreceptor defect in the brain similar to that seen in the beta cells was suggested as a cause for this neuroendocrine activation (Porte and Woods, 1983). Alternatively, glucose enters the brain at a reduced rate in diabetic animals (Gjedde and Crone, 1981; McCall et al., 1982). Thus either reduced entry of glucose or a glucoreceptor defect in the brain could result in prevailing hyperglycaemia being detected within neurones as neuroglycopaenia and result in a counterregulatory response which is inappropriate for the blood glucose level. Several authors have noted that a counterregulatory response occurs above the usual hypoglycaemic range in chronically hyperglycaemic diabetic subjects and have suggested that this is due to an "upward resetting of the glucostat" in these subjects (DeFronzo et al., 1980).

It has been considered for many years that psychological stress can cause hyperglycaemia in humans in a manner analogous to physical stress and that severe or chronic stress may actually cause diabetes or alter metabolic control in diabetic individuals. Implicit in this hypothesis is the consideration that subjects with diabetes are more sensitive to psychological stress. The

demonstration of such a relationship would be powerful support for a 'stress' model of NIDDM.

Patients with insulin dependent diabetes have greater glycaemic responses following adrenaline, glucagon or cortisol administration (Shamoon et al., 1980). The increased sensitivity of subjects with IDDM to adrenaline is due to the inability of these subjects to secrete insulin which normally modulates the glycaemic response to adrenaline (Berk et al., 1985). Thus it is likely that the excessive glycaemic responses often seen during severe stress in subjects with IDDM are due to the inability of these subjects to mount an insulin response. Studies of this nature have not as yet been carried out in subjects with NIDDM. However, as subjects with NIDDM have impaired beta cell responses to glucose and also have a reduced insulin secretory capacity (Ward et al., 1984), these subjects may also have increased sensitivity to stress or to stress hormones (Porte and Woods, 1983).

Epidemiological studies utilising life stress analyses have generally found a positive correlation between adverse life events and metabolic control in both IDDM and NIDDM (Hinkle and Wolf, 1952; Jacobson et al., 1985; Chase and Jackson; 1981). However these studies were not able to determine whether the association was causal or due to factors such as motivation or compliance with therapy.

Several groups have attempted to induce metabolic changes in the laboratory using psychological stress. Early studies appeared to demonstrate that stress could cause elevations in free fatty acid and ketones, however the effects on blood glucose were variable, the glucose level either not changing, rising or even falling (Hinkle and Wolf, 1949; Vandenbergh et al., 1966; Baker et al.,

1969). A recent study failed to demonstrate that acute psychological stress in the laboratory caused hyperglycaemia in subjects with IDDM (Kemmer et al., 1986). Studies of this nature have not as yet been carried out in NIDDM.

The other approach which has been used to investigate the role of stress in diabetes has been to attempt to reduce stress or improve methods of coping with psychological stress and examine metabolic control. Several case reports reported an apparent benefit from biofeedback techniques. However any benefit was usually only in terms of a reduction in insulin requirements (Fowler et al., 1976; Guthrie et al., 1976). One controlled study found that relaxation therapy improved glucose tolerance and reduced fasting blood glucose and cortisol levels in a group of NIDDM subjects. The beneficial effect was considered to be consistent with a reduction in hepatic glucose production (Surwit and Feinglos, 1983). Interestingly, when the same study design was applied to a group of subjects with IDDM, relaxation therapy failed to influence glycaemia (Feinglos et al., 1987). In a longer term study, the combination of relaxation therapy and coping strategies was found to cause a reduction in levels of glycosylated haemoglobin in a group of subjects with NIDDM (Henry et al., 1986).

Thus whilst most recent studies have found that psychological stress has little effect on metabolic control in subjects with IDDM, the data suggest that psychological stress could have adverse effects on metabolic control in NIDDM. However few studies have been performed which examine this issue. The treatment regimens prescribed for NIDDM are complex and compliance with treatment is likely to be an important determinant of metabolic control. The effect of psychological stress could be mediated by compliance with therapy rather than by alterations of autonomic activation.

1.3.4 Animal models of NIDDM

Research into NIDDM is hampered by the lack of good animal models. However a number of animal models combine some of the features seen in human NIDDM and may provide insights into the pathophysiology of the human disease.

Recent studies of hypothalamic obesity (VMH lesioned rats) demonstrate that abnormal hypothalamic control of the autonomic nervous system results in several defects characteristic of NIDDM in addition to the classical obesity and hyperphagia. Thus the syndrome of VMH obesity includes insulin resistance, excess liver glucose production and mild hyperglycaemia (Penicaud et al., 1986). These observations clearly demonstrate that defects in the central nervous system (albeit artificially produced) can result in metabolic abnormalities.

A pathophysiological role for the autonomic nervous system is also supported by studies of the Zucker "fatty" rat, a genetic model of animal obesity. The obese (homozygous) rats have several features similar to VMH lesioned rats including hyperinsulinaemia which has been shown to be due to increased parasympathetic tone (Rohner-Jeanrenaud et al., 1983) and possibly also reduced sympathetic tone to the pancreatic islets (Levin et al., 1981; Planch et al., 1983). These animals become severely insulin resistant (Terrettaz and Jeanrenaud, 1983), and develop basal and prandial hyperglycaemia as they get older (Ionescu et al., 1985). The hyperglycaemia and glucose intolerance has been shown to be due to increased rates of hepatic glucose production (Rohner-Jeanrenaud et al., 1986). Thus these animals naturally develop a number of metabolic abnormalities similar to those seen in NIDDM which could be related to demonstrable abnormalities of the autonomic nervous system.

Another genetically obese rodent, the ob/ob mouse also possesses a number of central nervous system defects and develops hyperglycaemia, hyperinsulinaemia and insulin resistance (Bray and York, 1979). It has been shown that blood glucose levels in these animals are hyperresponsive to environmental stress (Surwit et al., 1984). This appeared to be due to an exaggerated sensitivity to adrenergic stimulation and suggested that altered sympathetic function could be an aetiological factor in the development of diabetes in these animals (Kuhn et al., 1987).

Finally, it has been reported that the combination of VMH lesions, high fat feeding and low dose streptozotocin in rats resulted in a syndrome of obesity and hyperglycaemia with features similar to NIDDM whereas each treatment in itself did not produce significant hyperglycaemia (Inoue et al., 1982). This model lends support to the hypothesis that an abnormality of autonomic function could combine with other metabolic defects (some of which may be genetically determined and others due to environmental factors) to cause the syndrome of NIDDM.

1.3.5 Neural hypothesis for NIDDM

A synthesis of what is known about normal physiology and the pathophysiology of NIDDM suggests several possible hypotheses to explain how central neural mechanisms could be causative factors in NIDDM or secondarily contribute to the metabolic disturbance of the condition. It seems clear that many of the metabolic abnormalities associated with NIDDM (such as insulin resistance and impaired beta cell function) can occur secondarily to hyperglycaemia. Therefore consideration of how neural factors could cause or contribute to hyperglycaemia would appear to be central to any theory of the pathogenesis of NIDDM.

The closely integrated nature of the hypothalamic structures which control the two arms of the autonomic system suggests that an imbalance in the activity of several important hypothalamic nuclei could cause a reciprocal imbalance in autonomic nervous activity. Excess sympathetic drive and/or reduced parasympathetic activity to the liver and pancreas could cause excess liver glucose production and also cause an inhibition of insulin secretion. Thus an overactive VMH (increased sympathetic tone) and underactive LHA (reduced vagal activity) might produce the reciprocal defects in hepatic glucose production and pancreatic beta cell function typical of NIDDM. VMH overactivity could also explain several other defects of NIDDM such as the activation of other neuroendocrine systems.

It is possible that instead of having a primary role in the aetiology of NIDDM or a secondary role in the pathogenesis of hyperglycaemia, an abnormality in the autonomic nervous system could arise secondary to autonomic neuropathy. There is now evidence to suggest that subclinical abnormalities of parasympathetic and sympathetic function occur more frequently than conventional tests suggest (Pfeiffer et al., 1984). Metabolic deterioration occurs commonly after long duration of NIDDM and patients often eventually require insulin therapy. Abnormal autonomic control of the liver or islet cells secondary to neuropathy might contribute to this deterioration.

1.4 Aims of this thesis

The aims of this thesis are to investigate certain aspects of the autonomic nervous system and its control of metabolism with particular reference to NIDDM. The studies described in this thesis concentrated on two areas of metabolism, namely prandial insulin secretion and basal hepatic glucose production.

The following specific aims were pursued:

1. (a) to determine whether cephalic phase insulin secretion could be demonstrated in nondiabetic subjects and whether it has a significant influence on glucose homeostasis.

(b) to determine whether the deficiency in early prandial insulin secretion contributes to the hyperglycaemia of NIDDM.

2. (a) to determine whether basal hyperglycaemia and hepatic glucose production in NIDDM are altered by stimulation of the sympathetic nervous system.

(b) to determine whether subjects with NIDDM have altered sensitivity to the sympathetic neural activation of psychological stress.

CHAPTER TWO

Materials and methods

2.1 Introduction

This chapter describes the subjects who participated in the experiments and discusses the principles, methods and precision of the various substrate and hormone measurements; the preparation and delivery of administered substances and agents; the glucose clamp technique used in chapter 3; the glucose isotope techniques used in chapters 5 and 6; and the respiratory gas exchange measurements and indirect calorimetry used in chapter 5.

2.2 Subjects

Subjects with noninsulin dependent diabetes mellitus (NIDDM) and nondiabetic control subjects were studied in this thesis. All who took part gave written informed consent before the studies and all studies were approved by the Ethics and Research Committee of St. Vincent's Hospital.

In chapter 3, young healthy volunteers were studied. Most of these were students from the University of New South Wales. A number of young healthy men employed at the Garvan Institute were also studied. None had a family history of NIDDM.

In chapters 4, 5 and 6, subjects with NIDDM were studied and in chapters 4 and 6 age and weight matched nondiabetic subjects were also studied. The diabetic patients were recruited from either the St. Vincent's Hospital Diabetes Clinic, the private clinics of the visiting endocrinologists to St. Vincent's Hospital or from advertisements in the local media. These subjects all fulfilled published criteria for NIDDM (National Diabetes Data Group, 1979). All were treated with either diet or diet supplemented with sulphonylurea agents (either glibenclamide, gliclizide or tolbutamide). All were otherwise healthy and did not take medications or have other conditions known to interfere with carbohydrate

metabolism. The diabetic subjects who took part in the meal studies had previously received appropriate instruction from a dietitian regarding a diabetic (high complex carbohydrate, low fat) diet.

The nondiabetic subjects were recruited from hospital staff or were friends or relatives of staff and several responded to advertisements in the local media (radio and newspapers). One subject with treated hypothyroidism (euthyroid for more than 3 years at the time of study) was recruited from the general endocrine clinic. No subjects had other medical conditions or were on other medication. None had first degree relatives with NIDDM.

All studies were performed in the clinical investigation facility at the Garvan Institute of Medical Research. All studies were carried out in the morning after an overnight fast. Where appropriate, sulphonylurea therapy was suspended for 48 hours before each study so that there was virtually no sulphonylurea remaining in the circulation.

In all studies, arterialised venous blood samples were collected (McGuire et al., 1976). A Teflon catheter (22 or 18 gauge) was inserted retrogradely into a dorsal wrist or hand vein. The hand was warmed by a thermostatically heated electric blanket to a temperature in excess of 60^{o} Centigrade throughout the studies. The pO_2 of a venous sample was obtained to ensure adequate arterialisation of the venous blood before all studies.

Where substances were infused, separate venous cannulae were inserted into antecubital veins. In chapter 3, where 20% dextrose was infused, long lines (Cavafix, Braun, Melsungen, West Germany) were inserted via an antecubital vein into the axillary vein to minimise the risk of thrombophlebitis.

2.3 Hormone and substrate assays

The following assays were already established at the Garvan institute and were used in the studies contained in this thesis.

Glucose: Plasma and blood glucose levels were measured by an immobilised enzymatic method using glucose oxidase (Yellow Springs model 23 AM, Yellow Springs, Oh).

Insulin: Serum insulin was estimated using a double antibody radioimmunoassay using highly purified human insulin as standard. The intra- and inter-assay coefficients of variation for this assay are 6% and 7% respectively at 5 mU/l.

C Peptide: Serum C peptide was measured by double antibody radioimmunoassay using synthetic human C peptide as standard (intra- and inter-assay coefficients of variation are 4% and 6% respectively at 1 μg/l).

Glucagon: Blood samples for glucagon estimations were collected into tubes containing heparin and 1000 units/ml of blood of the protease inhibitor, aprotinin (Trasylol). These were collected on ice and immediately separated and frozen for later assay. Plasma glucagon was measured in a double antibody radioimmunoassay using the RCS5 antiserum (purchased from Dr Steven Bloom) and highly purified porcine glucagon as standard (intra- and inter-assay coefficients of variation are 6% and 15% at 100 ng/l respectively). The antiserum is specific for the C-terminal end of the glucagon molecule and the assay is thus specific for pancreatic glucagon and does not detect gut derived glucagon like material (Alford et al., 1977).

Free fatty acids: Serum free fatty acid levels were measured by an accurate and precise enzymatic colorimetric method (NEFA C, Wako Chemical Industries, Osaka, Japan). Blood was collected, separated and the serum immediately frozen for later analysis. The intra-assay coefficient of variation for this assay is 1.1%.

Catecholamines: Plasma catecholamines were determined by two methods.

1. Plasma adrenaline and noradrenaline were determined by a sensitive radioenzymatic method (CAT-A-KIT, Amersham Int, UK). This assay utilises the enzyme catechol-O-methyltransferase to catalyse the transfer of a [^{3}H]-methyl group to the hydroxyl in position 3 of the catecholamine ring. Separation of the resulting products is achieved by thin layer chromatography and the products of noradrenaline and adrenaline ([^{3}H]normetanephrine and [^{3}H]metanephrine respectively) are counted after prior elution and periodate oxidation to [^{3}H]vanillin. The intra- and inter-assay coefficients of variation for noradrenaline are 4.2% and 7.5% and for adrenaline are 3.6% and 10%.

2. Plasma levels of free noradrenaline and noradrenaline metabolites were determined by a highly specific and precise gas chromatograph/mass spectrometry method (GC/MS) using deuterated internal standards (Smythe et al., 1982; 1983). Blood for this assay was collected on ice and immediately separated and frozen for later assay. The intra- and inter-assay coefficients of variation for plasma free noradrenaline are less than 3%. Plasma free levels of the primary metabolites of noradrenaline, 3,4-dihydroxyphenylethylene glycol (DHPG) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), were also measured by GC/MS using deuterated internal standards. The inter- and intra-assay coefficients of variation for these analytes are less than 4%. In the tyramine administration studies, tyramine levels were estimated in the samples

by GC/MS by reference to the deuterated MHPG standard. Tyramine levels were estimated in arbitrary units and responses compared with baseline, i.e. before any agent had been delivered. This provided a semi-quantitative index of the timing and magnitude of tyramine absorption after oral administration of the agent.

Cortisol: Serum cortisol was measured by RIA using a highly specific antiserum, a tritium labelled tracer and a charcoal separation step. The inter- and intra-assay coefficients of variation for this assay are 6.8% and 8.5% respectively.

Adrenocorticotrophin (ACTH): Blood was collected into plastic tubes containing EDTA and chilled on ice before rapid separation. The plasma was frozen immediately for later assay. Plasma ACTH was assayed, after a preliminary extraction step, by RIA using a highly specific antiserum and a charcoal separation step. The standard used was human corticotrophin (National Institute for Biological Standards and Control, London). The inter- and intra-assay coefficients of variation for this assay are 18% and 11% respectively.

Growth Hormone (GH): Serum GH was measured by radioimmunoassay. The inter- and intra-assay coefficients of variation for this assay are 11.4% and 5.8% respectively.

Prolactin: Serum prolactin levels were measured by a double antibody radioimmunoassay using a highly specific antibody against purified human prolactin calibrated against the first International Reference Preparation (1st IRP 75/504) as standard. The inter- and intra-assay coefficients of variation for this assay are 7% and 5% respectively at 400 mIU/l.

Pancreatic polypeptide: Serum pancreatic polypeptide levels were measured by radioimmunoassay using an ^{125}I-labelled tracer, a specific antibody and a second antibody precipitation step. The inter- and intra-assay coefficients of variation for this assay are 13.3% and 9.5% respectively.

2.4 Administration of hormones and pharmacological agents

In chapters 4 and 5, exogenous neutral porcine insulin (Actrapid MC, NOVO Laboratories, Copenhagen) was delivered intravenously during standard mixed meals to subjects with NIDDM. The insulin was delivered in polygeline solution (Haemacel, Boeringwerke, West Germany), 54 units in 500 mls, which has been shown to reliably deliver 10 units per 100 mls (Kraegen et al, 1975) by means of an electronic pump (IVAC 531, Ivac Corporation, San Diego, Ca).

Intranasal insulin administration to subjects with NIDDM was also studied during standard mixed meals in chapter 4. The nasal insulin solution was prepared in conjunction with the St. Vincent's Hospital pharmacy. Crystalline porcine monocomponent insulin (donated by CSL-NOVO, Novo Research Institute, Copenhagen) was dissolved in 1% sodium glycocholate (Sigma Chemical Co, St Louis, USA) in an 0.14 molar phosphate buffer solution, pH 7.0, to make a solution containing 250 units/ml insulin. Intranasal insulin was delivered using a hand held nebuliser (Pfeiffer pump, donated by Boehringer Ingelheim, Artarmon, NSW) which delivered 60±9 µl/spray (mean±SD, n=10). Each spray therefore delivered 15 units of insulin intranasally. The nasal insulin solution prepared in this way may lose potency within 8 weeks of preparation (Frauman et al., 1987); therefore the solution was freshly prepared each month.

In chapter 6, noradrenaline was delivered intravenously to both nondiabetic and diabetic subjects via an electronic pump (IVAC 531, Ivac Corporation, San Diego, Ca). Noradrenaline (Levophed, Winthrop Laboratories, Sydney, Australia) was administered at a rate of 60 ng/kg.min for 60 minutes. The noradrenaline solution was prepared on the morning of each study and the solution protected from light before and during the infusions. The noradrenaline (4 ml ampoules containing noradrenaline acid tartrate, equivalent to 4 mg base) was added to 500 mls normal saline containing 250 mg ascorbic acid as an antioxidant to make a solution containing 8 μg/ml noradrenaline.

Also in chapter 6, tyramine hydrochloride (Sigma Chemical Co, St Louis, USA) was administered orally at a fixed oral dose of 800 mg/person. Capsules containing 200 mg tyramine were prepared by the St. Vincent's Hospital pharmacy.

2.5 Hyperglycaemic clamp (chapter 3)

During the studies in chapter 3, intravenous dextrose (20%) was administered to acutely elevate the blood glucose and to obtain a hyperglycaemic clamp. The dextrose was delivered via an electronic pump (IVAC 531, Ivac Corporation, San Diego, Ca) which was calibrated before each study. Blood glucose measurements were obtained at 5 minute intervals and the dextrose infusion rate was varied by means of an algorithm to achieve a target blood glucose level and maintain the desired blood glucose level for a period of 90 minutes. The following published algorithm (Furler et al., 1986) was used to achieve the clamp:

$$R_i = 0.5x(R_{i-1} + R_{i-2}) + 12x(L - G_{i)} + 18x(G_{i-1} - G_i)$$

where R_i is the infusion rate at each time point, L is the target blood glucose level (8 mmol/l) and G_i is the blood glucose level at each time point.

2.6 Calculation of portal insulin concentration (chapter 4)

Portal venous insulin concentrations in the early insulin augmentation studies (chapter 4) were calculated using the non-steady state method suggested by De Feo et al (1986). This calculation makes use of estimations of insulin secretory rates based on changes in C peptide concentrations, with equations derived from a two-compartmental model (Eaton et al., 1980). The calculations of insulin secretory rates depend on the assumption that portal blood flow remains constant. Therefore in these prandial studies, when changes in portal vein blood flow are likely to occur, interpretations based on calculated portal vein insulin levels must be made with some caution.

Portal vein insulin concentrations were calculated using the equation:

$$PI_t = AI_t + [PI_o - AI_o].ISR_t/ISR_o$$

where PI_t and PI_o are the portal venous insulin concentrations at time t and basally respectively, AI_t and AI_o are the arterial insulin concentrations at time t and basally and ISR_t and ISR_o are the insulin secretory rates at time t and basally. Initial portal vein insulin concentrations were estimated with a value of 2.4 for the portal venous-systemic insulin gradient (De Feo et al., 1986).

2.7 Isotopic determination of glucose turnover (chapters 5, 6)

The studies in chapter 6 employed tracer methodology to determine glucose turnover in the basal state and after the administration of hormones and pharmacological agents. The techniques used in these studies have been previously used to study exercise physiology at the Garvan Institute (Chisholm et al., 1982; Jenkins et al.,1985; 1986).

In these studies, subjects received a constant infusion of [3-^{3}H]glucose (Amersham, Australia). The tritiated glucose was diluted in 50 mls 0.9% wt/vol saline, passed through a 0.22 μm Millipore filter (Millipore, Bedford, Ma) and delivered via an infusion pump (Braun Perfusor, Braun, Melsungen, West Germany) at an approximate delivery rate of 25 μCi/hour. A 90 minute baseline period allowed equilibration of tracer before the commencement of all studies.

Plasma samples for [^{3}H] glucose determination were deproteinised with $ZnSO_4$ and $Ba(OH)_2$ and counted in a liquid scintillation spectrometer after evaporation of ^3H_2O at 60^O Centigrade. Samples from the infusion syringe were similarly counted to obtain an accurate determination of the tracer infusion rate.

The data were analysed using a non-steady state pool fraction approach to give estimates of rates of systemic glucose appearance and disappearance. This method uses Steele's formula for non-steady state kinetics (Steele et al., 1956) as modified by De Bodo et al (1963) and assumes that the glucose pool is a single compartment and that there is a fixed rapidly equilibrating pool. The equations of Steele have been validated and found to give results similar to equations using multiple glucose pools (Radziuk et al., 1978). A pool fraction of 0.65 was used in these studies (Cowan and Hetenyi, 1971). Plasma glucose and [3-^{3}H]glucose concentration data were smoothed using a three-point moving average before tracer kinetic analysis. Plasma glucose concentration and [3-^{3}H]glucose concentrations were fitted using a cubic spline function which provides a continuous first derivative (Jenkins et al., 1986).

The equations used to calculate glucose production (R_a) and glucose disposal (R_d) are as follows:

$$R_a = R_a^*/SA - [pVC/SA].dSA/dt$$

$R_d = R_a - pV.dC/dt$

where R_a^* is the infusion rate of the glucose tracer, SA is the specific activity of glucose (C^*/C), p is the pool fraction (=0.65) and V is the volume of distribution (20% of body weight).

Glucose clearance was obtained by the calculation: glucose clearance = $R_d \div$[glucose]. Glucose clearance is directly measured during glucose tracer infusions with R_d being derived from it in the Steele equations. It is therefore a valid method of evaluating peripheral glucose disposal and takes into account the prevailing levels of glycaemia which influence glucose uptake by mass action (Radziuk and Lickley, 1985). Glucose clearance is useful when groups with differing levels of glycaemia are being compared.

A double isotope approach was used in chapter 5 to measure peripheral and hepatic contributions to oral glucose tolerance (Radziuk et al., 1978; Ferrannini et al., 1985) following augmentation of early prandial insulin secretion in NIDDM. A glucose isotope, [3-^{3}H]glucose was infused peripherally into an antecubital vein and a different glucose isotope [6-^{14}C]glucose (Amersham International, UK) was used to trace the absorption of an ingested glucose load. After the 90 minute equilibration period for the tritiated glucose infusion, the subjects drank 70 gm glucose in 100 mls water enriched with 50 μCi [6-^{14}C]glucose to which was added 100 gm of uncooked beaten egg to approximate the fat and protein content of the standard mixed meals used in chapter 4. An aliquot of the enriched glucose solution was obtained prior to addition of the egg, for accurate determination of the glucose concentration and the specific activity of the drink before ingestion.

Aliquots of plasma were obtained, deproteinised and evaporated (to remove ^3H_2O) as above and counted for both [^{3}H] and [^{14}C] radioactivity. Recycling of [^{14}C] label into glucose was estimated by determining the incorporation of [^{14}C] into the 1-position of glucose by enzymatic decarboxylation using the method of Kalhan et al (1977) as modified by Firth et al (1986). The ^{14}C counts obtained were multiplied by 4 to provide an estimate of total recycled glucose (Reichard et al., 1963; Insel et al., 1975).

The rates of appearance (R_a) in the systemic circulation of both ingested and total glucose were calculated (total glucose includes both ingested and endogenously produced glucose). The plasma concentration of glucose derived from the oral load was obtained by measuring the plasma radioactivity of [6-^{14}C]glucose and dividing this by the specific activity of the oral load. From the plasma concentrations of ingested glucose and those of the infused tracer ([3-^{3}H]glucose), the R_a of ingested glucose was calculated. From the concentrations of total glucose and those of the infused tracer, the R_a of total glucose was calculated. Endogenous hepatic glucose production after the oral load was calculated as the difference between systemic appearance of ingested glucose and total glucose appearance.

There are several potential sources of error in the use of glucose tracers to trace glucose turnover. Recycling of the labelled carbon in the glucose cycle (glucose/glucose 6-phosphate) would lead to an underestimate of rates of glucose turnover, however the isotope [3-^{3}H]glucose is not detritiated in the glucose/glucose-6-phosphate cycle (Altszuler et al., 1975; Katz and Rognstad, 1976). However, tritium at the third position could be incorporated into glycogen and released without releasing its label and this would cause erroneously low tracer-determined glucose disposal rates. Limitations in the

model and equations used could result in either under- or overestimations in tracer determined rates of glucose turnover depending on the experimental conditions (Cobelli et al., 1987). Discrepant metabolism of tracer labelled glucose in comparison with native glucose (i.e. an isotope effect) may also result in underestimates of tracer derived rates of glucose turnover (Bell et al., 1986; Argoud et al., 1987). The influence of different tracers has been restudied recently and [3-^{3}H]glucose may slightly underestimate true rates of R_a and R_d in both nondiabetic and insulin dependent diabetic man although alterations in turnover rates are estimated correctly (Bell et al., 1986). This has not as yet been studied in NIDDM.

The aim of the studies in chapter 6 was to examine the glucose turnover responses to several stimuli which would increase glycogenolysis and possibly also gluconeogenesis. Therefore several of the above potential sources of error may have been present. For instance, incorporation of tracer into glycogen and subsequent release following administration of noradrenaline or tyramine could have resulted in underestimates of true rates of glucose disposal and hepatic glucose production. The turnover data must therefore be considered to represent qualitative rather than quantitative changes and in particular comparisons between the diabetic and nondiabetic subjects must be made with some caution.

In the meal studies (chapter 5), subjects with NIDDM were studied under 2 conditions and each subject served as their own control, thus minimising several of the potential sources of error. The isotope [3-^{3}H]glucose was used to trace the rate of systemic glucose appearance. This isotope is not detritiated in the glucose/glucose-6-phosphate cycle, however it could be incorporated into glycogen and released without releasing its label and this could lead to an

underestimate of rates of glucose appearance and disappearance. Firth et al (1986) found that postprandial estimates of glucose turnover rates were essentially the same in subjects with NIDDM when using either [2-^{3}H]glucose (which does not retain its label during uptake and release of glucose from glycogen) or [3-^{3}H]glucose. Thus these studies suggested that cycling through glycogen is minimal in NIDDMs following glucose ingestion and also demonstrated that futile cycling (glucose cycle) which is present in NIDDMs in the preprandial state (Efendic et al., 1985) does not appear to be substantial after oral glucose.

After the ingestion of [6-^{14}C]glucose, radioactive glucose may appear in the systemic circulation as unchanged [6-^{14}C]glucose or as new glucose synthesised from labelled three-carbon intermediates which have been derived from glycolysis of the ingested labelled glucose (Cori cycle). This component has been shown to be small in NIDDM (Firth et al., 1986) however it is conceivable that this could have been influenced by the conditions of the study (early insulin augmentation); therefore recycling of ^{14}C in the sixth position to the first position was estimated in these studies. Assuming equivalent randomisation to positions 1, 2, 5 and 6 (Reichard et al., 1963), a qualitative estimate of glucose derived from this pathway can be obtained.

2.8 Respiratory gas analysis and indirect calorimetry (chapter 5)

Rates of energy expenditure and substrate oxidation can be accurately calculated from determinations of respiratory gas exchange (Frayne, 1983; Garlick et al., 1987). In the double isotope study (chapter 5), respiratory gas analysis was performed to obtain measurements of meal related energy expenditure and rates of carbohydrate and lipid oxidation were calculated.

The technique used was a modification of the method of Lindmark et al (1985). The equipment comprised a semi-opened circuit, ventilated hood system. This was operated by the negative-flow pressure of the gas flow through the system. A soft mask was loosely placed over the subjects mouth and nose inside a ventilated hood. A flow rate of 25-30 litre/minute prevented the local accumulation of CO_2. The expired air was drawn through a mixing chamber to avoid breath to breath gradients during recordings. An aliquot of the expired air was drawn from the mixing chamber by a separate pump with a flow of 1.5 litre/minute for analysis. The gas sample was dried with silica gel before analysis for carbon dioxide (Horiba, Kyoto, Japan, model PIR2000) and oxygen (Applied Electrochemistry, Sunnyvale, CA, model S-3A). Data logging and control were automated via an Apple IIe desktop computer with purposebuilt interface. The analysers were calibrated with analysed gases and room air oxygen and carbon dioxide levels were determined immediately before and after each test period. The computer collected values every 60 seconds from the instruments and processed the values after a complete run to give O_2 consumption, CO_2 production and RQ. Flow was measured by linear mass flowmeters (Teledyne Hastings Raydist, Hampton,VA, models ST-5K and ST-50K).

The subjects voided urine at the beginning and end of the study to obtain an estimate of urinary nitrogen and glucose excretion. Urine urea was measured by the St Vincent's Hospital Biochemistry Department, using a urease method on an Astra and Beckman analyser. Nitrogen excretion was then calculated using the method of Blackburn et al (1977). The amount of protein oxidised was estimated from the urinary nitrogen excretion assuming 1g nitrogen = 6.25g

protein. For the calculations of nonprotein substrate oxidation, it was assumed that protein oxidation occurred at a constant rate throughout the meal period.

Heat production following meal ingestion was calculated according to De Weir's equation (1949):

heat production = 16.5 x O_2 consumption + 4.62 x CO_2 production.

Rates of whole body carbohydrate and lipid oxidation were estimated using the equations:

$G = 4.55\ VCO_2 - 3.21\ VO_2 - 2.87\ N$

$L = 1.67\ (VO_2 - VCO_2) - 1.92\ N$

in which G denotes the carbohydrate oxidation rate (mg/min), L the lipid oxidation rate (mg/min), VCO_2 carbon dioxide production (ml/min), VO_2 oxygen consumption (ml/min) and N the rate of protein oxidation (Frayne, 1983).

CHAPTER THREE

Cephalic phase insulin secretion and glucose homeostasis.

3.1 Introduction

Cephalic responses are elicited by stimuli impinging on sensory receptors of the oropharynx and head and are mediated by a neural pathway which has a central nervous system relay (Powley and Berthoud, 1985). The study of the cephalic phase allows neurally based metabolic responses to be studied independently of the gastric and intestinal phases of digestion or effects due to absorbed nutrients. Cephalic responses may have a function in triggering digestive or metabolic processes before the arrival of food into the gut and may be important for both digestive and metabolic efficiency (Brand et al., 1982).

Cephalic phase insulin secretion has been demonstrated in a number of animal species including man (Hommel et al., 1972; Louis-Sylvestre, 1976; Sjostrum et al., 1980). In animals, various methods have been used to elicit cephalic phase insulin secretion. These include taking frequent blood samples during food ingestion and assuming that the earliest phase of insulin secretion before any detectable nutrient absorption is the cephalic response (Louis-Sylvestre, 1976; Berthoud et al., 1980); feeding the animals nonnutritive substances (Strubbe and Steffens, 1975) or using classical conditioning techniques (Storlien, 1985). With such methods, cephalic phase insulin secretion has been routinely elicited in animals with amplitudes up to 200% from basal.

The situation in humans is less clear. A number of studies have reported cephalic phase insulin secretion in humans following the sight and smell of food (Parra-Covarrubias et al., 1971; Sahakian et al., 1981) and the hypnotic suggestion of eating (Goldfine et al., 1970). Many of these studies have been criticised either for being uncontrolled, reporting changes close to the sensitivity of the insulin assays or because responses were elicited in only a

small proportion of subjects (Powley and Berthoud, 1985). Two recent studies failed to elicit cephalic phase insulin release in nonobese subjects (Sjostrum et al., 1980; Taylor and Feldman, 1982). Obese humans appear to exhibit a stronger cephalic insulin response (Parra-Covarrubias et al., 1971; Sjostrum et al., 1980) and in the study of Sjostrum and colleagues (1980) reliable insulin responses were found only in obese and not in lean subjects. Thus the human studies appear to document the existence of cephalic phase insulin secretion although the responses have been relatively small and inconsistent.

The efferent arc of the reflex responsible for the cephalic insulin response is mediated by the vagus nerves to the pancreas and cephalic phase insulin secretion is abolished by vagotomy (Louis-Sylvestre, 1976; Storlien, 1985). Cephalic phase insulin secretion is also affected by manipulations of the hypothalamus. VMH lesions (Storlien, 1985) or the administration of procaine to the VMH (Berthoud et al., 1980) have been shown to abolish the response whereas in another study VMH lesions resulted in an augmentation of cephalic phase insulin secretion (Louis-Sylvestre, 1976). The reasons for this discrepancy are unclear but the animals in the latter study bore chronic VMH lesions and hence may have been hyperphagic and may have had beta cell hyperplasia. At any rate, it is clear that the hypothalamus is involved in mediating the cephalic insulin response.

A function for cephalic phase insulin secretion has not been determined although several possible have been suggested (Powley and Berthoud, 1985). Cephalic responses may provide information about food before it is ingested or absorbed and could serve as a regulator of either appetite or satiety; cephalic phase insulin secretion may occur merely as a result of adventitious conditioned stimuli and have no function; alternatively, cephalic phase insulin

secretion may play a role in metabolism and act to trigger metabolic processes in anticipation of the inflow of nutrients following meal ingestion.

Support for a metabolic function for cephalic phase insulin secretion comes from studies in rats where the reflex has been eliminated. When the cephalic phase is bypassed by intragastric feeding (Louis-Sylvestre, 1978) or the efferent arc is interrupted surgically or pharmacologically (Strubbe and van Wachem, 1981; Trimble et al., 1981; Steffens, 1976), early insulin secretion is abolished and a worsening of glucose tolerance results. Replacement of the deficient early insulin response with intravenous insulin restored glucose tolerance to normal in one study (Berthoud et al., 1981). These experiments therefore suggest that cephalic insulin secretion has an important role in minimising prandial deviations of glucose from basal. The observation that rats chronically fed intragastrically accumulate more fat than pair-fed controls supports the contention that cephalic phase insulin secretion has a role in metabolic efficiency (Cox and Powley, 1981).

No studies have been directed at assessing the physiological significance of cephalic responses in humans. However studies of the timing of insulin delivery to insulin dependent diabetic subjects produce results remarkably similar to the above animal studies. When preprandial insulin delivery is delayed even slightly to such patients, a substantial worsening of glucose tolerance is seen (Kraegen et al., 1981; Dimitriadis and Gerich, 1981). The presence of insulin antibodies in patients with insulin dependent diabetes causes plasma free insulin levels to rise slowly after subcutaneous injection and this appears to cause prandial hyperglycaemia (Van Haeften et al., 1987). These studies clearly indicate that an early rise in insulin levels is important in prandial glucose metabolism.

A large number of peptides, hormones and substrates have been shown to be influenced by cephalic responses although the function of most of these neural reflexes is unknown. Free fatty acid levels (FFA) have been shown to fall rapidly with the sight and smell of food (Parra-Covarrubias et al., 1971; Penick et al., 1966) and there is evidence that blood glucose is influenced directly by hypothalamic nervous activity and could conceivably also be subject to a cephalic response (Shimazu, 1981; Smythe et al., 1984). Thus it is possible that there are a number of cephalic reflexes which may influence the flux of nutrients during feeding.

The aim of the studies in this chapter was firstly to demonstrate cephalic phase insulin secretion in normal human subjects using a variant of the tease meal technique and then to determine whether cephalic responses would alter the disposal of an intravenous glucose load. It was considered possible that cephalic phase effects on the beta cell might alter subsequent glucose stimulated insulin secretion in a manner analogous to the potentiation of insulin secretion by gut hormones (Andersen et al., 1978) and the experiments were designed to test for this.

In addition, the effects of cephalic stimuli on FFA and pancreatic polypeptide (hPP) were examined. Both substances have been shown to be influenced by cephalic responses (Penick et al., 1966; Schwartz et al., 1979). The relationship between a cephalic FFA response and cephalic phase insulin secretion has not previously been examined. Secretion of hPP is mediated by the vagus nerve (Schwartz et al., 1978) and thus could serve as an independent measure of vagal activity.

3.2 Subjects and methods

Seventeen healthy normal subjects aged 19-23 years, and within 15% of ideal body weight, participated in the studies. Six subjects (4 women and 2 men) participated in study 1, seven men in study 2 and five men in study 3 (one subject participated in studies 2 and 3).

Each subject was studied on two occasions in the morning after an overnight fast. The studies were performed in random order. The subjects were informed about the general nature and aims of the study. On one occasion (food stimulus study), they were told to expect a breakfast meal, the composition of the meal and the approximate time the meal would be presented. On the control study day they were informed that they would not receive a meal. In study 2 (see below), the subjects were given the additional information that the effect of a sweet taste presented immediately before a meal was being studied.

On each occasion (studies 1 and 2 only), an antecubital vein was cannulated and the catheter advanced to the subclavian vein to allow dextrose infusion and a vein on the dorsum of the ipsilateral hand was cannulated retrogradely for blood sampling. The hand was warmed to 60^{o}C using a thermostatically controlled electric warming blanket to enable sampling of arterialised venous blood (as detailed in chapter 2.2). Subjects were studied sitting comfortably at a table and at least 30 minutes was allowed to elapse before commencement of the study to minimise effects due to the stress of cannulation. Blood samples were taken for baseline values (3 samples before food stimulus presentation) and at varying intervals thereafter for blood glucose, serum insulin, C peptide, hPP, and FFA levels (FFA taken in study 2 only).

Blood and serum samples were assayed as described in Chapter 2.3. All samples from the paired studies of each subject were included in the same assay to avoid inter-assay variation.

During each study, after the initial baseline period (± presentation of food stimuli), 20% dextrose was delivered (via a calibrated, electronic pump) at a fixed rate of 83.3 µmol/(kg.min) (15 mg/(kg.min)) to elevate the blood glucose rapidly from basal to a level of 8 mmols/l, after which the dextrose infusion rate was varied according to 5 minutely estimations of blood glucose, using a previously published algorithm (Furler et al., 1986) in order to maintain a clamped blood glucose level of 8 mmols/l for a period of 90 minutes (hyperglycaemic clamp - see chapter 2.5 for details).

Study 1. The subjects were exposed to the sight and smell of a meal which they were expecting to eat. After the basal period, a freshly cooked, appetising and odoriferous meal (bacon, eggs, tomato, croissants, jam and percolated coffee) was presented to them 3 minutes before the dextrose infusion. They were asked to delay eating for various fictitious reasons (e.g. until a further blood sample was taken) but remained under the impression that they were about to eat until at least 5 minutes after the start of the dextrose infusion. They were then informed of the true nature of the study, the food was removed and they were informed that they could eat the meal at the end of the study.

Following the study the subjects completed a questionnaire in which they were asked to indicate whether they expected to eat the food before it was withdrawn and (i) their degree of hunger before the food was presented, and (ii) how appetising the food appeared, on a 5 point scale (e.g. from very hungry to not hungry).

Study 2. During this study, subjects underwent a protocol similar to study 1 except that a sweet taste was provided as well as the sight and smell of food. As a sweet taste was presented the breakfast meal was chosen to complement sweetness (freshly prepared croissants, butter, jam, orange juice and coffee). The sweet taste was provided by the non-caloric artificial sweetener aspartame (Nehrling et al., 1985) which was taken in unflavoured chewing gum (19 mg aspartame/piece of gum) and supplemented by 19 mg aspartame dissolved in 10 mls water. The breakfast was presented 5 minutes before the start of the dextrose infusion, subjects were asked to chew the gum to extract the sweet taste and to sip the sweetened water to supplement the sweet taste. As in the first study the true explanation and withdrawal of food took place 5 minutes after the start of the dextrose infusion. During the control study for study 2, subjects chewed unflavoured gum and sipped 10 mls of unsweetened water from 5 minutes before commencement of the dextrose infusion.

Following these studies the subjects completed a questionnaire as detailed in study 1. This included an additional question, where they were asked to indicate (on a 5 point scale) how pleasant they found the sweet taste.

Study 3. This study was performed to determine whether aspartame administration alone could stimulate insulin secretion or influence blood glucose. On one occasion, the subjects sipped 15 ml water containing 38 mg of dissolved aspartame for a period of five minutes and on the control occasion they sipped 15 ml water alone. Blood samples were drawn for blood glucose and serum insulin as in the previous studies. These studies were not followed by a dextrose infusion.

3.3 Data analysis

An "anticipation score" was obtained by summing the scores from the 5 point scales for each of the questions from the questionnaires. Thus in study 2 there was a possible maximum score of 15. This score gave an estimate, for each subject, of the hedonic qualities of the presented stimuli as well as the subjects' degree of anticipation of the meals.

A baseline value for each metabolite or hormone was taken from the mean of 3 samples taken before the time of food stimulus presentation or the corresponding control period. Changes in hormone and substrate levels were compared with control studies. Individual integrated hormone or substrate responses were obtained and used for regression analyses. Because there was an overshoot of blood glucose above the intended clamp level of 8 mmols/l during the first 30 minutes of the hyperglycaemic clamp, an index of glucose metabolism was obtained to quantify glucose disposal during that time period for each individual. This index was obtained by dividing the mean blood glucose level by the mean dextrose infusion rate during the first 30 minutes of the clamp. Statistical comparisons were made using Student's paired t tests.

3.4 Results

There was no difference in baseline values determined for each hormone or substrate between control and study days (Table 3.1). Baseline hPP values in all subjects were below the sensitivity of the assay (<6 pmol/l).

Study 1. All subjects reported feeling either hungry or moderately hungry when the food was presented to them. All reported that the food appeared appetising or very appetising and all expected to eat the food before it was withdrawn.

Following the meal presentation, there were no significant changes seen in blood glucose, insulin, C peptide or hPP levels (see fig 3.1). There was also no difference in dextrose infusion rates and stimulated insulin or C peptide levels, during the hyperglycaemic clamp between the food stimulus and control studies.

Study 2. As in study 1, all subjects reported that the food appeared either appetising or very appetising and that they expected to eat the meal before it was withdrawn. One subject did not feel hungry before the study, the others felt either hungry or very hungry. Five of the seven subjects did not enjoy the sweet taste of the aspartame.

Table 3.1 Mean (±SEM) baseline values before control and food-stimulus study for each of the three conditions.

	Study 1 (Tease meal)		**Study 2 (Tease+ aspartame)**		**Study 3 (Aspartame)**	
	Control	Food stimulus	Control	Food stimulus	Control	Food stimulus
Glucose (mmol/l)	4.7±0.1	4.6±0.1	4.5±0.1	4.6±0.	4.4±0.2	4.4±0.2
Insulin (mU/l)	10.1±1.9	7.7±0.5	6.8±1.2	6.2±0.9	3.9±0.7	3.4±1.0
C peptide (ng/ml)	0.8±0.1	1.0±0.3	1.0±0.2	1.0±0.1	-	-
FFA (mmol/l)	-	-	0.61±0.07	0.64±0.10	-	-

There was a significant mean fall in blood glucose levels (0.20±0.03 mmols/l, $p<0.005$) compared to control levels, within 3.5 minutes of the presentation of the combined food stimulus (fig 3.1). There was also a significant mean per cent rise in serum insulin levels (38±15%, $p<0.05$) compared to control levels which occurred within 5 minutes of the food stimulus presentation. Mean C peptide levels rose and mean FFA levels fell (both non-significantly) following the food presentation (Table 3.2) and no change was seen in hPP levels. There was a significant inverse correlation between the rise in insulin levels and the fall in blood glucose ($r=-0.75$, $p<0.05$). There was a strong negative correlation between the individual blood glucose response following the food and taste presentation and the "anticipation score" ($r=-0.90$, $p<0.01$; fig 3.2) but there was no significant correlation between the "anticipation score" and the insulin responses ($r=0.61$, $p=0.2$).

During the hyperglycaemic clamp there was no difference from the control studies in mean glucose stimulated insulin or C peptide secretion, in mean dextrose infusion rates or in the mean blood glucose increment due to the initial fixed rate dextrose infusion. However there was a significant negative correlation between the individual cephalic insulin responses and the delta (i.e. the difference between the food-stimulus and control studies) initial blood glucose increment induced by the fixed rate dextrose infusion ($r=-0.87$, $p<0.02$; fig 3.3). In addition there was a significant negative correlation between the individual cephalic insulin responses and the delta of an index of blood glucose metabolism (mean blood glucose/mean dextrose infusion rate, see Data analysis above) during the first 30 minutes of the clamp ($r=-0.87$, $p<0.02$).

Study 3. The administration of aspartame alone had no effect on blood glucose or serum insulin levels when compared with control (Table 3.3).

Fig 3.1 Blood glucose, serum insulin and per cent insulin responses immediately following tease meals (expectation of eating, sight and smell of food; left panel) and following tease meals combined with a sweet taste (right panel), *p<0.05, † p<0.02, ‡ p<0.005.

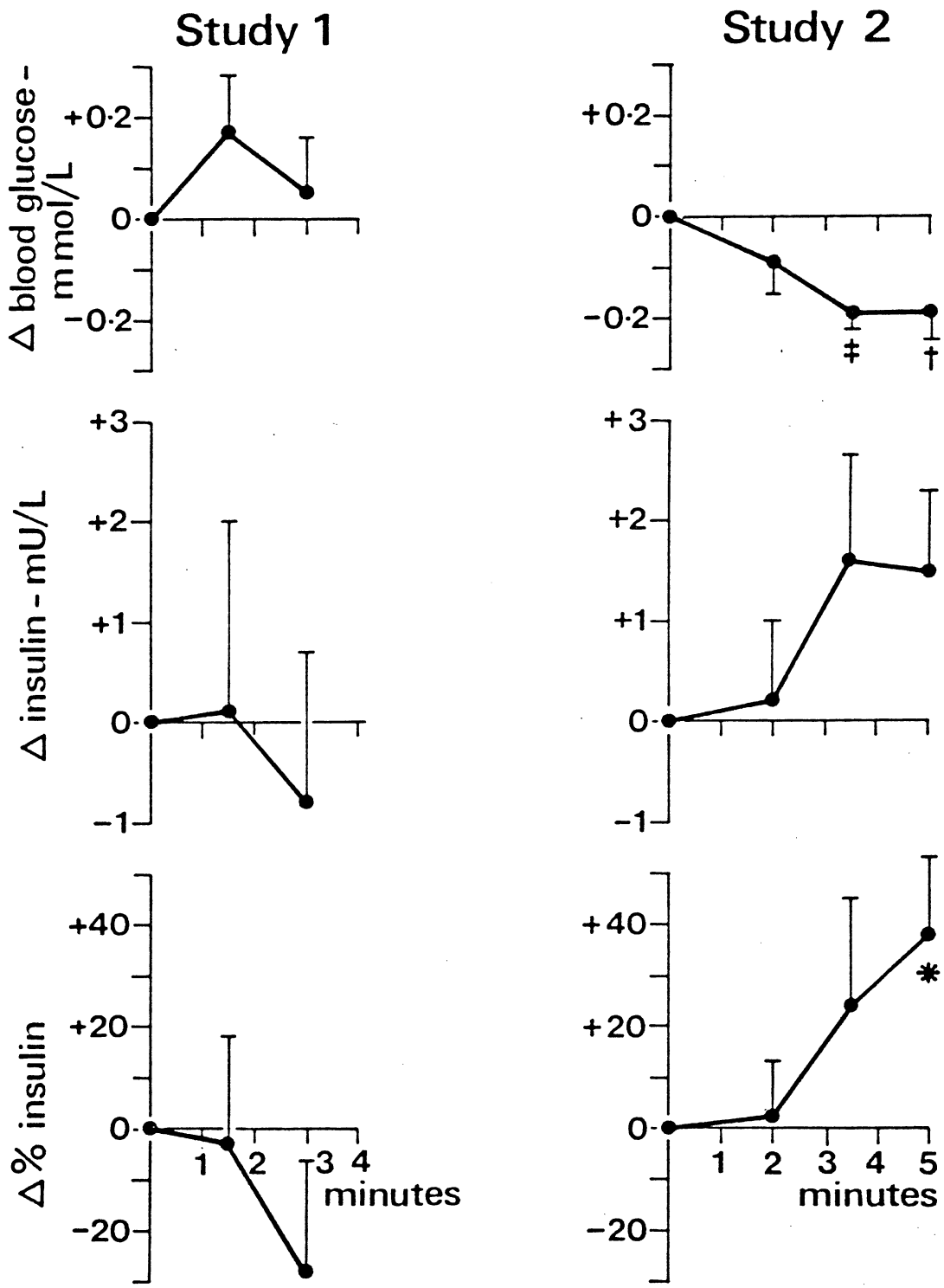


Table 3.2 Mean (±SEM) C peptide and FFA incremental responses to the combined stimuli of a tease meal and a sweet taste (study 2) in seven men compared with control.

	2 minutes	**3.5 minutes**	**5 minutes**
C peptide (ng/ml)	-0.03±0.03	+0.05±0.07	+0.09±0.06
FFA (mmol/l)	-0.01±0.03	-0.02±0.05	-0.06±0.10

Table 3.3 Mean (±SEM) incremental blood glucose and serum insulin responses of five men to aspartame (38 mg) dissolved in 15 ml water compared with control (water alone).

	2 minutes	**3.5 minutes**	**5 minutes**
Blood glucose (mmol/l)	-0.04±0.06	0±0.14	-0.10±0.09
Insulin (mU/l)	-0.3±0.5	-0.5±0.6	0±0.3

Fig 3.2 The correlation between individual blood glucose responses following tease meals combined with a sweet taste and an "anticipation" score derived from their subjective responses to the food stimulus.

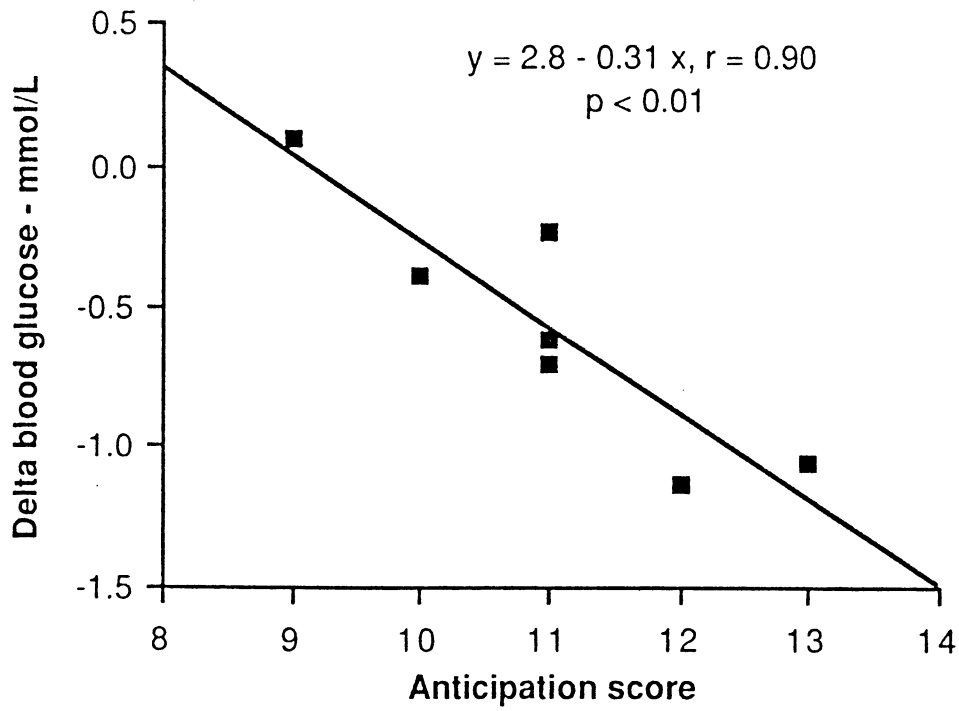
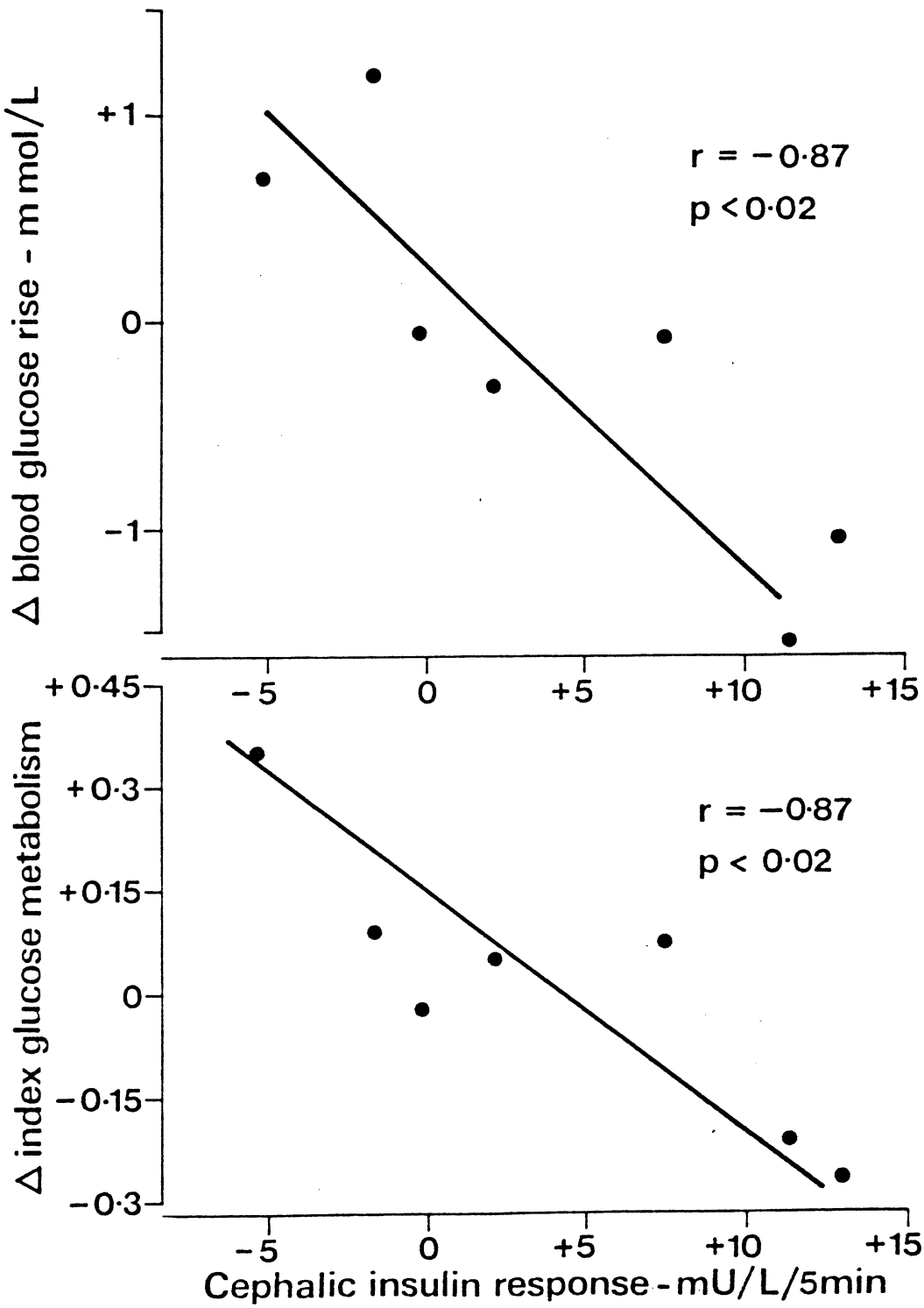


Fig 3.3 Correlations of individual cephalic insulin responses in study 2 (tease meals + sweet taste) with the delta of the blood glucose rise induced by intravenous dextrose, 15 mg/kg for 5 minutes (upper panel), and with the delta of an index of glucose metabolism (blood glucose increment/dextrose infused) during the first 30 minutes of a hyperglycaemic clamp.



3.5 Discussion

The initial aim of these studies was to elicit cephalic phase insulin secretion in normal subjects and then to determine whether cephalic insulin release has a role in glucose metabolism.

3.5.1 Cephalic responses

Cephalic responses proved to be difficult to elicit and could only be demonstrated by a combination of food-related stimuli, namely the sight, smell and expectation of a meal plus a sweet taste. Isolated segments of the above composite stimulus failed to elicit demonstrable cephalic responses. There is ample evidence from the animal literature that stronger cephalic responses can be elicited by combined rather than isolated oro-pharyngeal stimuli (Nilsson et al., 1974; Berthoud, 1982).

Although the addition of a sweet taste to the other stimuli was necessary to elicit cephalic phase insulin secretion, several subjects did not find the taste enjoyable. Despite this, four of the five subjects who reported adverse feelings to the taste had demonstrable cephalic insulin or glucose responses. Thus it seemed that recognition of the sweet taste and not necessarily enjoyment of the taste was the necessary stimulus.

The results of this study are consistent with those of Sjostrum et al (1980) who were unable to demonstrate cephalic phase insulin secretion in nonobese subjects using only the sight and smell of food. However, another group failed to elicit cephalic phase insulin release using a composite stimulus where subjects tasted, chewed and expectorated an appetising meal (Taylor et al., 1982). In that study the first blood sample was not taken for 15 minutes and an early rise in insulin levels may have been missed. Furthermore, the subjects in

that study bore naso-gastric tubes and may have been subject to adversive stimulation sufficient to extinguish cephalic responses.

It should be pointed out that the sex difference between the subjects in studies 1 and 2 could be a confounding variable and therefore direct comparison of the results between the studies should be made with some caution. However, there was no suggestion of a difference between the responses of the men and the women in study 1 to indicate a sex effect.

A number of factors are likely to influence cephalic responses in human subjects and make their demonstration difficult. Psychological stress associated with the study situation is likely to diminish cephalic responses. Thus the strong relationship between the subjective feelings of the subjects ("anticipation score") and the metabolic responses in this study suggests that those who did not respond did not perceive a sufficient stimulus. This may have been due to the stress associated with the experimental situation. If this is indeed the case then it is likely that with normal meal taking greater cephalic responses would result.

The magnitude of the cephalic insulin responses seen with the composite stimulus was not large. However as portal insulin levels are approximately 2.5-3 times higher than peripheral levels (Blackard and Nelson, 1970; Berger et al., 1973) the mean rise of approximately 40% peripherally with a range of response of from 0-100% above basal, suggests that substantial elevations in portal insulin levels would have occurred. In addition, for reasons which have been detailed above, it seems likely that with normal stress-free eating and optimal oro-pharyngeal stimulation the rise in insulin levels due to cephalic stimulation could be substantially greater.

C peptide levels also rose following presentation of the composite stimulus although the rise was not statistically significant. It has been shown that insulin and C peptide exhibit different kinetics particularly in the non-steady state. Peripheral C peptide levels increase more slowly than C peptide secretion rates and therefore do not accurately reflect increasing rates of insulin secretion (Polonsky et al., 1986).

The most powerful and rapid effect was on blood glucose levels rather than on insulin and there was only a modest correlation between the glucose and insulin responses. The blood glucose may have fallen because of a rapid rise in portal insulin levels inhibiting hepatic glucose production. However, two subjects demonstrated a clear fall in glucose levels and did not exhibit an insulin response suggesting the possibility that the fall in glucose levels was not due to insulin release. There is good evidence that the hypothalamus is able to directly alter hepatic glucose production via direct neural pathways (Shimazu, 1981; Smythe et al., 1984). It is therefore possible that a direct cephalic response exists which can cause a reduction in peripheral glucose levels by a neurally mediated effect on the liver.

Whatever the explanation, the demonstration that cephalic responses rapidly reduce basal blood glucose levels is consistent with a homeostatic function for the cephalic phase. These neural reflexes may act to reduce hepatic glucose production (assuming that the effect is hepatic rather than peripheral) before the influx of nutrients consequent on food ingestion and thus limit prandial hyperglycaemia.

In contrast to previous studies (Penick et al., 1966) no significant alteration in FFA levels was seen. In the study of Penick et al, a large number of subjects

were studied on repeated occasions and FFA responses were not always seen. Thus subject variability or differences in study design may account for the lack of apparent FFA responses.

It is of considerable interest that insulin levels rose when no change in hPP levels was detected. It should be pointed out that although hPP levels did not increase above the limit of sensitivity of the assay, the assay is sufficiently sensitive to detect normal prandial hPP responses (Schwartz et al., 1979) and therefore the lack of hPP response in this study was not artifactual. Both insulin and hPP are under vagal (cholinergic) control and cephalic hPP secretion has been reported (Taylor et al., 1982; Schwartz et al., 1979). It has been suggested that the swallowing reflex is a particularly salient stimulus for hPP secretion (Brand et al., 1982) and in the present studies swallowing was not a prominent feature, perhaps explaining the lack of hPP response. This difference in insulin and hPP responses suggests that a functional organisation of the vagal innervation of the pancreatic islet cells may exist.

3.5.2 Effects on glucose homeostasis

No effects on overall glucose disposal or on glucose stimulated insulin secretion were demonstrated during the intravenous dextrose infusion following cephalic phase insulin secretion. However, the strong negative relationship between the cephalic insulin responses and the initial glucose increment secondary to the fixed-rate dextrose infusion suggests that cephalic phase insulin secretion had a restraining effect on the rise in blood glucose levels. Furthermore the presence of cephalic insulin responses was followed by a lower index of glucose metabolism (mean blood glucose/mean dextrose infusion rate) during the initial 30 minutes of the clamp suggesting that the restraining effect of cephalic phase insulin secretion on blood glucose levels

had a longer duration. These effects were independent of the initial fall in basal blood glucose levels. Thus the data suggest that where an adequate cephalic insulin response was stimulated, there was a restraining effect on the subsequent glycaemic response to the parenteral glucose load.

It can be argued that with normal meal taking, these effects of cephalic phase insulin secretion on glucose disposal would be reliable and of greater magnitude. It is also possible that the effects of cephalic responses would be greater following an oral rather than a parenteral glucose load. Thus with normal meal taking, cephalic phase insulin secretion may have an important role in limiting the rise in blood glucose consequent on the influx of nutrients from the meal.

There are certain inconsistencies in the data which are worth considering. As noted above the most consistent effect of the composite food stimulus was on blood glucose rather than insulin levels, whereas the restraining effect on the rise in glucose levels induced by the dextrose infusion was associated with the cephalic insulin response and not with the initial blood glucose decrement. Finally the "anticipatory score" correlated with the glucose decrement and not with the insulin response. These apparent inconsistencies suggest the possibility that the food-associated stimuli may have resulted in independent effects on blood glucose and on insulin secretion. This is consistent with studies which have demonstrated direct neural control of hepatic glucose output independent of effects due to insulin (Shimazu, 1981; Smythe et al., 1984). Thus it may be that cephalic responses initiate a series of metabolic events which include a direct neural suppression of hepatic glycogenolysis as well as stimulation of pancreatic insulin release.

In summary, the results of this chapter show that cephalic phase insulin release can be stimulated in normal weight human subjects using a composite food-associated sensory stimulus. Cephalic responses cause a rapid fall in basal blood glucose levels and cephalic phase insulin secretion has a modest restraining effect on the blood glucose rise induced by a dextrose infusion. These metabolic effects are strongly related to the subjective response to the stimulus which suggests that cephalic responses may have greater importance in the normal situation free from the stressful conditions of a research study.

CHAPTER FOUR

Prandial insulin secretion in noninsulin dependent diabetes mellitus

4.1 Introduction

Numerous studies of prandial insulin secretion in subjects with noninsulin dependent diabetes mellitus (NIDDM) have demonstrated that there is a delay or a deficiency in the initial rise in insulin levels and that insulin levels are subnormal during the first 30-60 minutes after the start of a meal (Yalow and Berson, 1960; Perley and Kipnis, 1966; Seltzer et al., 1967; DeFronzo and Ferrannini, 1982). Subsequent insulin levels are often greater than normal and this later hyperinsulinaemia probably occurs secondary to the prandial hyperglycaemia which is seen in NIDDM and is due to hyperglycaemic stimulation of the beta cells (Perley and Kipnis, 1966).

Delayed prandial insulin secretion has been reported in nondiabetic relatives of subjects with NIDDM who may be at risk of subsequently developing NIDDM (Colwell and Lein, 1967) and in one longitudinal study appeared to be a marker for the development of NIDDM (Kosaka et al., 1977). Thus, this abnormal pattern of prandial insulin secretion may occur as an early abnormality in the natural history of NIDDM and could even be a primary cause of the condition.

Other workers failed to find evidence of abnormal insulin secretion in subjects with "chemical" diabetes and championed the concept that insulin resistance rather than abnormal insulin secretion was probably the important metabolic defect in NIDDM (Reaven et al., 1971; 1983). Some of the controversy which has surrounded this issue may have reflected different criteria for patient selection. Many patients classified as having chemical diabetes or prediabetes would now be classified as having "impaired glucose tolerance" (National Diabetes Data Group, 1979). So far, follow-up studies have found that impaired glucose tolerance is a poor predictor for the development of NIDDM

(Kosaka et al., 1977; Sartor et al., 1980; Keen et al., 1982) and thus the significance of metabolic abnormalities in this patient group remains unclear.

Recently, several groups have examined nondiabetic subjects considered to be at high risk for eventually developing NIDDM. Subclinical abnormalities of both insulin secretion and peripheral insulin action were found in normoglycaemic women with a history of gestational diabetes (Ward et al., 1985) and in nondiabetic offspring of subjects with NIDDM (O'Rahilly et al., 1986). In both studies, first phase insulin secretion following parenteral secretagogues was deficient, however prandial insulin responses were not determined. These findings are consistent with the earlier findings of Cerasi and Luft (1967) who considered that reduced insulin secretion (during their glucose infusion test) was an early marker for NIDDM. However, these researchers have recently concluded that both impaired insulin secretion and insulin resistance usually coexist in early diabetes (Efendic et al., 1984).

It should be pointed out that, despite certain similarities, there is unlikely to be any direct relationship between first phase insulin secretion to intravenous glucose and early prandial insulin secretion. First phase insulin secretion is a function of the beta cell response to direct stimulation by glucose, whereas as discussed in detail below, the early prandial insulin response is due to a combination of stimuli including neural and gut-hormone factors. An abnormality of glucose-stimulated insulin secretion may only play a minor role in the deficient early prandial insulin response in diabetic subjects (Nauck et al., 1986).

It has recently been shown that therapeutic correction of hyperglycaemia results in improvements in both insulin resistance and secretion. The

therapeutic modality utilised appears not to be important and improvements occur following treatment with diet (Henry et al., 1986), sulphonylureas (Kolterman et al., 1985) and insulin (Garvey et al., 1985). This has led to the "glucotoxicity" hypothesis which states that hyperglycaemia itself is responsible for causing or contributing to impaired insulin secretion and reduced peripheral insulin sensitivity (Unger and Grundy, 1985; Olefsky, 1985). In the study of Henry et al (1986, fig 1) the deficiency in early prandial insulin secretion persisted following the correction of hyperglycaemia, although there was an overall improvement in beta cell function. The persistence of the defect, despite correction of prevailing hyperglycaemia, suggests that abnormal early prandial insulin secretion may not occur secondary to glucose toxicity but has a different aetiology.

The cause of the abnormality in early prandial insulin secretion is unknown. Possible causes include abnormalities of the neural component of insulin secretion, abnormalities of insulinotropic gut hormone secretion or intrinsic abnormalities of beta cell function.

Beta cell function in NIDDM is characterised by both qualitative and quantitative abnormalities (Ward et al., 1984; 1985). These include decreased responsiveness to glucose stimulation (Halter et al., 1979; Turner et al., 1976); deficient first phase insulin secretion following intravenous glucose (Brunzell et al., 1976); a moderately reduced number of pancreatic beta cells (Westermark et al., 1978; Gepts and LeCompte, 1981) and a reduced total insulin secretory capacity (Ward et al., 1984). Therefore abnormal function of the beta cells could be the cause of the defect in early prandial insulin secretion. However, Nauck et al (1986) used intravenous glucose infusions to simulate the prandial glycaemic response of diabetic and nondiabetic subjects to oral glucose in

order to quantify the proportion of insulin released due to direct stimulation of the beta cells by glucose or due to other (incretin) factors (i.e. gut hormone or neural factors). They found that the "incretin" effect (i.e. insulin secretion due to gut hormone and/or neural factors) was markedly diminished in NIDDM and appeared to be predominantly responsible for the deficient insulin responses. They concluded that beta cell dysfunction was unlikely to be responsible for the early deficiency in prandial insulin secretion in NIDDM.

An abnormality in insulinotropic gut hormone regulation may be the cause of the abnormality in early insulin secretion. A large number of potential gut hormones have been investigated for insulinotropic activity. Gastric inhibitory polypeptide (GIP) remains the strongest candidate although several other gut associated peptides e.g. cholecystokinin, may participate in the net "incretin" effect (Ahren et al., 1983). The insulin stimulatory action of GIP requires the intestinal absorption of monosaccharides (Creutzfeldt and Ebert, 1985) and GIP potentiates glucose stimulated insulin secretion but does not directly stimulate the beta cells (Creutzfeldt, 1979). In NIDDM, prandial levels of GIP are often elevated (Crockett et al., 1976; Takemura et al., 1981), do not correlate with the insulin responses and therefore GIP does not appear to be responsible for the abnormal insulin secretion (Creutzfeldt et al., 1983). There is evidence for the existence of additional insulinotropic factors and thus the deficiency in prandial insulin secretion could be related to an as yet unidentified gut hormone or factor (Creutzfeldt and Ebert, 1985). This unidentified factor could be the neural component of insulin secretion.

Studies of cephalic phase insulin secretion, which by definition occurs before ingested nutrients are digested or absorbed, suggest that this phase could have important regulatory effects on the glycaemic response to a meal. When

cephalic phase insulin responses are eliminated or bypassed in rats, the early rise in insulin levels is reduced and both prandial hyperglycaemia and hyperinsulinaemia result (Louis-Sylvestre, 1976; Trimble et al., 1980; Strubbe and van Wachem, 1981). Thus a deficiency in cephalic phase insulin secretion in animals produces prandial metabolic abnormalities not dissimilar from those seen in NIDDM. In a study in rats, replacing the deficient early insulin response with intravenous insulin essentially corrected these prandial abnormalities (Berthoud et al., 1981).

There are several ways in which abnormal neural control of prandial insulin secretion could arise in NIDDM. An increase in sympathetic nervous activity and/or a reduction in vagal activity to the pancreatic beta cells could cause such an abnormality. Robertson et al (1976) found that the alpha-adrenergic blocking drug phentolamine caused a greater increase in basal and stimulated insulin secretion in subjects with NIDDM compared with nondiabetics and concluded that increased alpha-adrenergic activity to the beta cell existed in NIDDM. In support of this hypothesis, a new hypoglycaemic agent which has potent alpha$_2$-adrenergic blocking activity has been shown to have beneficial effects in patients with NIDDM (Kashiwagi et al., 1986). This drug has been shown to cause an increase in early prandial insulin secretion and an improvement in prandial hyperglycaemia in subjects with NIDDM. Whether the therapeutic action of the drug is related to its adrenergic blocking activity is not currently known. Thus, there is evidence for abnormal sympathetic regulation of pancreatic insulin secretion in NIDDM.

There is considerable circumstantial evidence that the deficiency in early prandial insulin secretion may be a contributory cause of prandial hyperglycaemia in NIDDM. The cephalic studies detailed above demonstrate

that the absence of an early insulin response during meals causes prandial hyperglycaemia. This has also been demonstrated in humans. The administration of somatostatin to nondiabetic humans during the first fifteen minutes of an oral glucose drink suppressed the initial rise in insulin levels and caused a worsening of glucose tolerance (Calles-Escandon and Robbins, 1987). In addition, several studies of the timing of insulin delivery to insulin dependent diabetic subjects have shown that a short delay in preprandial insulin delivery results in prolonged and often severe prandial hyperglycaemia (Kraegen et al., 1981; Dimitriadis and Gerich, 1981).

Theoretically, as hyperglycaemia itself can worsen insulin resistance and impaired insulin secretion, (according to the "glucotoxity" hypothesis, Unger and Grundy, 1985), prandial hyperglycaemia could cause a worsening of existing abnormalities of insulin secretion and action in subjects with NIDDM which could in turn cause a worsening of hyperglycaemia. By a similar mechanism, prandial hyperglycaemia could cause a worsening of subclinical metabolic abnormalities in subjects who are at risk of developing NIDDM and therefore the deficiency in early prandial insulin secretion could play a part in the pathogenesis of NIDDM.

Both glucagon and free fatty acids (FFA) are abnormally regulated in NIDDM (Fraze et al., 1985; Muller et al., 1979). Glucagon levels often rise abnormally during meals in subjects with NIDDM and this has been considered to be a possible contributory cause of prandial hyperglycaemia (Gerich et al., 1975). As both glucagon and FFA are regulated by circulating insulin levels, the delay in prandial insulin secretion could contribute to their abnormal regulation in NIDDM.

In summary, the early deficiency in prandial insulin secretion is a characteristic metabolic abnormality of NIDDM which may be due to an abnormality of the neural component of prandial insulin secretion. There is controversial data to suggest that this abnormality may occur early in the pathogenesis of the condition. The physiological importance of early prandial insulin secretion remains uncertain but there is sufficient evidence to suggest that the deficient early response could contribute to prandial hyperglycaemia in NIDDM. Furthermore, the "glucotoxicity" hypothesis provides a mechanism whereby prandial metabolic abnormalities could in the long term cause significant functional abnormalities of insulin secretion and action and contribute to or even cause fasting hyperglycaemia.

4.2 Aims

The aim of the studies in this chapter were to determine whether:

1. correcting the deficiency in early prandial insulin secretion with a physiological dose of exogenous insulin would result in an improvement in prandial hyperglycaemia in NIDDM.

2. the use of intranasal insulin delivery is likely to be a useful therapeutic agent for correcting the deficiency in early prandial insulin secretion.

4.3 Physiological insulin replacement in NIDDM

In this study, the deficiency in early prandial insulin secretion in subjects with NIDDM was corrected using a physiological dose of exogenous intravenous insulin given in a manner designed to simulate normal early prandial insulin secretion. The effects of this physiological correction of the early insulin response on prandial glucose, insulin, C peptide, glucagon and FFA levels were studied.

4.3.1 Subjects and methods

Eight subjects with NIDDM (7 men, 1 woman) and 9 nondiabetic subjects (6 men, 3 women) participated in the studies (Table 4.1). The diabetic subjects had a history of stable hyperglycaemia for at least 1 year. All had received instruction regarding a standard diabetic diet (high complex carbohydrate - 45-55% of total diet composition) in the preceding year and they were instructed not to change their diet during these studies. One subject was treated with diet alone and the remainder with diet supplemented with sulphonylurea therapy (glibenclamide). Sulphonylureas were ceased 48 hours before each study. No subjects were taking other therapy except for 2 normokalaemic patients who took thiazide diuretics for essential hypertension. None of the nondiabetic subjects had other illness, were taking medications or had a history of diabetes in either siblings or parents. Both groups of subjects were of similar age and degree of obesity and both groups included obese and nonobese subjects.

Table 4.1 Clinical details of the diabetic and nondiabetic subjects. *p<0.001.

SUBJECT	AGE	BODY MASS INDEX (kg/m^2)	DURATION DIABETES (years)	FASTING BLOOD GLUCOSE (mmol/litre)
Diabetic Subjects				
1	58	38.1	9	9.1
2	52	37	19	8.8
3	58	41.5	15	8.3
4	62	32	6	6.7
5	63	23	1	6.1
6	67	23.1	13	10.0
7	63	24.7	7	10.9
8	75	27.8	3	7.2
Mean±SD	62±6.8	30.9±7.3	9.1±6.2	8.4±1.7
Nondiabetic Subjects (n=9)				
Mean±SD	59.2±8.3	27.2±4.1	_	5.1±0.3*

Studies were performed after an overnight fast and were performed in random order. Arterialised venous blood samples were obtained as described in chapter 2.2. The sampling catheter was kept patent with a slow saline (0.9%) infusion. A second catheter was inserted into an ipsilateral antecubital vein for infusion of neutral porcine insulin as described in chapter 2.

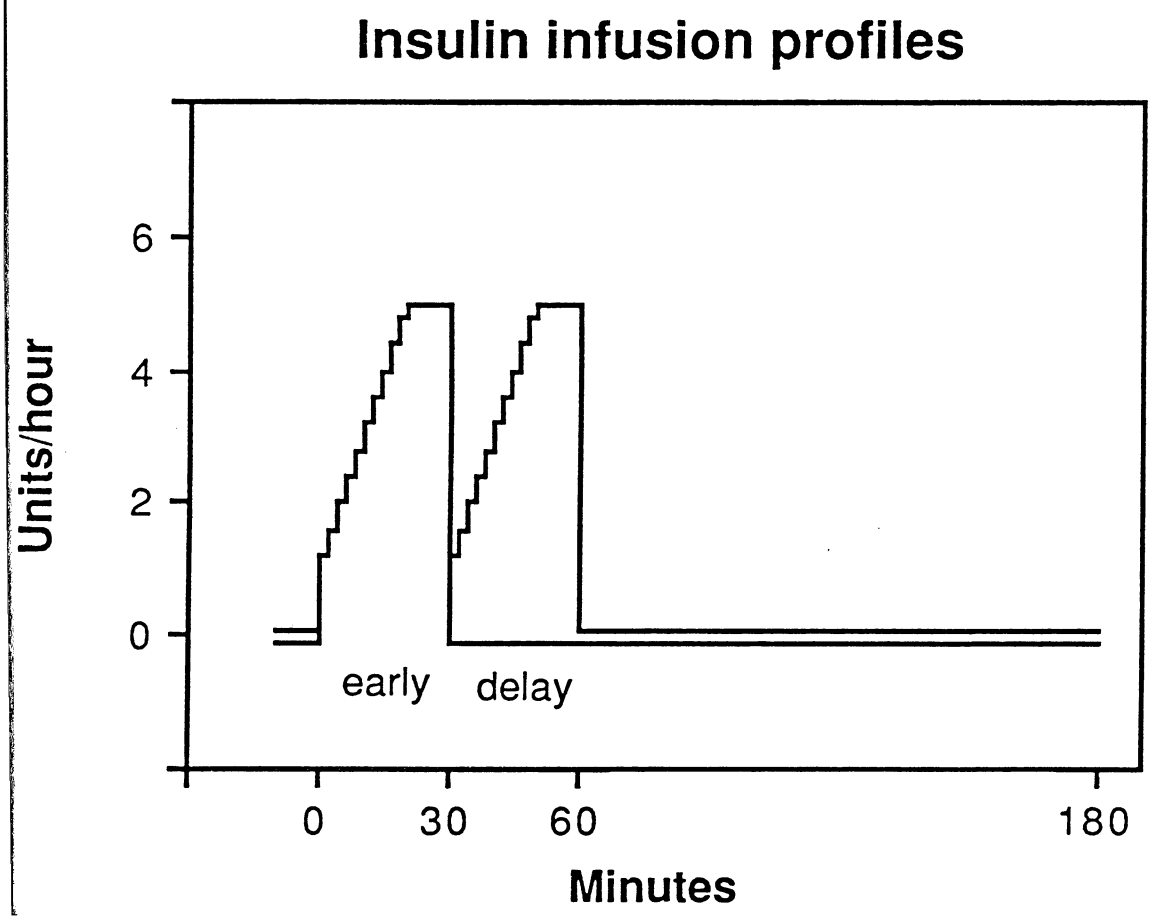
Each diabetic patient underwent a maximum of 4 studies and each nondiabetic subject was studied once. All subjects arrived early in the morning, intravenous lines were inserted and they were seated comfortably at a table. After at least 30 minutes, they were presented with a standard breakfast meal which was consumed within 15 minutes. The meal consisted of breakfast cereal (30g), milk (150ml), orange juice (150ml), 2 slices of toast (30g each), butter (10g) and 2 cups of decaffeinated coffee (total energy content 1840 kjoules, 62% (68g) carbohydrate, 27% fat, 11% protein). Blood samples were drawn for baseline values before, during and for 180 minutes after the meal was commenced for blood glucose, serum insulin, C peptide, FFA and plasma glucagon. Time zero was taken as the time of the first mouthful of food.

The studies in the diabetic subjects were performed at intervals of at least one week and were performed in random order. They were studied without exogenous insulin delivery during the meal (control condition) and on 3 occasions with intravenous insulin supplementation. On one occasion, 1.8 units neutral insulin was delivered intravenously in a stepwise incremental fashion during the first 30 minutes after commencing eating in order to reproduce the normal early phase of prandial insulin secretion (see fig 4.1, "early" profile). This profile is the initial 30 minute segment of a 3 hour profile which has been shown to simulate the normal insulin response to a mixed meal in nondiabetic subjects (Kraegen and Chisholm, 1981). This segment of the

profile delivers 1.2 units of insulin and a basal delivery component (0.6 units) was included in order to slightly overcorrect the peripheral insulin response in an attempt to approach normal portal insulin levels. On a separate occasion, the same total dose of insulin (1.8 units) was delivered using the same incremental profile but given between 30 and 60 minutes after commencing the meal (see fig 4.1, "delayed" profile). Finally, on another occasion the same total dose of insulin (1.8 units) was delivered but as a constant infusion (0.6 units/hour) from 0-180 minutes.

All eight diabetic subjects underwent the control (no insulin) and early (0-30 minutes) insulin protocols. However two subjects completed only 3 of the 4 protocols. One subject became psychologically stressed due to a family problem and became metabolically unstable before undergoing the fourth study (constant insulin infusion) and the other patient declined to return for a repeat study after a problem with blocked sampling lines invalidated his fourth study (delayed insulin infusion). Nondiabetic subjects were studied on a single occasion without insulin infusion for comparison with the diabetic responses to the meal.

Fig 4.1 Diagrammatic representation of the insulin delivery profile used to simulate normal early insulin secretion in the diabetic subjects. On one occasion, this profile was given between 0 and 30 minutes and on another occasion between 30 and 60 minutes.



Blood samples were processed and assayed as detailed in chapter 2. Samples for serum insulin from the studies undergone by each diabetic patient were stored and assayed in the same assay to avoid inter-assay variation.

Calculations and statistics: Portal venous insulin concentrations were calculated as described in chapter 2.6. Substrate and hormone responses were compared using both absolute and incremental responses from baseline. The data are mainly presented as increments from baseline to minimise the variance due to minor variations in baseline values. Baseline values were calculated as the mean of determinations obtained before the meal commenced. Hormone and substrate integrated responses were estimated (area under the curve) using a computer program. Simple linear regression analysis was performed using the method of least squares. Statistical comparisons used paired and unpaired t tests as appropriate. Analysis of variance followed by Dunnett's multiple group comparison's test were used where more than 2 means were compared (Dunnett, 1955). Where paired tests were used, the data for 7 or 8 subjects is reported depending on the number completing the 2 conditions; in fig 4.5, for the sake of clarity, control data for 8 subjects is presented alongside the data for the subjects who completed the delayed and constant insulin conditions (7 in each).

4.3.2 Results

Prior to meal ingestion (table 4.2), blood glucose levels were greater in the diabetic than in the nondiabetic subjects, whereas serum insulin, C peptide, FFA and plasma glucagon levels were not different between both groups. There were no significant differences in fasting levels of blood glucose, serum insulin, C peptide, FFA or plasma glucagon in the diabetic subjects before each study condition (ANOVA, table 4.2).

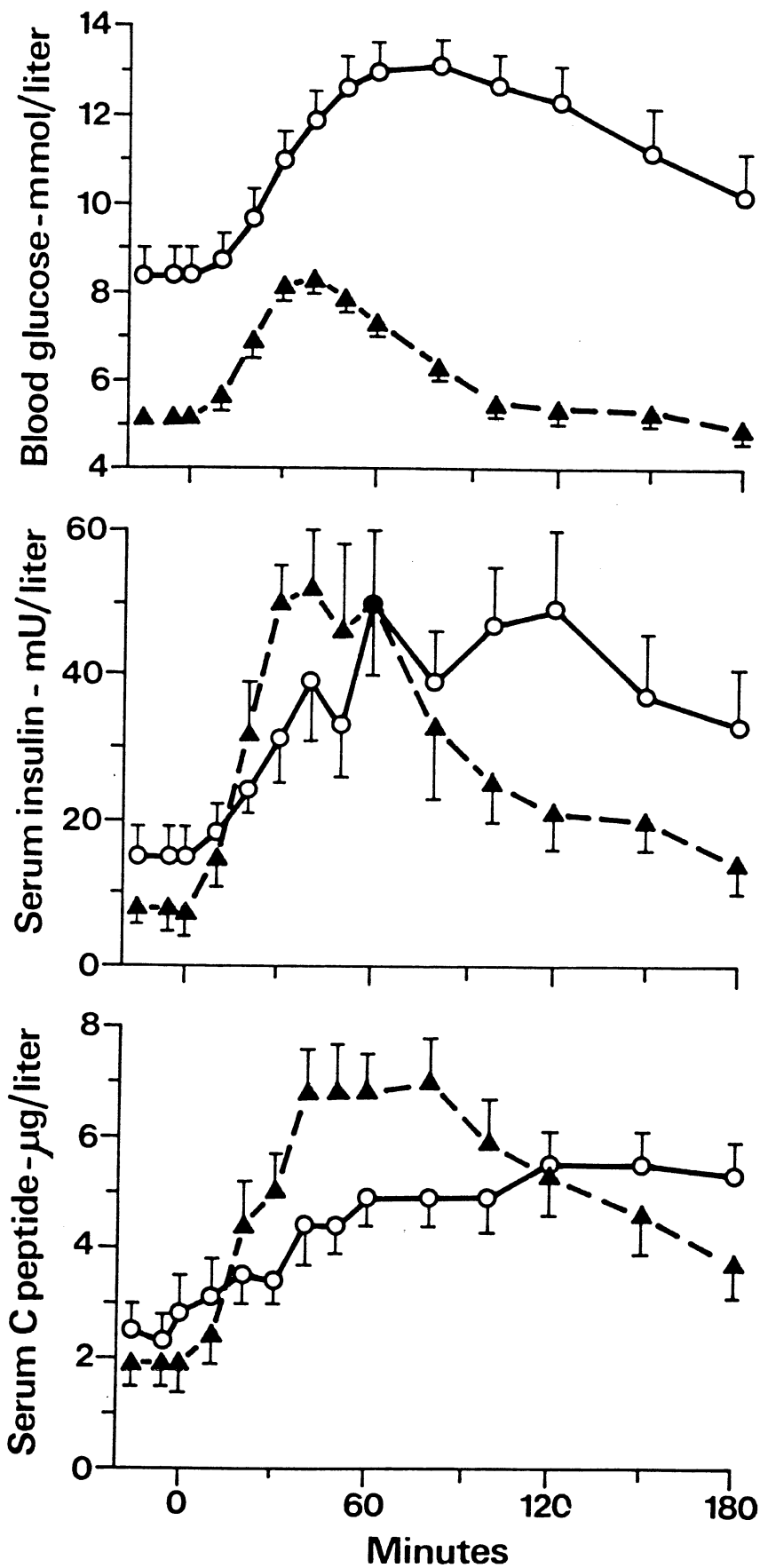
Diabetic and nondiabetic responses to the meal (fig 4.2): Following meal ingestion, the peak blood glucose concentration (13.3±0.6 vs 8.1±0.3 mmol/l, $p<0.001$), the glycaemic excursion from baseline (4.9±0.1 vs 3.2±0.2 mmol/l, $p<0.001$) and the integrated glucose response above baseline (576±31 vs 189±18 mmol/l.180 min, $p<0.001$) were greater in the diabetic subjects. There was a significant positive correlation between the fasting blood glucose level and the integrated glycaemic response above baseline in the diabetic subjects ($r=0.76$, $p<0.05$) but there was no such correlation in the nondiabetic subjects ($r=-0.30$). Serum insulin levels in the nondiabetic subjects rose promptly to a peak 40 minutes after the start of the meal and returned to baseline by 180 minutes. In contrast, serum insulin levels in the diabetic subjects rose slowly, were significantly lower at 25 and 30 minutes ($p<0.05$), peaked significantly later and remained significantly higher (from 100 minutes, $p<0.05$) than in the nondiabetic subjects. The C peptide responses to the meal also showed a delayed rise and C peptide levels were significantly lower between 40 and 80 minutes ($p<0.05$) in the diabetic subjects followed by elevated levels at the end of the study. The integrated insulin responses above baseline to the meal were not different between the diabetic and nondiabetic subjects (4142±848 vs 3862±585 mU/l.180 min) whereas integrated C peptide responses were

greater in the nondiabetic subjects (376±57 vs 606±84 μg/l.180 min, $p<0.05$). FFA levels in the nondiabetic subjects were significantly lower between 30 and 60 minutes after the meal ($p<0.05$) although there were no significant differences in the fall in FFA levels or the integrated fall in FFA (0-60 minutes) from baseline between both groups of subjects (fig 4.7, upper panel). There was a significant rise in glucagon levels in the diabetic subjects during the initial 60 minutes after meal ingestion which was not seen in the nondiabetic subjects (fig 4.7, lower panel).

Table 4.2 Mean (±SEM) fasting levels of substrates and hormones in the diabetic subjects before each study condition and in the nondiabetic subjects. *p<0.001 vs control.

	NONDIABETIC	DIABETIC SUBJECTS		
	control	early insulin	delayed insulin	constant insulin
Glucose (mmol/l)	5.1±0.1*	8.4±0.6	9.1±0.9	9.3±0.9	8.6±0.8
Insulin (mU/l)	8±3	15±4	13±4	17±4	15±3
C peptide (μg/l)	1.9±0.4	2.6±0.6	2.7±0.6	2.8±0.7	3.0±0.8
FFA (mmol/l)	0.57±0.06	0.63±0.06	0.68±0.02	0.73±0.08	0.62±0.07
Glucagon (ng/l)	28±9	33±7	22±4	32±7	38±8

Fig 4.2 The mean (±SEM) blood glucose, serum insulin and C peptide responses to a standard mixed meal in subjects with NIDDM (n=8, O) and nondiabetic subjects (n=9, ▲).



Responses to early insulin augmentation in NIDDM (fig 4.3): Exogenous insulin given to augment early prandial insulin secretion in the diabetic subjects resulted in significantly lower blood glucose increments above baseline than control (i.e. no exogenous insulin) from 30 minutes after the start of the meal until the end of the study. The peak blood glucose excursion was 1.4 mmol/l lower ($p<0.005$), the integrated glucose response above baseline was 33±4% less than control (383±28 vs 576±31 mmol/l.180 min, $p<0.005$) and the final mean blood glucose level remained 0.9 mmol/l lower than control ($p<0.05$). Peripheral insulin levels rose rapidly due to the intravenous insulin infusion and reached a peak level of 81±11 mU/l after 30 minutes. Thereafter, serum insulin levels promptly fell and were significantly below control levels for most of the duration of the study (final insulin level: 22±5 vs 33±8 mU/l at 180 minutes, $p<0.05$). Total integrated insulin levels after the meal were the same as control (4125±656 vs 4142±848 mU/l.180 min) whereas integrated insulin levels during the final hour of the study were significantly lower (982±258 vs 1466±377 mU/l.60 min, $p<0.05$). C peptide levels were similar to control during the initial 120 minutes and then fell below control during the final hour of the study (final C peptide level: 4.0±0.5 vs 5.3±0.6 µg/l, $p<0.02$). There was a non-significant tendency for C peptide levels to be suppressed after the intravenous insulin infusion at 50 minutes ($p<0.1$). However the difference in C peptide levels at the end of the study was not due to beta cell suppression by insulin infusion as integrated C peptide levels above baseline were the same during each of the first 2 hours of the study and lower only during the final hour (first hour: 51±13 vs 64±16 µg/l.60 min, NS; second hour: 123±20 vs 142±23 µg/l.60 min, NS; third hour: 110±17 vs 170±23 µg/l.60 min, $p<0.01$). Total integrated C peptide responses above baseline were significantly reduced following early

insulin augmentation compared with control (283±43 vs 376±57 μg/l.180 min, $p<0.01$).

Baseline calculated portal insulin concentrations were similar in the diabetic subjects during the control and early insulin augmentation studies (37±10 and 34±10 mU/l respectively); these levels were non-significantly greater than in the nondiabetic subjects (20±6 mU/l). The absolute peak portal insulin concentrations achieved during early insulin augmentation (at 30 minutes after meal ingestion) was calculated to be 123±24 mU/l. This compares with a peak level of 105±22 mU/l seen at 60 minutes in the diabetic control study and a peak level of 95±11 mU/l (non-significantly different from the peak during early insulin augmentation) seen at 40 minutes in the nondiabetic subjects. The corresponding peak increments from baseline were 81±17 mU/l following early insulin augmentation, 68±18 mU/l in the diabetic control study and 74±8 mU/l in the nondiabetic subjects. The incremental portal insulin responses to the meal are displayed in fig 4.4.

Fig 4.3 The mean (±SEM) incremental blood glucose, serum insulin and C peptide responses above baseline to a mixed meal in 8 diabetic subjects following delivery of intravenous insulin (1.8 units, given between 0 and 30 minutes, Δ) and without insulin delivery (control, O). The shaded area represents the mean±1SEM incremental responses in 9 nondiabetic subjects.

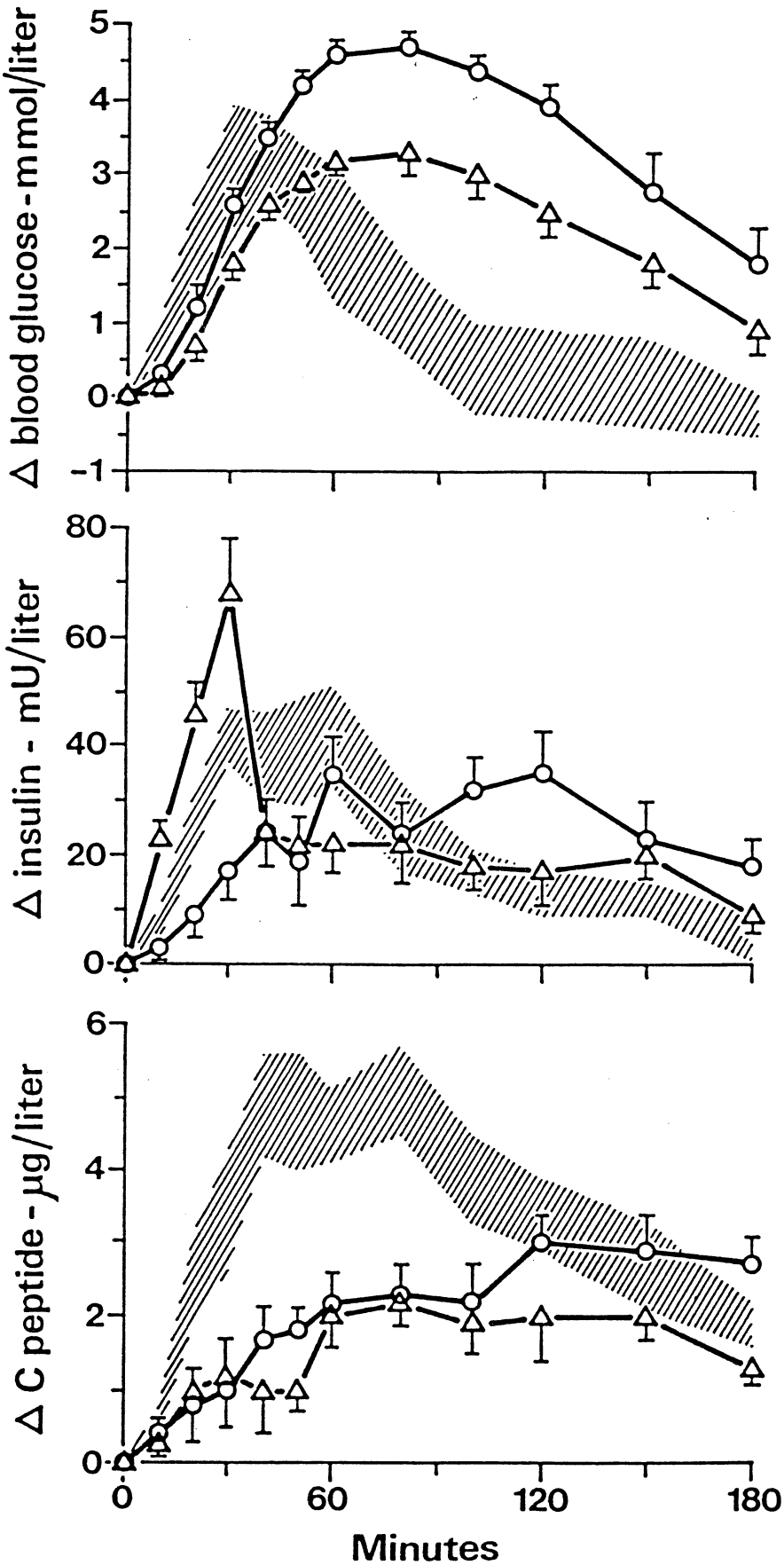
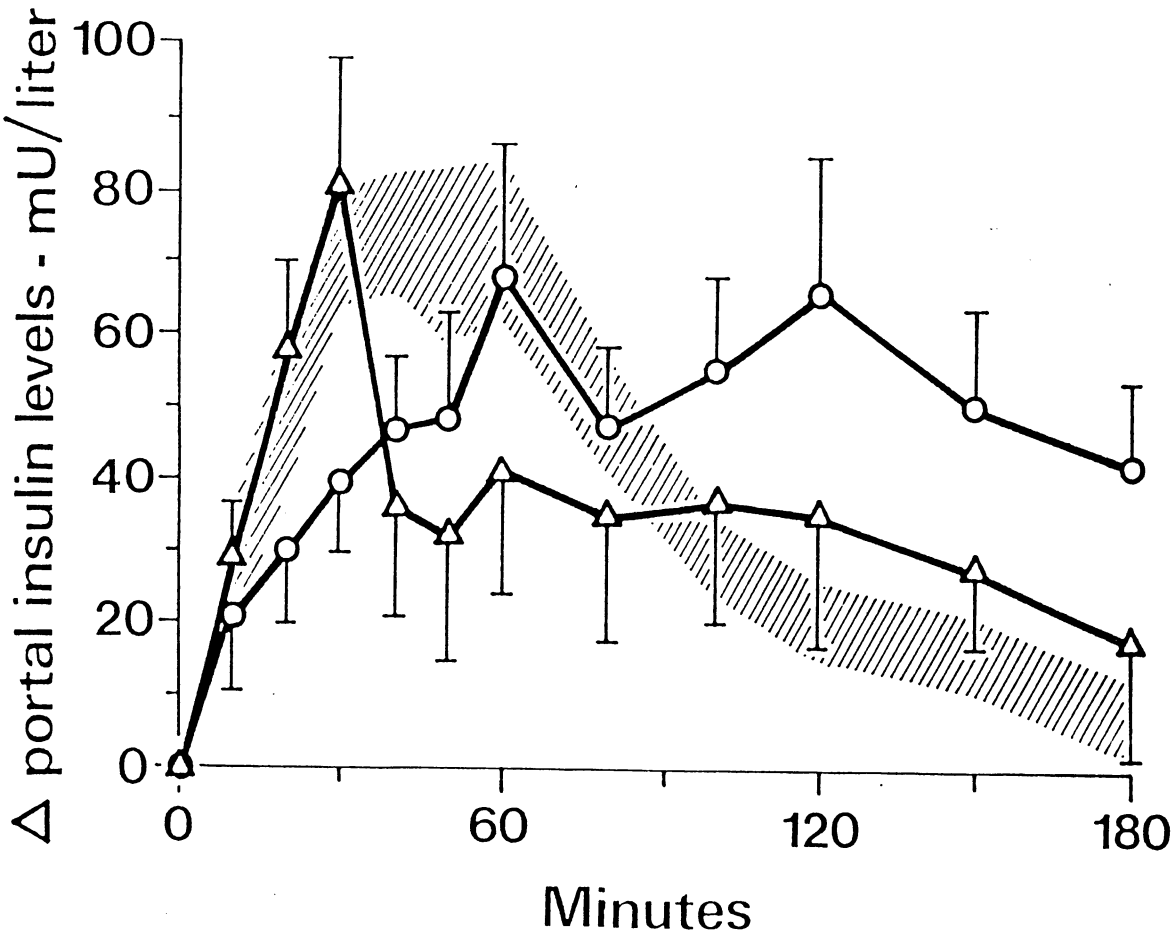


Fig 4.4 The mean (±SEM) incremental (calculated) portal insulin responses above baseline to a standard mixed meal following early insulin augmentation (from 0-30 minutes, Δ) and without exogenous insulin (control, O). The shaded area represents the mean±1SEM incremental responses in 9 nondiabetic subjects.



Delayed insulin augmentation (fig 4.5): Exogenous insulin given between 30 and 60 minutes after the start of the meal had no significant effect on the peak blood glucose excursion compared with control (4.4±0.2 vs 4.7±0.2 mmol/l) and subsequent blood glucose increments were the same as control except at the 120 minute time point (3.0±0.4 vs 3.9±0.4 mmol/l, $p<0.02$). The total integrated blood glucose response above baseline was similar to control (503±36 vs 574±30 mmol/l.180 min). Peak serum insulin levels (80±13 mU/l) achieved approximated those seen after early insulin delivery and occurred at 50 minutes. The total integrated insulin and C peptide responses above baseline were not significantly different from control (insulin: 4631±839 vs 4249±971 mU/l.180 min; C peptide: 316±64 vs 335±46 µg/l.180 min).

Constant insulin augmentation (fig 4.5): Exogenous insulin delivered as a constant infusion had no significant effect on the peak blood glucose increment (4.4±0.3 vs 4.7±0.2 mmol/l), subsequent blood glucose increments or the total integrated glycaemic response to the meal (509±53 vs 570±35 mmol/l.180 min). Serum insulin and C peptide levels were the same as control and there was no difference between total integrated insulin responses (5847±986 vs 4415±927 mU/l.180 min) and total integrated C peptide responses (384±74 vs 382±66 µg/l.180 min).

When the integrated glucose, insulin and C peptide responses above baseline to the four meal conditions were analysed together (ANOVA, fig 4.6), there was a significant difference in the blood glucose responses ($F=4.35$) which was due to the effect of early insulin delivery ($p<0.01$). There were no significant differences in the insulin ($F=0.74$) or C peptide responses ($F=0.53$) among the four treatments.

Fig 4.5 The mean (±SEM) incremental glucose, insulin and C peptide responses to the meal in 7 diabetic subjects following delayed delivery of intravenous insulin (given from 30 to 60 minutes, ●) and following constant insulin delivery (from 0-180 minutes,□) compared with control (O, error bars omitted for clarity). The shaded area represents the mean±1SEM incremental responses in 9 nondiabetic subjects.

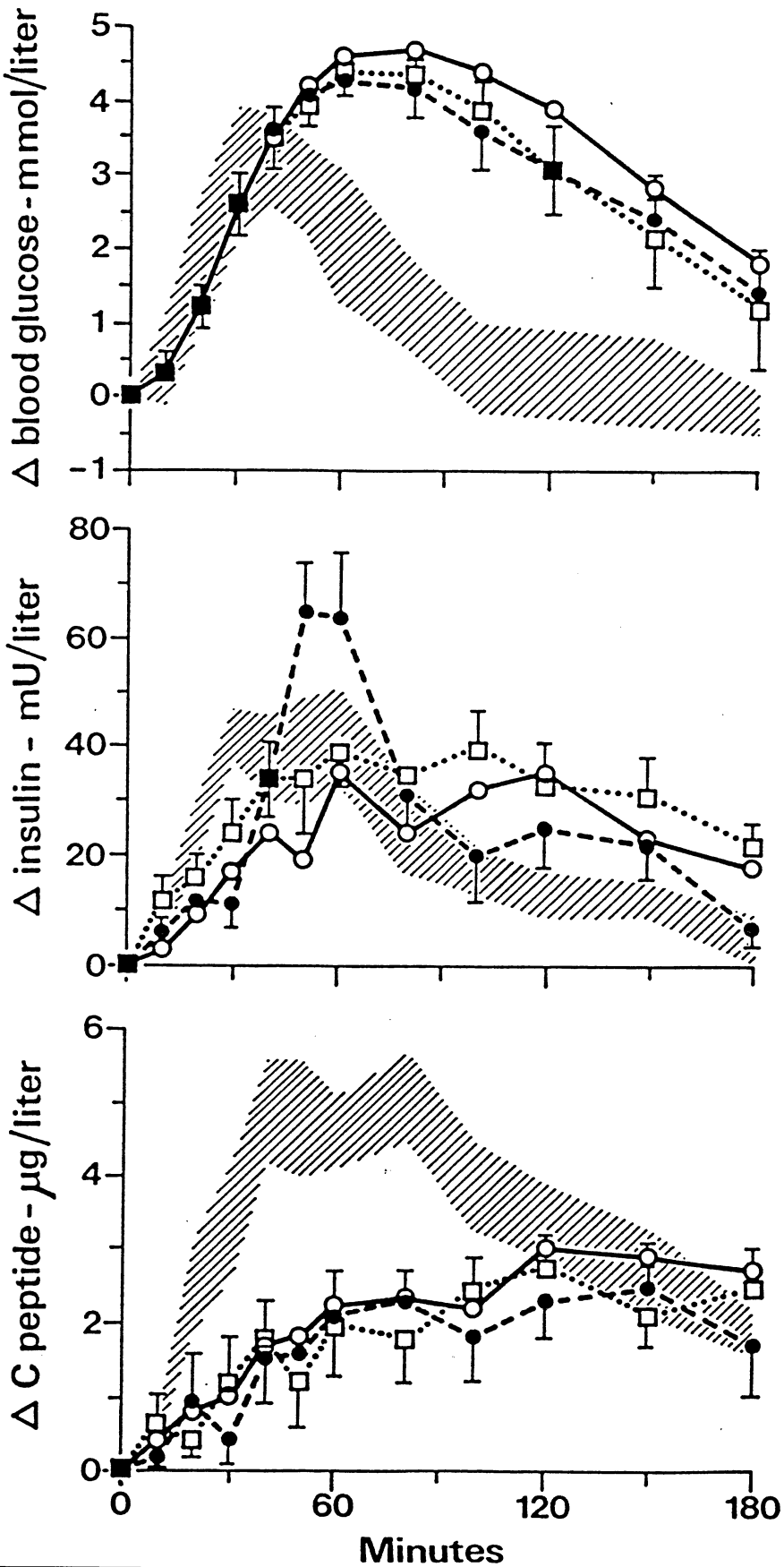
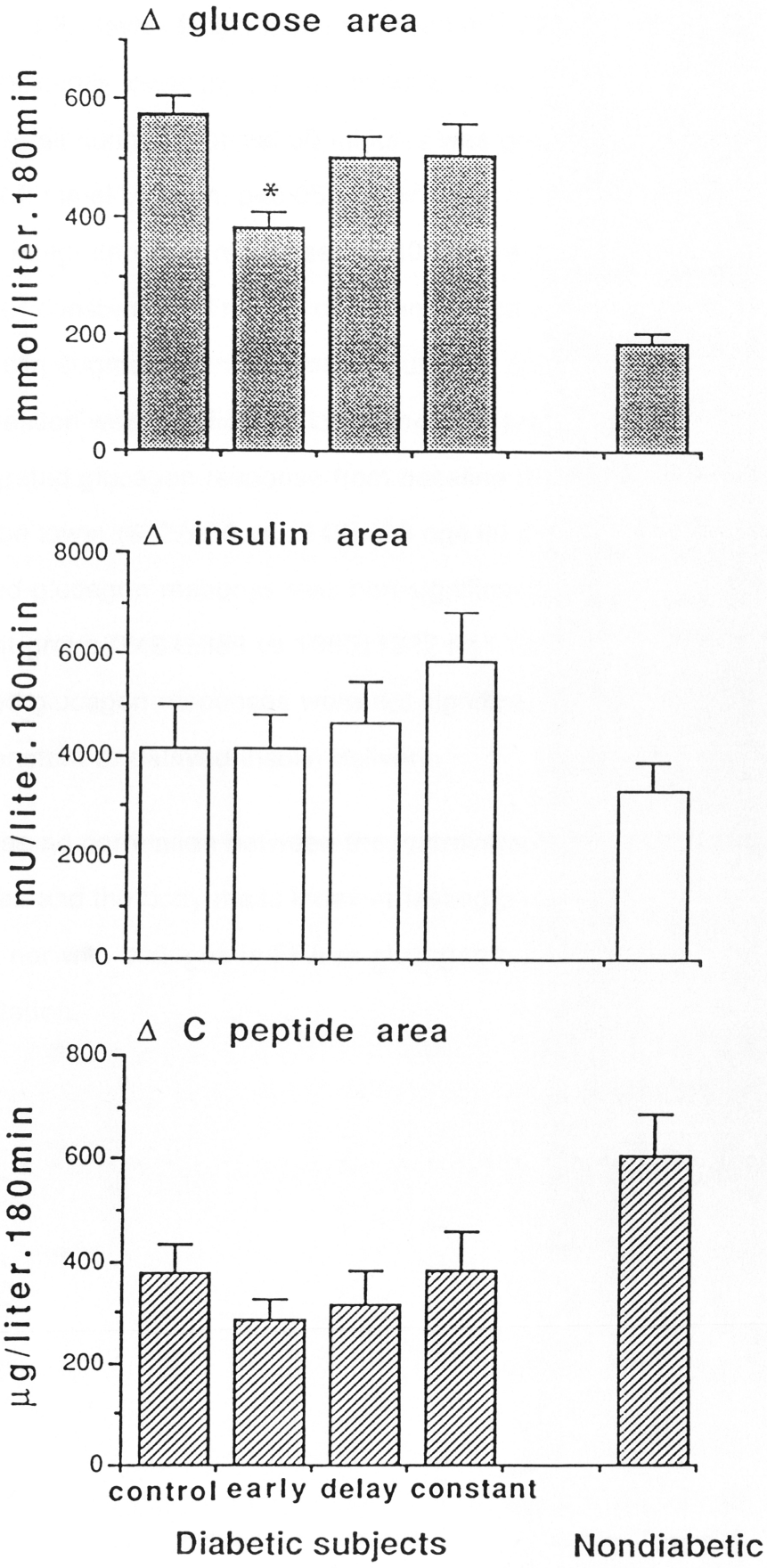


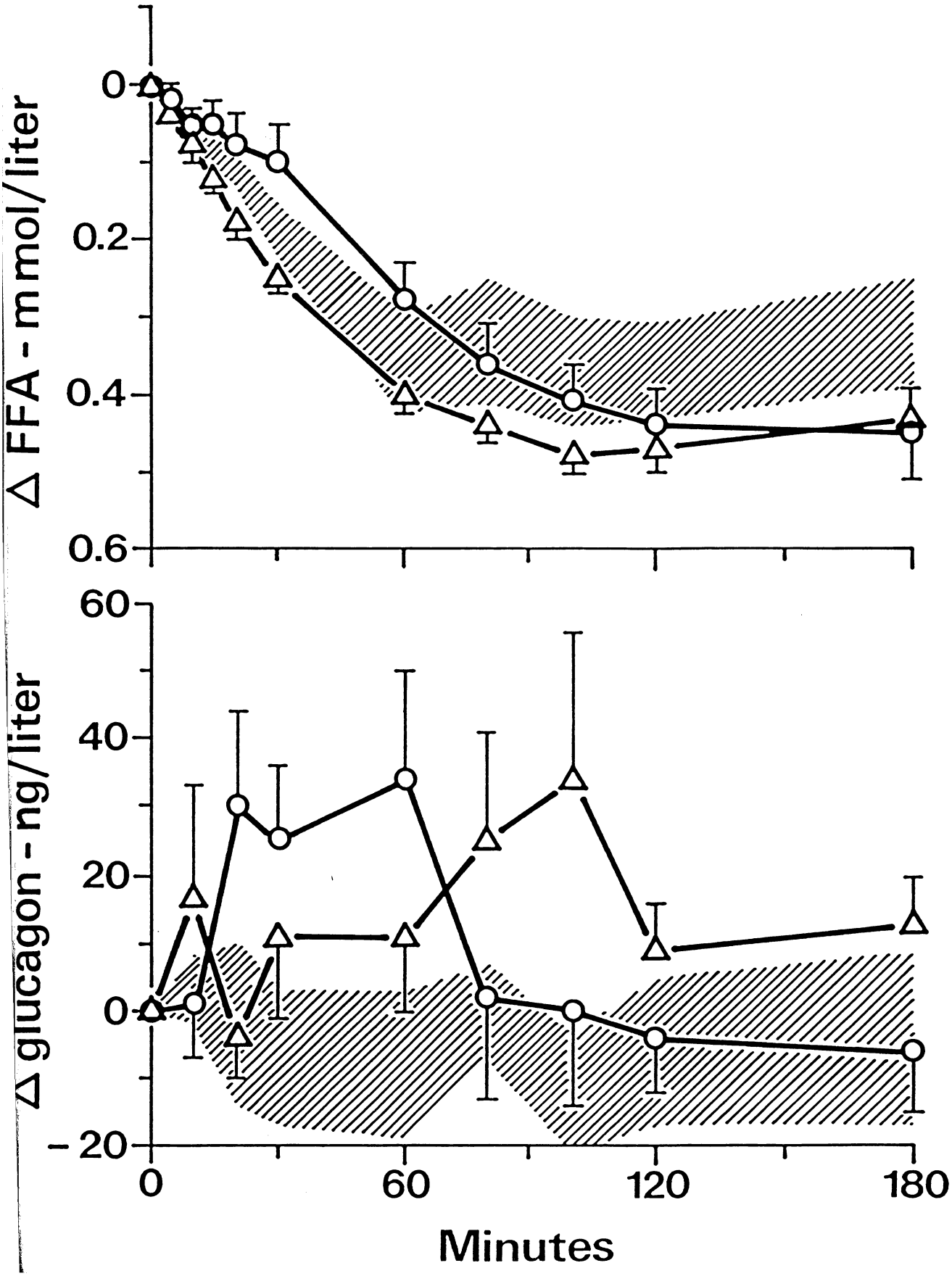
Fig 4.6 The mean (±SEM) integrated total glucose, insulin and C peptide responses above baseline to the standard meal in the diabetic subjects under each of the four conditions: no insulin (control, n=8), early insulin delivery (n=8), delayed insulin delivery (n=7) and constant insulin delivery (n=7). Integrated responses of the nondiabetic subjects are also displayed for comparison. *p<0.02 among the diabetic subjects only.



FFA and glucagon responses (fig 4.7): Early exogenous insulin administration resulted in FFA levels falling faster than control. FFA decrements from baseline were significantly lower from 15-80 minutes after meal ingestion and the integrated fall during the initial 60 minutes was greater than control (-13.5±0.7 vs -7.3±2.2 mmol/l.60 min, $p<0.05$). There was no significant difference in FFA levels or integrated FFA responses (0-60 minutes) following delayed insulin delivery or constant insulin infusion when compared with control. Following early insulin augmentation, the mean glucagon concentration 20 minutes after meal ingestion was significantly lower ($p<0.05$) and there was a tendency for the integrated glucagon response from baseline during the first hour after the meal to be lower (672±650 vs 1347±498 ng/l.60 min, $p<0.1$) although the total integrated glucagon response was non-significantly higher in the early insulin treatment group (2434±889 vs 1385±1222 ng/l.180 min). Glucagon levels and integrated glucagon responses were not significantly different from control with either constant or delayed insulin delivery.

There was no correlation between the improvement in individual glycaemic responses and the body mass index or fasting blood glucose level of the subjects nor with changes in FFA or glucagon responses following early insulin augmentation.

Fig 4.7 The mean (±SEM) incremental FFA and glucagon responses to a standard mixed meal in 8 diabetic subjects following early insulin augmentation (1.8 units from 0-30 minutes, Δ) and without exogenous insulin (O). The shaded area represents the mean±1SEM incremental responses in 9 nondiabetic subjects.



4.3.3 Discussion

In the present investigation, correction of the deficiency in early prandial insulin secretion in subjects with NIDDM with a physiological dose of intravenous insulin resulted in a substantial improvement in prandial hyperglycaemia. The peak blood glucose increment was reduced to normal, the total glycaemic increment was reduced by a third and the improvement in glycaemia lasted for at least 180 minutes. In contrast, the same dose of insulin given 30 minutes later or given as a constant infusion had only a minor influence on prandial hyperglycaemia and had no effect on the peak blood glucose increment. This study demonstrates that the initial rise in insulin levels following meal ingestion has an important physiological role in limiting the prandial rise in blood glucose and that the delay in insulin secretion causes or at least contributes substantially to prandial hyperglycaemia in NIDDM.

The improvement in prandial hyperglycaemia which followed early insulin augmentation was accompanied by changes in endogenous insulin secretion with lower insulin and C peptide levels in the final hour of the study period. Thus the improvement in glycaemia was achieved without any change in total peripheral insulin levels. This finding indicates that the apparently near-normal total prandial insulin response which was seen in these subjects during the control studies may be related to hyperglycaemic stimulation of the beta cells.

The effect of the very small (1.8 units) dose of insulin used in this study contrasts with the very large doses of conventionally administered insulin required to achieve normoglycaemia in other studies (e.g. a mean daily dose in excess of 100 units in the study of Garvey et al (1985)). However, it should be pointed out that the patients studied had relatively mild hyperglycaemia and fairly intact endogenous insulin secretion. Their responsiveness to the small

dose of insulin may suggest that they were also relatively sensitive to insulin and it is possible that supplementation of early prandial insulin secretion may have less marked effects on prandial hyperglycaemia in severely insulin resistant diabetic patients.

Portal insulin concentrations are 2-3 times higher than peripheral concentrations (Blackard and Nelson, 1970). The insulin infusion rate chosen in this study was designed to overcorrect normal early peripheral insulin levels in order to simulate the normal early portal insulin response which is likely to be a key factor in controlling prandial hyperglycaemia. This resulted in higher peak peripheral insulin levels following the infusion (80 vs 52 mU/l) and higher estimated portal insulin concentrations (123 vs 95 mU/l) compared with the nondiabetic subjects. The improvement in prandial glycaemia could therefore be due to the presence of supernormal insulin concentrations. However, there are several reasons why this is unlikely to explain the improved glucose responses to the meal. Firstly, similar insulin concentrations were achieved when the insulin infusion profile was delayed, yet there was little improvement in glycaemia. In fact, only 20 minutes separated the time of peak insulin concentrations following the early and delayed insulin infusions, further emphasising the importance of the timing of insulin secretion. Secondly, it has been suggested that insulin responses to oral glucose are dependent on the previous basal insulin level (Bagdade et al., 1967) and that percent incremental responses rather than absolute insulin responses may allow better comparison between groups with differing basal insulin levels (Bagdade et al., 1967; Ionescu et al., 1985). Using this form of analysis, the percent peak insulin increments following the insulin infusion were non significantly lower than the insulin increments in the nondiabetic subjects (862±276% vs 1236±299%).

Furthermore, the increments in estimated portal insulin levels were quite similar in the two groups (81 vs 74 mU/l).

Early insulin augmentation resulted in FFA levels falling more rapidly in the diabetic subjects suggesting that abnormal FFA responses reported in other studies (high fasting levels and a reduced rate of fall with meals, Fraze et al., 1985) may also be related to differences in early insulin secretion. However, the FFA responses of the diabetic subjects to the meal in this study were not greatly abnormal in that fasting levels were the same as in the nondiabetic subjects and fell at a similar rate following meal ingestion. This difference from previous reports may be because both groups in the present study exhibited a range of obesity and the diabetic subjects exhibited a range of fasting hyperglycaemia, and both factors influence FFA metabolism (Fraze et al., 1985; Golay et al., 1986).

The paradoxical rise in glucagon levels following meals which is seen in NIDDM (Muller et al., 1979) may also be related to a failure of early insulin secretion as early insulin augmentation partially suppressed or delayed the abnormal rise in glucagon levels in these subjects. This finding is in agreement with Schichiri et al (1979) who found that abnormal glucagon responses to oral glucose could be normalised by insulin when given in a near physiological manner, whereas in other studies insulin given as a constant infusion failed to correct the abnormal glucagon response (Aydin et al., 1977).

The observed changes in FFA and glucagon metabolism may be relevant to the improvement in prandial hyperglycaemia resulting from augmentation of the early insulin response. Prandial hyperglycaemia in NIDDM is due to both an impaired suppression of liver glucose production and reduced peripheral

glucose disposal (Firth et al., 1986). Elevated FFA and glycerol levels promote gluconeogenesis (Ferrannini et al., 1983) and elevated FFA levels in certain circumstances cause insulin resistance (Ferrannini et al., 1983). The accelerated fall in FFA levels following early insulin augmentation could have contributed to the improvement in prandial hyperglycaemia due to a reduction in liver glucose production and/or an improvement in peripheral glucose disposal. The reduction in glucagon levels following early insulin augmentation could likewise have caused a reduction in endogenous liver glucose production (Gerich et al., 1975).

Direct effects of insulin on the liver or peripheral tissues are alternative possible explanations for the improvement in hyperglycaemia. However total peripheral insulin concentrations were unchanged by exogenous insulin delivery. An early rise in insulin levels (before the rise in blood glucose) following a meal may act to switch off liver glucose production or alternatively could act as a signal in the hypothalamus (Storlien et al., 1975; Iguchi et al., 1981) which may (indirectly) cause a reduction in liver glucose production (Shimazu, 1981; Smythe et al., 1984).

It is interesting that there was a correlation between fasting blood glucose and prandial hyperglycaemia in the diabetic subjects. Basal levels of hepatic glucose production correlate strongly with fasting blood glucose in NIDDM (Bogardus et al., 1984; Kolterman et al., 1981). Thus overproduction of glucose by the liver may be responsible for prandial as well as fasting hyperglycaemia.

4.4 Intranasal insulin delivery

4.4.1 Introduction

The studies detailed in the preceding part of this chapter demonstrated that augmentation of the deficiency in early prandial insulin secretion in NIDDM with intravenous insulin had beneficial effects on prandial hyperglycaemia and hyperinsulinaemia. This finding suggests that delivery of insulin during the early part of a meal might be therapeutically useful in subjects with diabetes. However, conventional methods of insulin delivery do not achieve sufficiently rapid delivery of insulin with maximum insulin levels being reached approximately 1-2 hours after injection of soluble insulin at a time shown in the previous studies to have relatively little effect on prandial hyperglycaemia. Dimitriadis and Gerich (1981) found that injecting soluble insulin subcutaneously 60 minutes before meals improved prandial hyperglycaemia but at the expense of preprandial hypoglycaemia.

Recently, intranasal delivery of insulin has become feasible because of the development of adjuvant agents which facilitate absorption across the nasal mucosa (Hirai et al., 1978). The adjuvant materials used have included hydrophobic bile salts, which act by forming micelles with the insulin molecules which are then transported across the nasal mucosa (Gordon et al., 1985). Several short-term clinical studies have indicated that this method of insulin delivery is safe and reasonably effective (Moses et al., 1983; Salzman et al., 1985; Frauman et al., 1987; 1988).

The intranasal delivery of an aerosol containing an insulin/bile salt mixture results in a rapid increase in circulating insulin levels, with peak levels occurring within 10 to 15 minutes after administration and a maximum duration of the effect no longer than 60 minutes (Moses et al., 1983). These properties

suggest that this form of insulin delivery could be used to selectively augment early prandial insulin secretion in diabetic subjects.

Intranasal insulin has been tested with mixed meals in subjects with NIDDM and large doses of insulin administered preprandially were found to reduce the prandial rise in blood glucose levels (Frauman et al., 1987). In other studies, the use of intranasal insulin given preprandially in combination with daily injections of ultralente insulin was found to be reasonably effective in controlling blood glucose levels in patients with IDDM (Salzman et al., 1985).

There are several problems associated with the use of intranasal insulin delivery. Firstly, the bile salt adjuvants are not efficient promoters of insulin absorption and intranasal insulin delivery is approximately 10-20% as efficient as intravenous delivery (Moses et al., 1983). Secondly, there appears to be a fair degree of intersubject variation in the absorption of insulin although there is reasonably good reproducibility in an individual's response to repeated administration (Moses et al., 1983). Thirdly, the different bile salts have differing degrees of potency in terms of insulin absorption but they also exhibit different degrees of local toxicity.

The aim of the present studies was to test whether intranasal insulin delivery could be used to augment the early phase of prandial insulin delivery in subjects with NIDDM and cause improvements in prandial metabolism similar to those seen following intravenous insulin delivery. The study was designed so that the effects of intranasal insulin administration on circulating insulin levels and prandial hyperglycaemia could be compared with the data from the intravenous supplementation studies.

The bile salt glychocholate was used as the adjuvant agent in these studies. This agent was the second most potent of the bile salts examined by Gordon et al (1981) in promoting insulin absorption and caused little local toxicity. This agent has been used in short and longer term studies in subjects with NIDDM and IDDM (Frauman et al., 1987; 1988).

4.4.2 Subjects and methods

Six subjects with NIDDM (4 men and 2 women) participated in these studies (table 4.3). The subjects were otherwise healthy, were free of serious nasal pathology and had a history of stable hyperglycaemia for at least one year. All had received instruction regarding a standard diabetic diet within the preceding year and they were instructed not to change their diet during the studies. Five of the subjects were on sulphonylurea therapy (0.5-1 tablet of either glibenclamide or gliclizide); one subject also took metformin. All subjects ceased their medication 48 hours before the studies. These subjects were of similar age and had similar degrees of fasting hyperglycaemia, obesity and duration of diabetes to the subjects with NIDDM in the previous (intravenous augmentation) study.

The subjects were each studied on two occasions, after an overnight fast in random order. On each occasion, a single sampling catheter was inserted for collection of arterialised venous blood as previously described (chapter 2.2). The subjects were seated at a table and after at least 30 minutes were presented with the same standard breakfast meal as in the previous study (see chapter 4.3.1). They received a single spray intranasally of either insulin/glychocholate aerosol or placebo (glychocholate alone) in a single-blind fashion immediately before they commenced eating. Blood samples were drawn for arterialised venous glucose, insulin, C peptide, FFA and glucagon levels before, during and for 180 minutes after the meal. The insulin samples from each pair of studies were estimated in the same assay to avoid inter-assay variation.

Table 4.3. Clinical details of the diabetic subjects studied with intranasal insulin administration.

Subject	Age (years)	BMI (kg/m^2)	Duration of diabetes (years)	Fasting glucose (mmol/l)
1	66	27.1	7	7.6
2	68	22.8	2.5	7.9
3	74	27.4	3	7.5
4	75	27.8	3	8.3
5	61	32.4	17	6.1
6	65	31	5	10.6
mean	68.2	28.1	6.3	8.0
SD	5.4	3.3	5.5	0.6

4.4.3 Preparation of the intranasal solution

The insulin/glychocholate solution was prepared in conjunction with the St Vincent's Hospital Pharmacy. The method has been described in detail in chapter 2.4. Briefly, crystalline porcine insulin was dissolved in 1% sodium glychocholate to make a solution containing 250 units/ml insulin. The insulin was delivered as a fixed volume spray using a hand held nebuliser which delivered 60±9 µl/spray (mean±SD). Each spray therefore delivered approximately 15 units of insulin onto the nasal mucosa. The absorption into the circulation of insulin in the more potent bile salt, sodium deoxycholate has been estimated to be between 10-20% compared with intravenous insulin (Moses et al., 1983) and with sodium glychocholate to be approximately 12% (Frauman et al., 1987). Therefore a spray of 15 units into the nose was likely to deliver the equivalent of between 1.5 and 3 units of intravenous insulin. This compares with 1.8 units delivered intravenously in the previous study.

The efficacy of the preparation was tested in a nondiabetic fasting subject who received a single spray intranasally (15 units) without receiving a subsequent meal. This resulted in a rapid increase in venous serum insulin levels, with peak levels of 32 mU/l occurring 5 minutes after the spray and levels returning to baseline by 25 minutes. Fasting blood glucose levels fell to a nadir of 2.7 mmol/l at 30 minutes which was associated with mild hypoglycaemic symptoms.

4.4.4 Results

There was no difference in baseline levels of arterialised venous blood glucose, serum insulin, C peptide, FFA or plasma glucagon levels before the placebo or intranasal insulin studies (table 4.4).

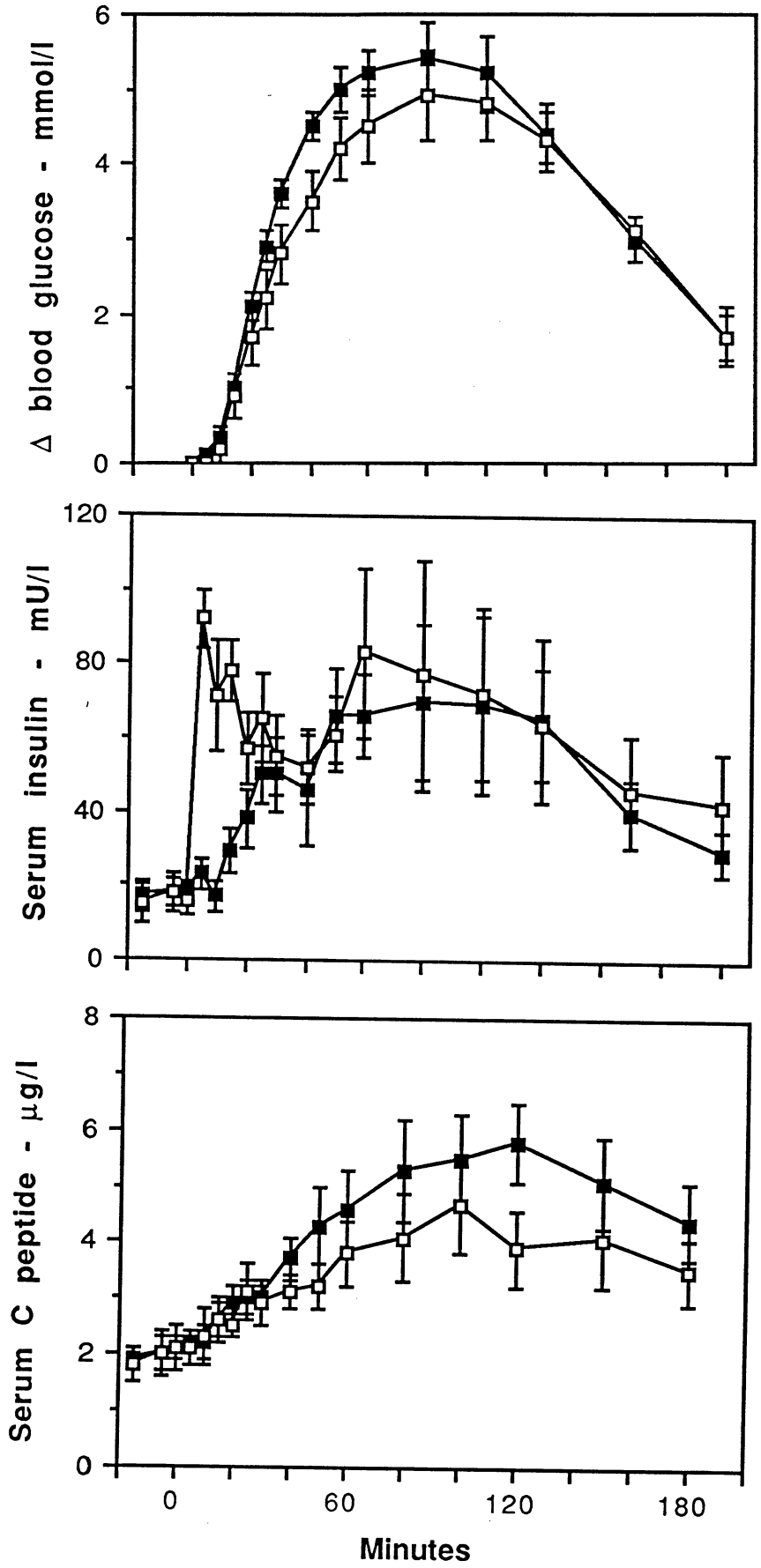
Intranasal administration of either glychocholate alone or the insulin/glychocholate mixture caused transient nasal symptoms in 5 of the 6 subjects. These were described as a mild tickling sensation or irritation and lasted no longer than 5 minutes in any subject. There was no difference in the severity or duration of symptoms caused by either aerosol.

Glycaemic and beta cell responses (fig 4.8): The intranasal administration of insulin resulted in a rapid rise in serum insulin levels with peak levels of 92±8 mU/l occurring five minutes after administration. Insulin levels subsequently fell, remained significantly greater than the placebo study from 5-20 minutes after administration and thereafter were not different compared with the placebo study. Intranasal insulin administration had only a minor effect on the prandial glycaemic response compared with placebo administration. The mean glycaemic increment was lower at 40 minutes after the meal ($p<0.05$). However, there was no significant improvement in the peak glycaemic increment (4.9±0.6 vs 5.4±0.5 mmol/l) or the total glycaemic responses to the meal compared with placebo (611±53 vs 668±41 mmol/l.180 min). C peptide levels were significantly lower after intranasal insulin than after placebo at several time points after the meal (80, 120 and 150 minute time points, $p<0.05$) and total incremental responses were also significantly lower after intranasal insulin (307±48 vs 461±75 µg/l.180 min, $p<0.05$).

Table 4.4. Mean ± SEM fasting levels for substrates and hormones in the diabetic subjects before the intranasal placebo and insulin studies.

	Glucose (mmol/l)	Insulin (mU/l)	C peptide (μg/l)	FFA (mmol/l)	Glucagon (ng/l)
IN placebo	8.0±0.6	17±4	2.0±0.2	0.62±0.11	46±10
IN insulin	8.3±0.6	16±4	1.9±0.4	0.64±0.05	59±21

Fig 4.8 Mean (±SEM) blood glucose increments (top panel) and insulin and C peptide concentrations after the standard mixed meal following intranasal placebo (■) and insulin administration (□) in 6 subjects with NIDDM.



FFA and glucagon responses (fig 4.9): Intranasal insulin administration resulted in nonsignificantly lower FFA levels compared with placebo ($p<0.1$ at the 40 min time point) and a nonsignificant fall in the integrated decrement (-13.93 ±1.90 vs -8.56±3.38 mmol/l.60 mins, $p<0.1$). There was no difference in the glucagon responses to the meal between the intranasal insulin or placebo studies.

For comparison purposes, the total integrated glycaemic and insulin responses during the intranasal studies have been plotted alongside the responses to the same standard meal during the intravenous augmentation study (fig 4.10). Intranasal insulin delivery resulted in a significantly greater total integrated insulin response compared with placebo (8293±1966 vs 5969±1816 mU/l.180 min, $p<0.01$) without any significant change in the integrated glycaemic response. In addition, the integrated insulin responses during the first 40 minutes after the meal are illustrated. There was no significant difference between the calculated amount of insulin delivered into the circulation (integrated area during insulin delivery minus appropriate control) due to exogenous insulin delivery during the first 40 minutes after the meal whether given by the intravenous or the intranasal route (1138±156 vs 1167±231 mU/l.40 mins). In terms of percent change, intravenous insulin augmentation resulted in a 33±4% improvement in the glycaemic responses ($p<0.005$) compared with control whereas intranasal insulin administration resulted in no significant change (92±8%) and individual glycaemic responses ranged from 75-126% of the placebo study.

Fig 4.9 Mean (± SEM) incremental FFA (top panel) and glucagon (bottom panel) responses to a standard meal after intranasal placebo (■) and insulin administration (□) in 6 subjects with NIDDM.

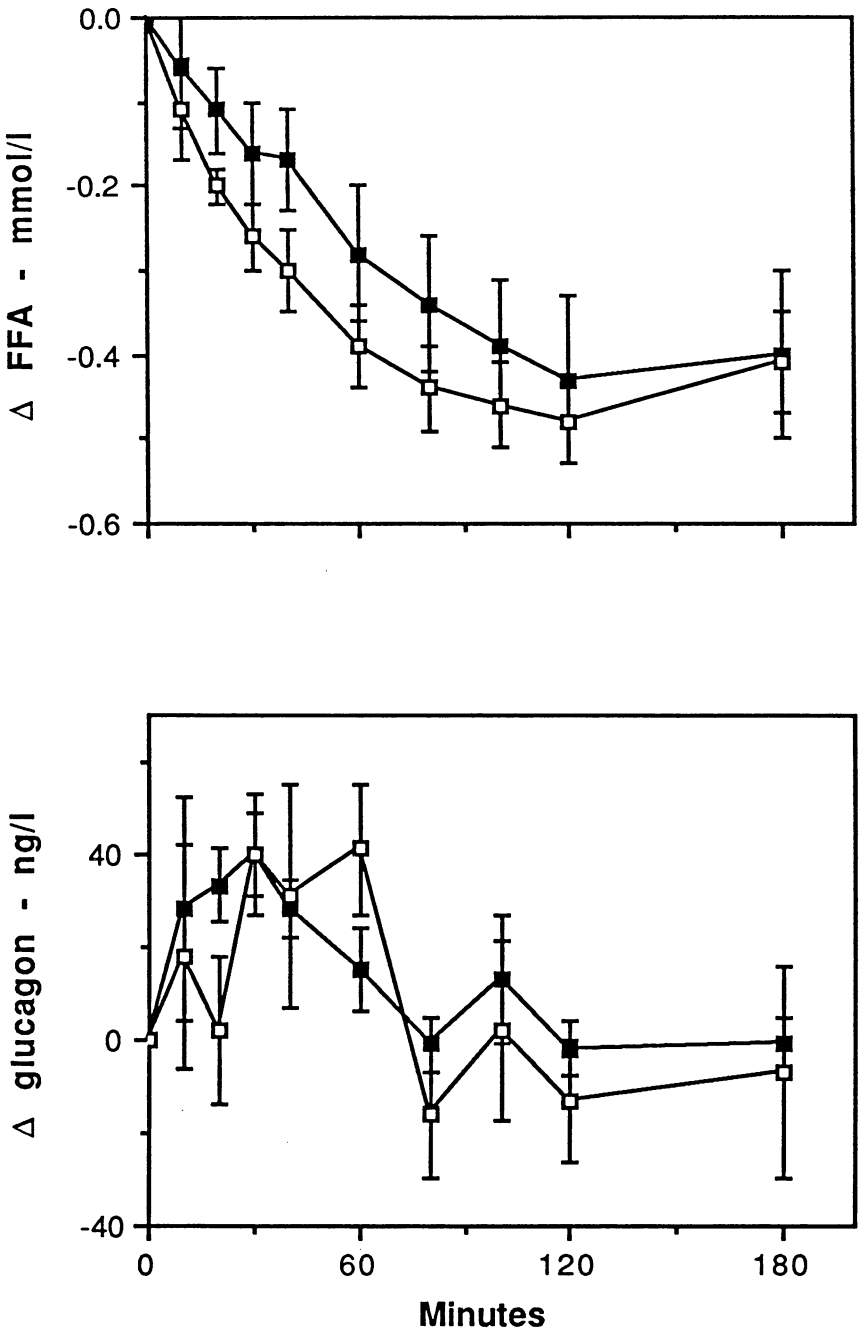
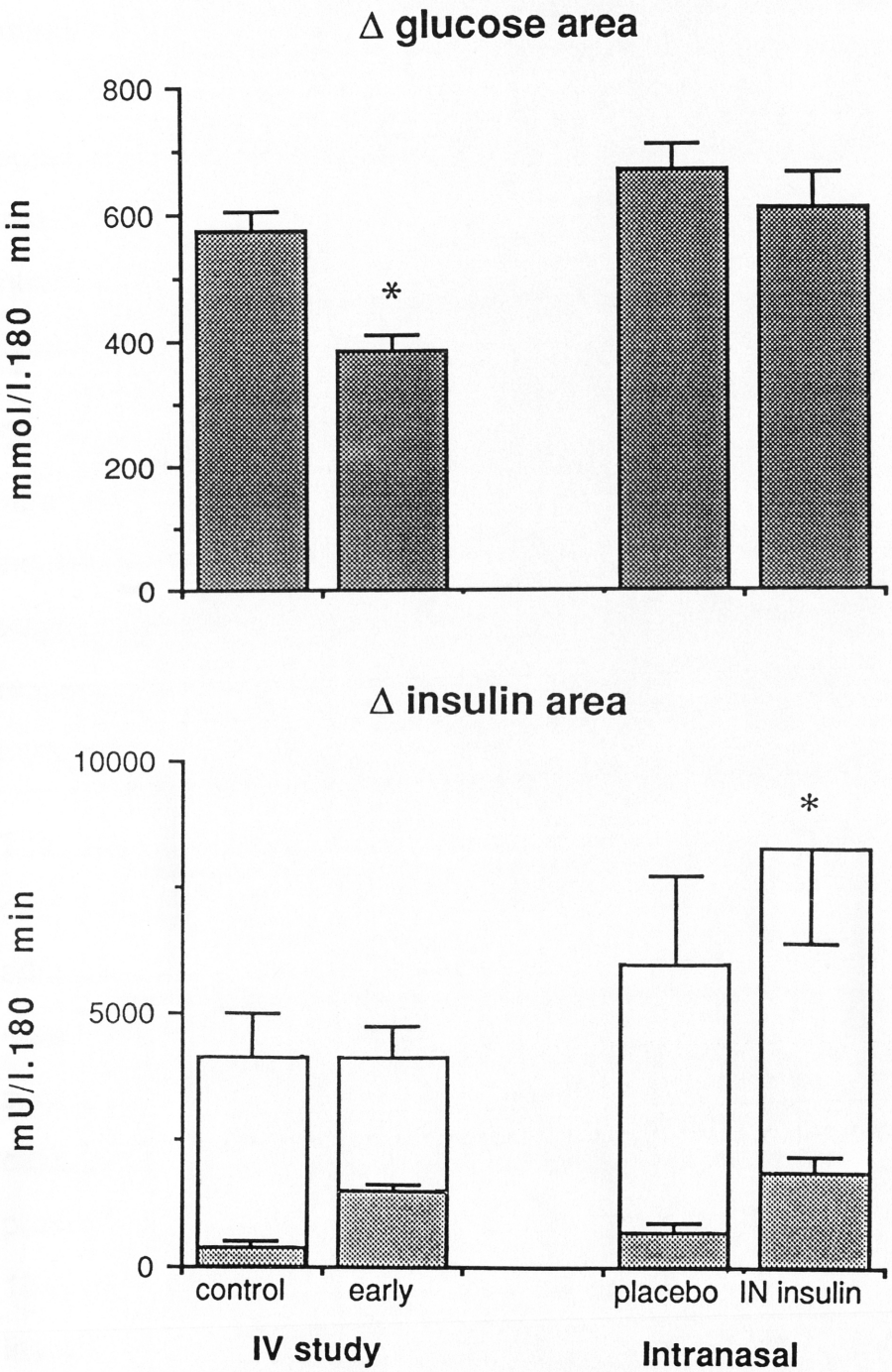


Fig 4.10 Mean (±SEM) total integrated glycaemic (top panel) and insulin responses (bottom panel) to the standard mixed meal in the intranasal and intravenous augmentation studies. The hatched bars (bottom panel) represent the initial 40 minutes after the meal.



4.4.5 Discussion

The major findings of this study were that the administration of intranasal insulin resulted in the rapid delivery of insulin into the circulation with peak arterial insulin levels being achieved comparable to those seen in the previous intravenous augmentation study. However, relatively little improvement in prandial hyperglycaemia occurred. There was an initial transient improvement in prandial hyperglycaemia but this was unsustained and there was no significant improvement in either the mean peak blood glucose level or the mean overall glycaemic profile after the meal. In addition, in contrast to the intravenous augmentation study, no improvement in subsequent hyperinsulinaemia or FFA and glucagon responses were seen.

Some subjects did show some improvement in prandial hyperglycaemia after intranasal insulin. However, the range of individual responses after intranasal insulin was considerably less than that seen after early intravenous augmentation (total glycaemic responses: 75-125% vs 40-80% of the respective control conditions) further emphasising the relatively poor effect of intranasal insulin delivery at the commencement of the meal.

The reason for the lack of efficacy of intranasal insulin cannot be definitely determined from these studies. Peak insulin levels achieved after intranasal administration were similar to those seen in nondiabetic subjects after the same meal (see fig 4.2) and similar to what was achieved during the intravenous augmentation study. In addition, there was no difference between the calculated amount of insulin delivered into the circulation during the early prandial period following intranasal and intravenous delivery. The delivery of 15 units intranasally was very similar to the intravenous delivery of 1.8 units of insulin and it can be estimated that approximately 12% of the insulin delivered

intranasally was absorbed systemically. This estimate of insulin absorption after intranasal administration agrees closely with the 10-20% estimated by Moses et al (1983) (with deoxycholate as adjuvant) and the 12% estimated by Frauman et al (1987).

The lack of effectiveness of the intranasal insulin could have been due to chemical alteration during preparation which rendered the insulin less biologically active. This seems unlikely as other groups have demonstrated significant biological activity using an identical preparation (Frauman et al., 1987; 1988); the preparation had considerable hypoglycaemic activity in a nondiabetic subject; and some hypoglycaemic effect was seen in the early prandial period after intranasal insulin delivery in the diabetic subjects.

The subjects who participated in the intranasal studies may have been relatively insulin resistant. However this seems unlikely as, although insulin sensitivity was not measured in these studies, the subjects who participated in the two sets of studies (intranasal and intravenous insulin studies) were selected from the same diabetic population, had similar clinical characteristics and both groups had similar degrees of basal hyperglycaemia with comparable basal insulin, FFA and glucagon levels.

It seems most likely that the lack of efficacy of the intranasal insulin was due to the timing of insulin absorption. Peak insulin levels were seen within five minutes of delivery and insulin levels were no longer significantly elevated 25 minutes after commencement of the meal. In contrast, insulin concentrations increased gradually and peaked at 30 minutes during the intravenous administration studies; this profile closely simulating the normal prandial rise in insulin levels. Therefore the lack of efficacy of intranasal insulin delivery is

likely to have been due to peak insulin levels occurring too early after the meal. If this is the correct explanation for the ineffectiveness of the intranasal preparation, then this finding further emphasises the importance of correct timing of insulin delivery to diabetic subjects in order to achieve control of prandial hyperglycaemia. In normal physiology, prandial insulin secretion appears to be precisely regulated with respect to timing as well as to the quantity of secretion.

There was no improvement in subsequent hyperinsulinaemia and no significant improvement in the abnormal prandial FFA and glucagon responses following intranasal insulin delivery. It should be pointed out that the FFA responses in these studies, approached statistical significance. With greater numbers it is possible that FFA levels would have been shown to fall at a faster rate following intranasal insulin delivery. This would be consistent with the notion that insulin sensitivity is relatively normal with respect to FFA metabolism in subjects with NIDDM (Howard et al., 1979).

Intranasal insulin delivery resulted in a significant reduction in C peptide levels and a significant reduction in the overall C peptide responses. The reason for this effect on C peptide levels in the absence of effects on the glycaemic responses or insulin concentrations is difficult to explain. The possibility that intranasal insulin delivery alters insulin metabolism or suppresses beta cell function in the absence of effects on blood glucose requires further study.

Several groups have demonstrated that large doses of insulin delivered intranasally with meals have some effect on glycaemic control in subjects with IDDM and NIDDM (Salzman et al., 1985; Frauman et al., 1987; 1988). However, these studies have not demonstrated that intranasal insulin delivery

has any advantage, in terms of glycaemic control, over conventional forms of insulin therapy. The results of the present studies indicate that attention to the timing of intranasal insulin delivery may improve its therapeutic usefulness.

CHAPTER FIVE

Prandial insulin secretion in NIDDM: pilot study utilising a dual isotope technique and respirometry

5.1 Introduction

The studies carried out in chapters 3 and 4 clearly indicate that the initial phase of insulin secretion is important in the regulation of prandial glucose disposal. The delayed rise in insulin levels seen in subjects with NIDDM appears to be responsible for a considerable proportion of prandial hyperglycaemia in this condition. The main aim of the study detailed in this chapter was to determine the mechanism for the improvement in prandial hyperglycaemia after augmentation of early prandial insulin secretion. In the time available, only a small number of experiments could be performed and the data presented herein represents a supplementary pilot study.

NIDDM has been shown to be associated with a reduction in meal-related energy expenditure. The second aim of the studies in this chapter was to determine whether the improved prandial glycaemic response to a meal after correction of the deficiency in early insulin release was associated with an improvement in meal-related thermogenesis. Such a finding would implicate the early phase of prandial insulin secretion in the regulation of meal-related thermogenesis.

5.1.1 Dual isotope technique

Normal oral glucose tolerance depends on a number of physiological processes, several of which could be influenced by prandial insulin secretion. These include glucose absorption from the gut, glucose uptake by the liver, reduction of endogenous hepatic glucose production and glucose uptake by the peripheral tissues.

Several components of oral glucose tolerance may be studied using labelled glucose tracers. In the dual isotope technique, a quantity of glucose enriched

with a known quantity of labelled glucose tracer is ingested to trace the rate of appearance into the systemic circulation of the oral glucose load. At the same time, a second glucose tracer (bearing a different label) is infused peripherally to trace peripheral glucose disposal and the total rate of appearance of glucose from the splanchnic circulation. Total glucose appearance derives from both the ingested load and endogenous hepatic glucose production. Endogenous hepatic glucose production can be calculated by subtracting the rate of appearance of the ingested load from the rate of total glucose appearance. This technique has been used to study oral glucose tolerance in nondiabetic subjects (Radziuk et al., 1978; Ferrannini et al., 1985; Jackson et al., 1986), subjects with IDDM (Pehling et al., 1984) and subjects with NIDDM (Firth et al., 1986).

In normal humans, the majority of a glucose load is not taken up by the liver as earlier studies suggested (Felig et al., 1978) but appears in the peripheral circulation where it is taken up by various peripheral tissues (Radziuk et al., 1978; Ferrannini et al., 1985). In the fasting state, only approximately 25% of total glucose utilisation takes place in insulin-dependent tissues since 75-80% occurs in brain and other non-insulin dependent tissues which have an obligatory need for glycolytic metabolism (Gottesman et al., 1984). After glucose administration, the majority of the increase in peripheral glucose disposal occurs in insulin-dependent tissues, primarily muscle (Katz et al., 1983; Jackson et al., 1986). Coincident with the prandial increase in peripheral glucose uptake, endogenous glucose production declines and remains below postabsorptive levels for several hours (Radziuk et al., 1978; Ferrannini et al., 1985). It is possible that the early increase in prandial insulin concentrations

plays an important role in regulating the increase in peripheral glucose disposal and the decline in hepatic glucose production.

Impaired suppression of hepatic glucose production is a major cause of prandial hyperglycaemia in insulin deficient diabetic subjects (IDDM) (Pehling et al., 1984). In NIDDM, prandial hyperglycaemia appears to be due to a combination of impaired suppression of hepatic glucose production and a reduction in peripheral glucose disposal (Firth et al., 1986). In one animal model of diabetes, the genetically obese Zucker (fa/fa) rat, impaired glucose tolerance is due to a lack of suppression of hepatic glucose production (Rohner-Jeanrenaud et al., 1986) and, although hyperinsulinaemic, these animals may have a relative deficiency in the early prandial insulin response.

The improvement in prandial hyperglycaemia seen after augmentation of early insulin secretion in chapter 4, could therefore be due to either (or both) an improvement in peripheral glucose disposal or suppression of hepatic glucose production.

5.1.2 Respiratory gas analysis and indirect calorimetry

The metabolism of a carbohydrate meal is accompanied by an increase in energy expenditure greater than can be attributed to the energy costs of digestion, absorption and storage of glucose. This thermic effect of food, or meal-related thermogenesis, may be an important determinant of overall energy expenditure in mammalian species. Meal-related thermogenesis is reduced in obesity (Jung et al., 1979; Segal et al., 1985) and this reduction in energy expenditure may contribute in the pathogenesis of obesity (Schutz et al., 1984). In NIDDM the thermic response to meals (Golay et al., 1982; Nair et al., 1986) and to parenteral glucose (Ravussin et al., 1983) is also reduced.

This reduction in the thermic effect of food or glucose may be an important cause of obesity in NIDDM.

Meal-related thermogenesis is reduced in insulin deficient animals and restored to normal with insulin treatment (Rothwell and Stock, 1981). This has led to the hypothesis that insulin action is involved in the thermic effect of food and that the decreased thermic effect of food in NIDDM is related to insulin resistance (Felig, 1984; Ravussin et al., 1985).

Insulin may activate the sympathetic nervous system which may be the major mediator of thermogenesis (Acheson et al., 1983; Astrup et al., 1984). The site for thermogenesis in humans is controversial, and both brown adipose tissue and skeletal muscle have been advocated (Cunningham et al., 1985; Astrup et al., 1986). Alternatively, Christin et al (1986) found that the insulin-mediated component of glucose-related thermogenesis was relatively minor and concluded that most of the thermic effect was due to cellular glucose metabolism.

The reduced thermic effect of food in NIDDM could be related to the early deficiency in insulin secretion. In support of this hypothesis, a recent study in nondiabetic humans reported that a somatostatin infusion, given for 20 minutes immediately after glucose ingestion to inhibit early insulin secretion, caused a deterioration in glucose tolerance and a reduction in energy expenditure (Calles-Escandon and Robbins, 1987). Thus, augmentation of the deficiency in early prandial insulin secretion in NIDDM could result in improved meal-related energy expenditure. In order to test this hypothesis, the studies in this chapter included measurements of respiratory gas exchange to measure prandial energy expenditure.

The measurement of oxygen consumption and carbon dioxide production can also give information on the type and rate of fuel oxidation in the body (Frayn, 1983). In NIDDM, basal rates of carbohydrate and lipid oxidation are normal but the normal rise in carbohydrate oxidation and the reciprocal fall in lipid oxidation after a meal are both blunted (Boden et al., 1983; Meyer et al., 1980). In normal subjects, the increase in carbohydrate oxidation with meals is at least partly due to the increase in insulin secretion (Thiebaud et al., 1982). Therefore, rates of substrate oxidation were estimated in these studies to determine whether abnormal glucose and lipid oxidation in NIDDM could also be related to the deficiency in early prandial insulin secretion.

5.2 Subjects and methods

Five subjects with NIDDM were studied. These subjects were in a stable metabolic state before and during the studies and were treated with either diet and/or oral agents (table 5.1). All had received instruction regarding a diabetes diet within the previous year and they were instructed not to alter their usual diet during the studies. All subjects were otherwise healthy and none were taking any other medications. The aim of the study was to reproduce the effect of early insulin augmentation on prandial hyperglycaemia demonstrated in chapter 4. This aim was not fullfilled in one subject and the data from this study was deleted from analysis (see below).

Each subject was studied in the morning after an overnight fast. The studies were separated by at least two weeks and performed in random order. Oral hypoglycaemic agents were ceased 48 hours before each study. On each occasion, an antecubital vein was cannulated for infusion of [3-^{3}H]glucose. A second catheter was inserted into a dorsal vein of the ipsilateral hand for arterialised venous sampling. On one occasion, an antecubital vein in the contralateral arm was cannulated for insulin infusion. The subjects sat comfortably in a bed throughout these studies except during the respiratory gas measurements (see below).

A continuous infusion of [3-^{3}H]glucose (25 μCi/hour) was commenced between 8 and 9 am for isotopic determination of rates of total glucose appearance and uptake. After a 90 minute equilibration period, each subject ingested the equivalent of the standard mixed meal used in chapter 4. This consisted of 70 g of glucose, enriched with 50 μCi [6-^{14}C]glucose dissolved in 100 mls of water, to which was added 100 g of beaten uncooked egg mixture (10.9 g fat, 12.3 g

protein, total energy content of the meal 1705 kjoules). An aliquot (1 ml) of the enriched oral glucose solution was collected prior to mixture with the egg for accurate determination of the specific activity and glucose concentration of the drink. The mixture was ingested in 3 equal volume boluses given at 0, 5 and 10 minutes after the start of the meal in order to simulate more closely the timing of natural meal ingestion. Two subsequent water rinses of the flask (50 mls) were also ingested to ensure that all the radioactivity was ingested.

In one study, an insulin infusion (total dose, 1.8 units) was commenced at the start of the glucose ingestion and continued for 30 minutes only. This was delivered in a manner identical to the studies in chapter 4 (see fig 4.1, "early"). A stepwise incremental infusion of insulin was delivered, starting with a rate of 1.2 units/hour and increasing at 2 minute intervals by 0.4 units/hour until a maximum rate of 5 units/hour was reached after 20 minutes. The insulin infusion was then continued at the same rate for a further 10 minutes and then ceased.

Samples of arterialised venous blood were taken at 5-15 minute intervals from the commencement of the study and for 240 minutes after the meal for plasma glucose, plasma [^{3}H]glucose specific activity, plasma [^{14}C]glucose specific activity, serum insulin and C peptide levels. Rates of total glucose appearance, endogenous hepatic glucose production and peripheral glucose disposal were calculated as described in chapter 2.7. Recycling of the ^{14}C label to carbon-1 of the glucose molecule was estimated as described in chapter 2.7 to provide an estimate of new glucose synthesis from 3 carbon intermediates derived from the ingested labelled glucose (Cori cycle).

Table 5.1 Clinical details of the four diabetic subjects who participated in the dual isotope and respirometry studies.

Subject	Age (years)	BMI (kg/m^2)	Duration of diabetes (years)	Fasting glucose (mmol/l)
1	72	26.6	16	6.6
2	61	29.7	3	12.6
3	65	28.9	12	10.3
4	71	28.5	1	6.8
mean	67.3	28.4	8	9.1
SD	5.2	1.3	7.2	2.9

Respiratory gas exchange measurements were obtained before and after the meal on each occasion via a semi-opened circuit, ventilated hood system as described in chapter 2.8. This was operated by the negative-flow pressure of the gas flow through the system. Briefly, the subject lay semi-recumbent on the bed, a light transparent plastic hood was placed over the subject's head and a soft mask was held in place over the nose and mouth to collect the expired gases. A flow rate of 25-30 litre/minute prevented the local accumulation of CO_2 and the expired air was drawn through a mixing chamber to avoid breath to breath gradients during recordings. An aliquot of the expired air was drawn from the mixing chamber by a separate pump for analysis. All subjects practiced breathing in the hood system on a separate occasion before the studies began. Continuous sampling of respiratory gases was performed for periods of 10 minutes, with the data from the final five minutes used for analysis. Respiratory gas exchange measurements were taken 45 and 15 minutes before the meal (the first being at least 60 minutes after the start of catheter insertion) and at 15, 45, 75, 105, 165 and 225 minutes after the start of the meal.

The subjects voided urine before and at the end of each study and the post-study urine analysed for urinary glucose and nitrogen excretion. Energy expenditure and rates of carbohydrate and lipid oxidation were calculated as described in chapter 2.8. Increments of energy expenditure and substrate oxidation were calculated assuming that no change would have occurred in basal rates in the absence of food ingestion.

In one subject, no improvement in prandial hyperglycaemia occurred after insulin administration. As the aim of the study was to determine the mechanism

of the improvement in hyperglycaemia, the data from this subject has been deleted. The reason for the lack of improvement may have been due to an acute stress response related to a blocked sampling line requiring replacement of an intravenous cannula immediately after the glucose drink and during the insulin infusion. This subject also complained of claustrophobic feelings and became overtly stressed during the later respirometry measurements in the same study. This clinical impression was confirmed by the finding of a substantial increase in serum cortisol levels after the cannula replacement and RQ readings consistent with hyperventilation during the early and later part of the experiment.

As a result of the exclusion of this subject's data, the results of only four subjects are available for respiratory gas analysis. In addition, the [^{3}H]glucose counts during the control study of subject 1 were aberrant (for no clear reason) and considered unusable. Therefore only the data from three paired studies are available for glucose turnover measurements.

The results from these few studies can only be regarded as a pilot study to test the feasibility of the techniques to test the given hypotheses. The results are mainly presented in a descriptive fashion and any conclusions are preliminary and subject to verification by future studies. Statistics quoted used Student's paired t test.

5.3 Results

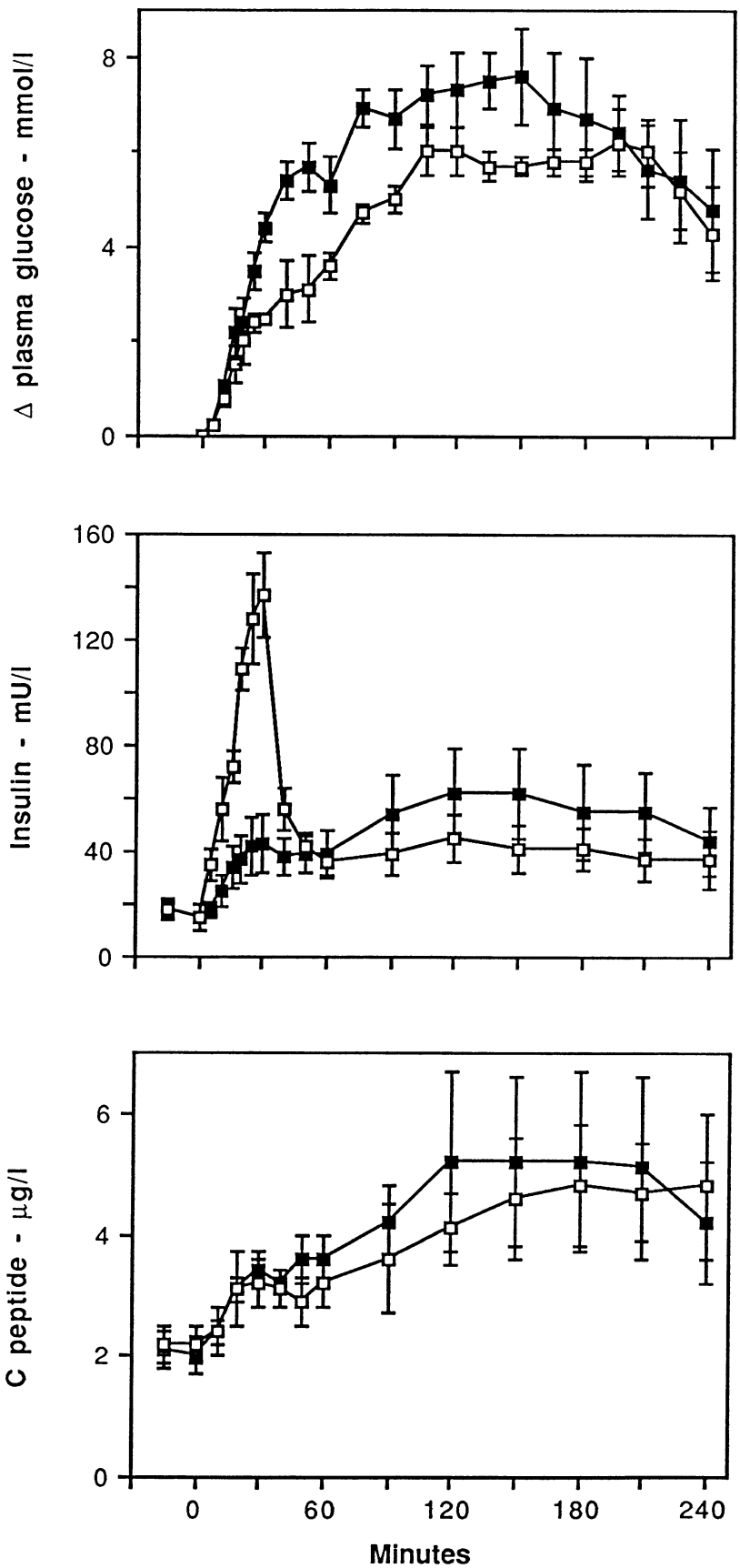
There was no difference in baseline plasma glucose, serum insulin or C peptide levels or in rates of glucose turnover, basal metabolic rate or substrate oxidation before the control and early insulin augmentation studies (table 5.2, figs 5.1-5.3).

Glucose, insulin and C peptide responses (fig 5.1): As in chapter 4, exogenous insulin administration given at the start of the meal resulted in a reduction in the prandial glycaemic response. Three of the four subjects had a sustained reduction in the glycaemic response whereas in one subject the improvement lasted for the first 100 minutes. Mean plasma glucose increments were significantly lower from 25-90 minutes after the meal commenced ($p<0.05$), the peak glycaemic increment was nonsignificantly lower (6.0±0.2 vs 7.6±0.6 mmol/l, $p<0.1$) and the incremental glycaemic increment was nonsignificantly lower (1086±44 vs 1397±124 mmol/240 mins, $p<0.05$ by one-tailed test) . The insulin infusion profile resulted in peak insulin levels being reached at 30 minutes after the meal commenced (137±16 mU/l), thereafter insulin levels promptly fell and were nonsignificantly lower than control for the rest of the study. Similarly C peptide levels were nonsignificantly lower than control following exogenous insulin delivery although the integrated C peptide response was significantly less than control (415±111 vs 572±178 μg/l.240 min, $p<0.05$).

Table 5.2 Mean (±SEM) basal plasma glucose levels, metabolic rate (BMR) and substrate oxidation rates before the control and insulin augmentation studies.

	Glucose (mM)	BMR Kj/min	Glucose Ox mg/min	Lipid Ox mg/min
Control Study	9.1±1.5	4.84±0.31	64.2±19.9	29.5±8.8
Insulin Study	9.0±1.5	4.51±0.25	47.6±13.8	35.4±8.9

Fig 5.1 Mean (±SEM) incremental plasma glucose responses (top panel) and serum insulin and C peptide concentrations after a simulated mixed meal in subjects with NIDDM (n=4) with (□) or without (■) early insulin augmentation with intravenous insulin (1.8 units between 0 and 30 mins).



Total glucose appearance and disappearance (fig 5.2): Early insulin augmentation reduced the rate of total glucose appearance in all three subjects. Peripheral glucose disposal increased to a similar extent in the control and insulin augmentation studies in all 3 subjects.

Entry of ingested glucose and endogenous glucose production (fig 5.3): There was a sustained reduction in endogenous glucose production in the control studies. After early insulin augmentation, endogenous glucose production was suppressed earlier compared with the control study in all three subjects. There was also a slight reduction in the systemic appearance of the ingested glucose (in 2 of the 3 subjects) initially after early insulin augmentation. Proportions of recycled ^{14}C in plasma [^{14}C]glucose were small but were reduced at the 60 minute time point in all four subjects after early insulin supplementation (table 5.3).

Fig 5.2 Mean (±SEM) rates of total systemic glucose appearance (Ra) and glucose disappearance (Rd) in subjects with NIDDM (n=3) after control (■) and early insulin augmentation (□).

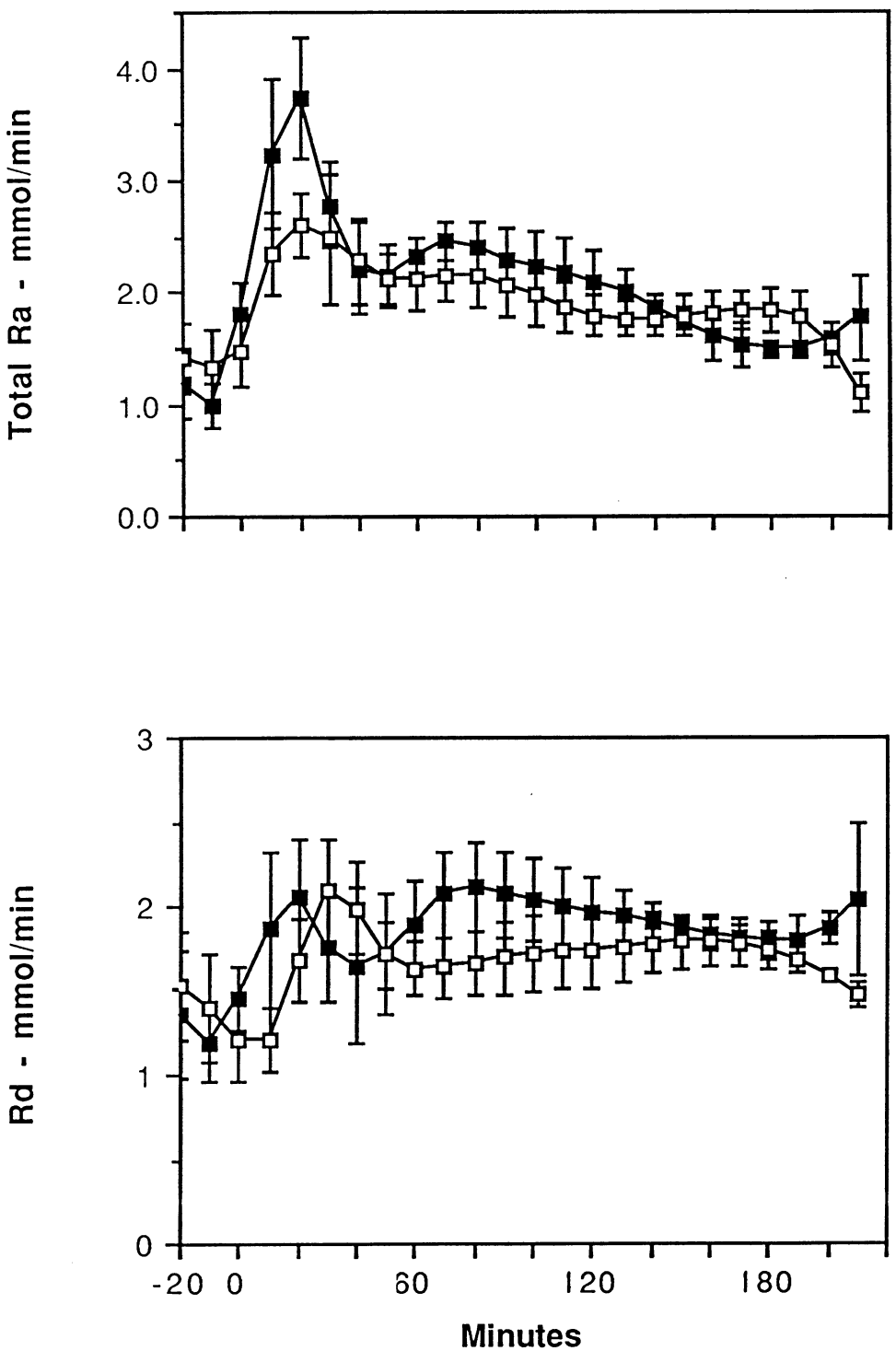


Fig 5.3 Mean (±SEM) rates of systemic appearance of meal derived glucose and endogenous hepatic glucose production (n=3) after control (■) and early insulin augmentation (□) in subjects with NIDDM.

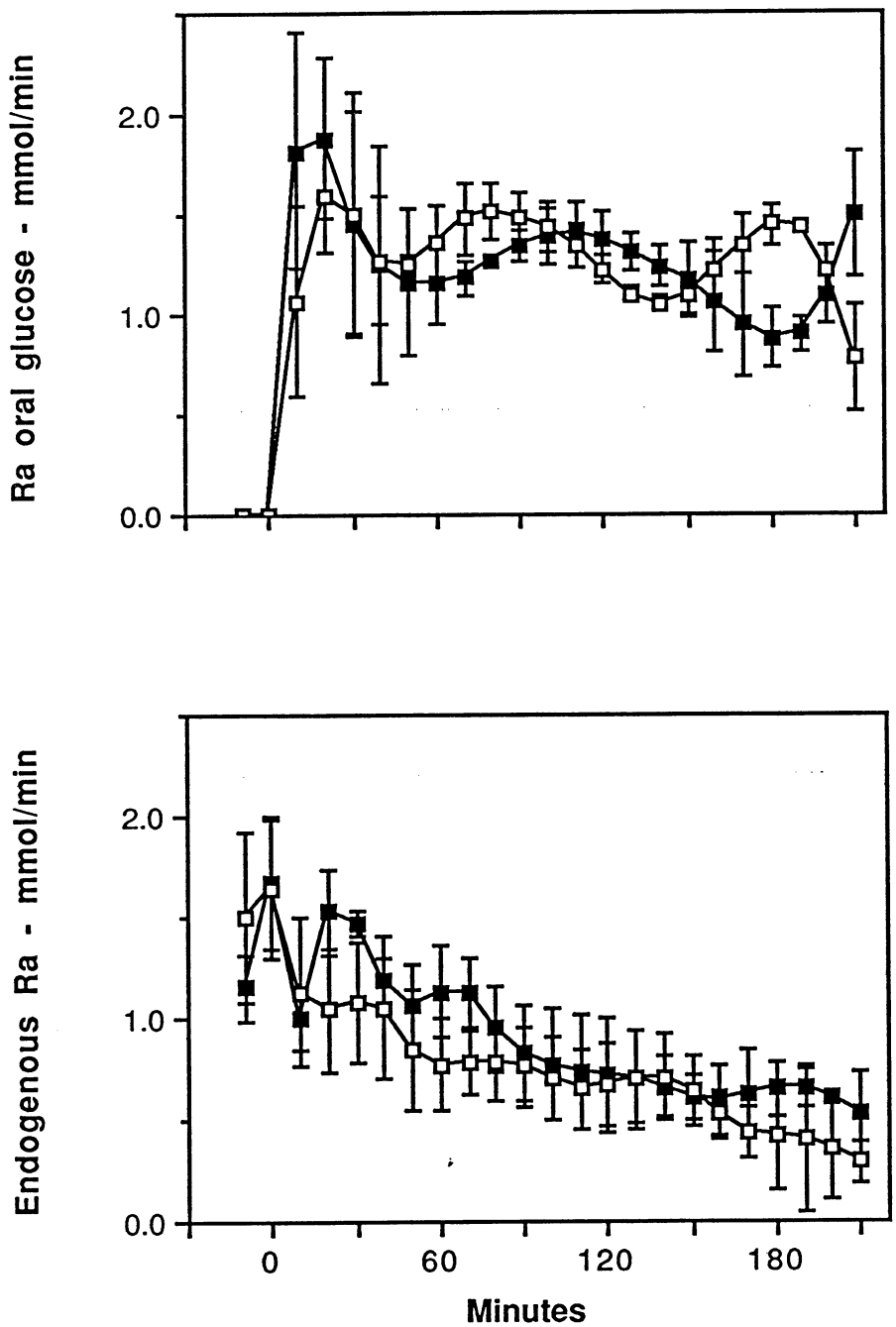


Table 5.3 Percent (%) recycling of ^{14}C label (Cori cycle) after the control and insulin augmentation studies in four subjects with NIDDM.

Time (mins)	60	105	165	225
Control Study	6.8±3.4	0.2±0.6	-0.7±0.6	1.6±0.6
Insulin Study	-1.0±1.0	0.4±0.1	0.7±0.6	1.5±0.9

Energy expenditure (fig 5.4): There was an increase in energy expenditure after the simulated mixed meal in all subjects. After early insulin augmentation, energy expenditure increased to a greater extent in all four subjects. In two subjects, the improved energy expenditure continued for the study duration, in one for 165 minutes and in one for only 45 minutes. This resulted in a significant increase in energy expenditure 15 minutes after the meal was ingested ($p<0.05$) and a nonsignificant overall increase in energy expenditure (integrated increase above basal, 117.39±22.71 vs 57.02±21.30 kjoule/225 mins).

CHO and lipid oxidation (fig 5.5): Basal rates of carbohydrate and lipid oxidation were the same before the control and insulin administration studies. After the mixed meal there was an increase in carbohydrate oxidation and a fall in lipid oxidation. Early insulin augmentation resulted in a greater increase in carbohydrate oxidation in all 4 subjects compared with control (integrated Δ CHO oxidation: control vs insulin, 7730±686 vs 16268±3915 mg/225 minutes, $P<0.1$). There was a slightly (nonsignificantly) increased rate of fall in lipid oxidation after early insulin augmentation (integrated Δ lipid oxidation: control vs insulin, -2000±653 vs -3758±1410 mg/225 minutes).

Fig 5.4 Mean (±SEM) increments in energy expenditure after the mixed meal in four subjects with NIDDM in the control (■) and early insulin augmentation studies (□).

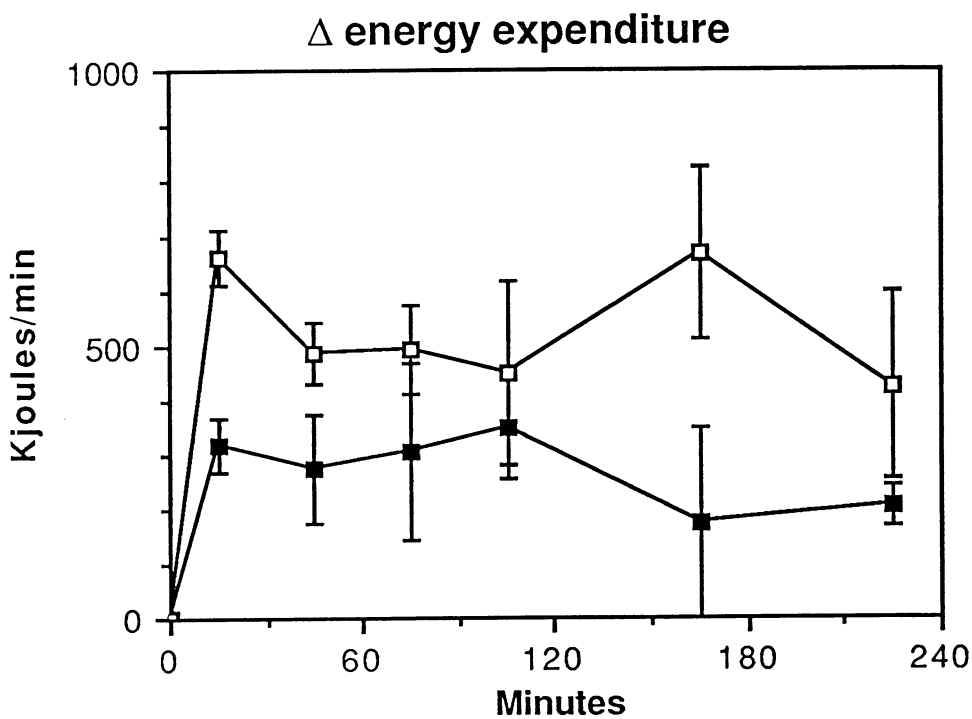
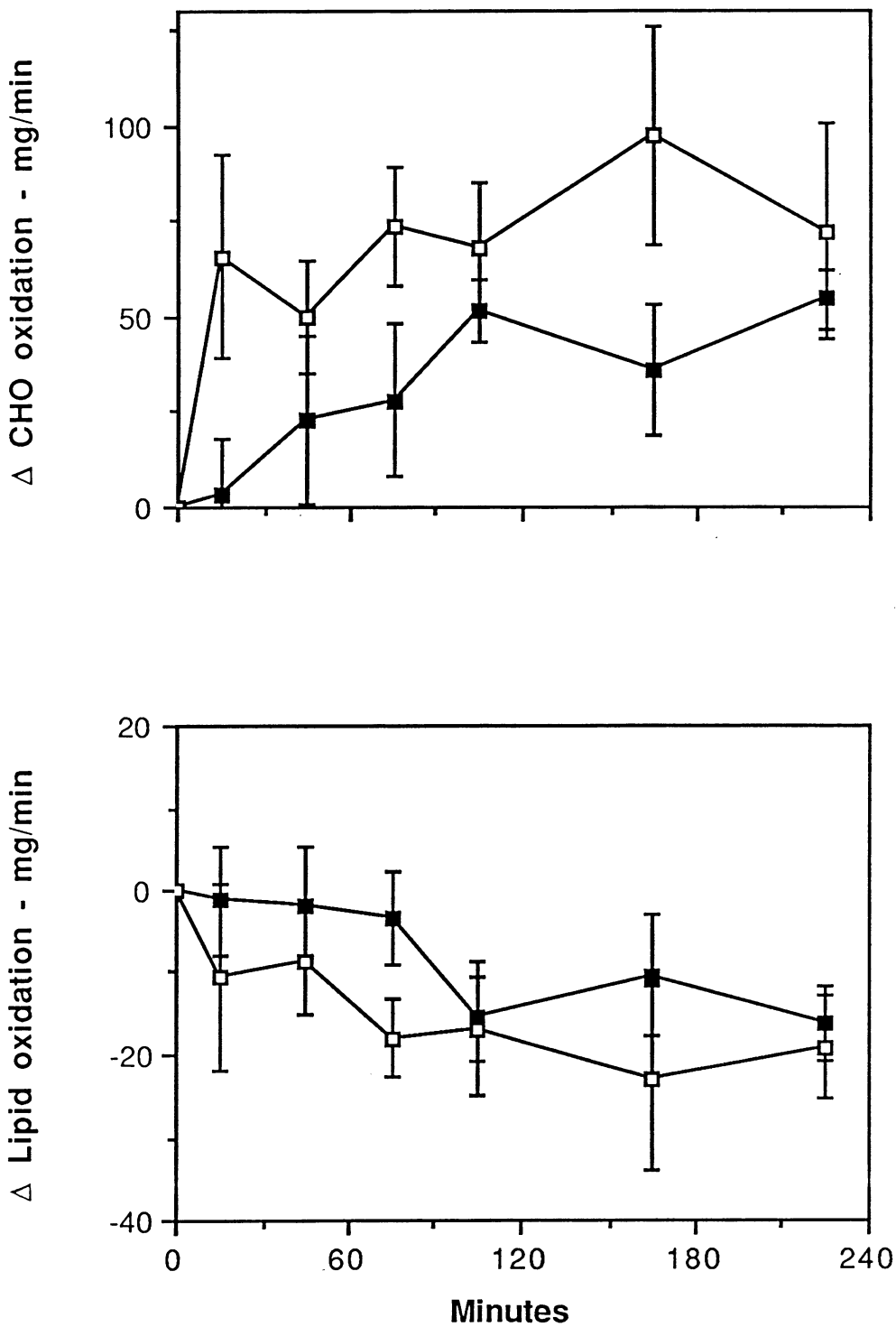


Fig 5.5 Mean(±SEM) incremental rates of glucose oxidation (top panel) and lipid oxidation following the mixed meal in four subjects with NIDDM in the control (■) and early insulin augmentation studies (□).



5.4 Discussion

In view of the small number of subjects studied, this study must be regarded as a pilot study and any possible conclusions are preliminary and depend on verification by further experiments. The major conclusion of this pilot study is that the dual isotope technique appears to be sufficiently sensitive to determine the mechanism for the improvement in prandial carbohydrate tolerance following augmentation of the early deficiency in insulin secretion.

The effect of the early insulin profile on the glycaemic response to the (predominantly glucose) meal was less sustained than was seen with the mixed meal studies in chapter 4. The glycaemic response to the meal was greater and more prolonged than with the mixed meal and therefore the insulin infusion may have undercorrected and not achieved a "normal" early insulin response. Alternatively the infusion may have produced a peak too early for the more prolonged hyperglycaemic response.

The studies suggest that the improvement in glucose tolerance after early insulin augmentation was due to an effect at the liver rather than at the periphery. After insulin augmentation, there was an earlier suppression of endogenous hepatic glucose production in all three subjects compared with the control condition. There was also a slight slowing of glucose entry from the gut in 2 of the subjects. Importantly, there was no suggestion of an alteration in peripheral glucose uptake following early insulin augmentation compared with the control studies, although the unchanged glucose disposal in the presence of lower blood glucose concentrations could be interpreted as relatively enhanced glucose uptake (clearance).

Thus, the preliminary data suggests that the major effect of the initial rise in insulin concentrations after a meal is to rapidly shut off hepatic glucose production. This minimises the prandial rise in blood glucose concentrations consequent on the entry of glucose from the gut. In contrast, the initial rise in insulin levels does not appear to have an important influence on the prandial increase in peripheral glucose disposal.

Interestingly, there was a reduction in the estimate of recycling of labelled carbon in the Cori cycle after early insulin augmentation. Recycling appears to be quantitatively quite small and the reduction after insulin does not appear to be of sufficient magnitude to explain the improvement in glucose tolerance. It should be stressed that only recycling of the labelled oral glucose and not total gluconeogenesis is measured by this technique and the results are qualitative only.

These preliminary studies also suggest that augmentation of the deficiency in early prandial insulin secretion caused an increase in the thermic effect of the meal. This suggests that the reduction in prandial energy expenditure documented in subjects with NIDDM is related to the deficiency in early prandial insulin secretion. An improvement in meal related thermogenesis could occur either because of an improvement in overall glucose disposal or due to the transient elevation in insulin levels *per se*.

Finally, calculation of substrate oxidation after correction for protein oxidation suggested that augmentation of the early phase of insulin secretion improved (at least partially) the blunted increase in prandial glucose oxidation which occurs in NIDDM and may also accelerate the suppression of lipid oxidation. These preliminary results are consistent with other studies in subjects with

NIDDM which indicate that improved glucose control is accompanied by improved carbohydrate oxidation (Boden et al., 1983). However these results implicate the deficiency in early prandial insulin secretion in the causation of abnormalities of glucose oxidation.

As stated above, further studies in diabetic individuals and in nondiabetic control subjects are required to verify the tentative conclusions in this chapter. However, the data in this chapter together with the findings in chapter four suggest that the early phase of insulin secretion influences a number of prandial metabolic processes. In NIDDM, the deficiency in early prandial insulin secretion appears to lead to impaired energy expenditure, thereby (possibly) contributing to excessive weight gain, in addition to accentuating prandial hyperglycaemia. Thus the loss of early prandial insulin secretion in NIDDM appears to be particularly important in contributing to abnormal prandial metabolism in NIDDM.

CHAPTER SIX

Sympathetic nervous system and glucoregulation

6.1 Introduction

The cause of basal hyperglycaemia in noninsulin dependent diabetes mellitus (NIDDM) is poorly understood. Abnormalities of insulin action and/or insulin secretion have usually been considered important (Ward et al., 1984; Olefsky et al., 1982; DeFronzo and Ferranini, 1982; Efendic et al., 1984), however recent evidence suggests that a third major metabolic defect, increased basal hepatic glucose production, is also an important cause (Olefsky, 1985).

Estimations of hepatic glucose production, using either tracer methodology or hepatic catheterisation techniques, have consistently shown that hyperglycaemic subjects with NIDDM have inappropriately elevated rates of hepatic glucose production (Bowen and Moorehouse, 1973; Kolterman et al., 1981). Furthermore, a strong correlation between fasting blood glucose levels and basal rates of endogenous hepatic glucose production in NIDDM has repeatedly been found (Revers et al., 1984; Bogardus et al., 1984; Best et al., 1982). Therapeutic correction of hyperglycaemia is accompanied by a reduction in hepatic glucose production irrespective of the treatment modality (Henry et al., 1986; Simonson et al., 1984; Scarlett et al., 1982) and in one study which employed weight reduction, there was a correlation between the improvement in fasting glucose levels and the reduction in hepatic glucose production (Henry et al., 1985). These data indicate that the rate of entry of glucose from the liver into the circulation closely regulates the level of glycaemia in subjects with NIDDM and that excess liver glucose production is a major determinant of hyperglycaemia.

The cause of increased liver glucose production in NIDDM is not known. Insulin deficiency is present in subjects with NIDDM when the prevailing levels of

glycaemia are taken into account although absolute levels are often normal (Ward et al., 1984) and thus inadequate insulinisation of the liver could be important. In addition, hepatic insulin resistance may play a role by diminishing the normal inhibitory effect of insulin on hepatic glucose production (DeFronzo et al., 1982).

The importance of basal insulin levels in the regulation of basal hepatic glucose production in NIDDM has been questioned (Olefsky, 1985). Baron et al (1985) estimated the proportion of basal glucose uptake mediated by basal insulin secretion by studying subjects during hypoinsulinaemia utilising somatostatin infusions. They demonstrated that insulin-mediated glucose uptake is responsible for approximately 30% of whole-body basal glucose uptake in both nondiabetics and subjects with NIDDM. Thus noninsulin-mediated glucose uptake appears to be responsible for the major proportion of glucose uptake in the basal state. From this data, Olefsky (1985) suggested that changes in insulin secretion and sensitivity are not likely to be the major cause of basal hyperglycaemia in NIDDM. However, subjects with NIDDM are less able to augment insulin-mediated glucose uptake, due to insulin resistance and insulin deficiency, and are less able to counteract an increase in hepatic glucose production. Thus an interaction among the three major defects commonly found in NIDDM may be important in the pathogenesis of hyperglycaemia.

Glucose production by the liver is potentially influenced by many hormonal, neuroendocrine and neural factors. In addition, the supply of gluconeogenic substrates to the liver (Wahren et al., 1972) and glucose itself have regulatory roles (Liljenquist et al., 1979). In NIDDM, excess hepatic glucose production could be due to an hypersensitivity of the liver or alternatively there could be excessive stimulation of the liver by one or more of these factors. In this regard,

hyperglucagonaemia has been considered important in the pathogenesis of hyperglycaemia in NIDDM (Baron et al., 1987).

Several areas of research indicate that the sympathetic nervous system can increase liver glucose production (Shimazu, 1981; Lautt, 1983; Smythe et al., 1984). Shimazu and coworkers demonstrated the importance of the hypothalamus in the regulation of hepatic enzyme systems important in glucoregulation (Shimazu and Fukuda, 1965; Shimazu and Amakawa, 1968). Other workers extended these observations by showing that stimulation of the hepatic sympathetic nerves causes hyperglycaemia in a number of animal species (Edwards, 1971; 1972). Although there may be indirect actions of sympathetic activation (changes in insulin and glucagon levels), there is evidence for a direct hepatic effect in that hyperglycaemia results after stimulation of the hepatic nerves of animals with denervated pancreases (Garceau et al., 1984) and following stimulation of nerves to isolated liver preparations (Lautt, 1983). In support of a direct effect in man, human hepatocytes are innervated by sympathetic nerves and hepatic nerve stimulation has been shown to increase plasma glucose levels in humans (Nobin et al., 1977).

These studies did not delineate a physiological role for the sympathetic nervous system in glucoregulation nor did they determine the relative importance of direct neural versus indirect hormonal regulation of liver glucose production. Shimazu suggested that glucose homeostasis may be under dual control. Direct neural innervation of the liver via sympathetic innervation is likely to be responsible for rapid and fine regulation of metabolic changes (e.g. during acute stress) whereas hormonal regulation may be responsible for prolongation or consolidation of metabolic changes (Shimazu, 1981). The hepatic

sympathetic nerves have been shown to modulate the actions of insulin and glucagon on the liver (Beckh et al., 1982) and the reverse may also be true.

Recently, a number of studies have extended the concept of CNS regulation of blood glucose and indicate that the hypothalamus and the hepatic sympathetic innervation may have a physiological role in normal glucoregulation.

Studies of central neuroglycopaenia following 2-deoxyglucose administration in both humans and rats have demonstrated that the peripheral hyperglycaemic response is mediated, at least in part, by direct sympathetic activation of the liver. The hyperglycaemic response to 2-deoxyglucose is attenuated in spinal man (Brodows et al., 1975) and following guanethidine administration (causing sympathetic blockade) in rats (Storlien et al., 1985). In the latter study, it was demonstrated that adrenomedullary adrenaline release modulated the response by inhibiting insulin secretion but was not the major cause of the hyperglycaemic response. The hyperglycaemic response to 2-deoxyglucose was accompanied by an increase in hypothalamic noradrenergic neurotransmitter activity which has previously been shown to correlate with peripheral glucose concentrations (Smythe et al., 1984). Following guanethidine administration, hypothalamic noradrenergic activity increased further, suggesting that activation of central noradrenergic nerves may drive hepatic glucose production via the sympathetic nerves (Storlien et al., 1985).

Moderate physical exercise is accompanied by a rapid increase in hepatic glucose output which balances the considerable increase in glucose uptake by exercising muscles. In humans this process is extremely precise and rapid and only minor changes in peripheral blood glucose levels are seen (Vranic and Berger, 1979). Hepatic glucose output during exercise increases before

changes in peripheral levels of glucoregulatory hormones occur, suggesting that the regulatory process is neural rather than hormonal (Chisholm et al., 1982; Richter et al., 1981).

Recent studies strongly support the view that the sympathetic nervous system directly promotes the increase in hepatic glucose output in exercising humans. Hoelzer et al (1986) demonstrated that when insulin and glucagon levels were prevented from changing during exercise (using somatostatin infusion combined with insulin and glucagon replacement), the hepatic response to exercise remained largely intact. However, hepatic glucose production and plasma glucose levels fell when alpha and ß-adrenergic receptor blockade were added. These data implicated either the sympathetic innervation or adrenomedullary adrenaline secretion in preventing hypoglycaemia during exercise. They replicated the study in adrenalectomised humans, found that glucoregulation was intact during exercise and concluded that it was the sympathetic nervous system that was critical in promoting the increase in hepatic glucose production during exercise. Thus, the sympathetic nervous system (and its higher regulatory centres) has an important role in regulating hepatic glucose production during exercise.

There is also evidence against the sympathetic nervous system having a role in glucoregulation. Glucose counterregulation following insulin induced hypoglycaemia is primarily dependent on the release of glucagon and adrenaline (Cryer and Gerich, 1985). Studies in both humans and rats indicate that the sympathetic nervous system does not appear to have a major role in this situation (Gerich et al., 1979; Rizza et al., 1979; Mikines et al., 1985). Thus the situation with insulin hypoglycaemia appears to be different from that seen following 2-deoxyglucose administration where direct sympathetic activation of

the liver is an important component of the counterregulatory response (Brodows et al., 1975; Storlien et al., 1985).

The difference between the counterregulatory responses induced by insulin and 2-deoxyglucose administration could be due to the different prevailing circulating levels of insulin. In normal physiology, low blood glucose levels always coincide with low insulin levels (e.g. during fasting) and the combination of hypoglycaemia and hyperinsulinaemia is never naturally experienced. The administration of 2-deoxyglucose, whilst not 'natural', produces a situation not dissimilar from normal with low cellular glucose concentrations and low circulating insulin levels. Insulin can act as a central signal within the brain (Szabo and Szabo, 1972) and detection of insulin within the CNS results in an inhibition of liver glucose production (Storlien et al., 1975; Iguchi et al., 1981). Thus, in the case of insulin hypoglycaemia, a sympathetic drive to the liver could be suppressed by the detection of high circulating insulin levels by the brain with counterregulation becoming dependent on (normally redundant) hormonal mechanisms.

In NIDDM, the cause of excess hepatic glucose production is unknown and few studies have as yet addressed this issue. However, there is some support for a potential role for the sympathetic nervous system in causing or at least contributing to the increased hepatic glucose output.

Several lines of evidence suggest that NIDDM may be characterised by a state of chronically increased basal sympathetic tone. Firstly, blockade of alpha-adrenergic receptors with phentolamine results in a greater increase in basal and stimulated insulin levels in subjects with NIDDM compared with nondiabetic subjects (Robertson et al., 1976; Broadstone et al., 1987). It has

been suggested that the effect of phentolamine on insulin secretion in NIDDM may be due to preferential blockade of presynaptic alpha$_2$-adrenergic receptors rather than postsynaptic receptors (Broadstone et al., 1987). Some studies have reported elevated plasma catecholamines in NIDDM (Robertson et al., 1976; Halter and Porte, 1977) and elevated noradrenaline levels in some non-ketotic diabetics (Cryer et al., 1978). However, increased noradrenaline levels have not been consistently found and such elevations could be related to poor metabolic control with alterations in fluid and electrolyte status (Christensen, 1979).

Secondly, treatment with guanethidine, a sympathetic blocking drug, has been reported to improve glucose tolerance in subjects with NIDDM (Gupta, 1969), thyrotoxicosis (Woeber et al., 1966) and nondiabetic subjects (Kansal et al., 1971). In addition, a new therapeutic agent which has prominent alpha$_2$-adrenergic receptor blocking activity has been found to be effective in subjects with NIDDM although whether its mechanism of action on hyperglycaemia is related to its adrenergic blocking activity is not currently known (Kashiwagi et al., 1986).

Finally, it has been demonstrated that during physical exercise, hepatic glucose output is regulated abnormally in NIDDM. Blood glucose levels rise in some subjects and fall in others despite prevailing hyperglycaemia and the presence of normal peripheral insulin and glucagon responses (Minuk et al., 1981; Jenkins et al., 1988). As hepatic glucose production during exercise is regulated by the sympathetic nerves (Hoelzer et al., 1986), an abnormality of sympathetic activation could explain the abnormal response to exercise in subjects with NIDDM.

Rather than having increased sympathetic nervous activity, subjects with NIDDM may have increased sensitivity to normal sympathetic tone. It has long been considered that psychological stress causes hyperglycaemia in subjects with diabetes whereas nondiabetic subjects are not prone to this (Surwit and Feinglos, 1984). Implicit in this assertion is the hypothesis that subjects with diabetes are abnormally susceptible to the autonomic and hormonal responses which accompany psychological stress (Surwit and Feinglos, 1988).

Psychological stress may cause metabolic changes similar to severe physical stress such as is seen with surgery, trauma or myocardial infarction. Stress induced hyperglycaemia following severe physical stress occurs secondary to increased hepatic glucose production and reduced peripheral glucose uptake (Porte and Woods, 1983). These metabolic changes are related to suppression of insulin secretion, elevated levels of stress hormones and activation of the sympathetic nervous system. Psychological stress can cause an increase in catecholamines (Levi, 1972), cortisol (Bliss et al., 1956) and possibly also growth hormone and glucagon levels (Miyabo et al., 1976).

Subjects with insulin dependent diabetes have been demonstrated to have an exaggerated glycaemic response to the administration of adrenaline, cortisol and glucagon (Shamoon et al., 1980) and are susceptible to severe physical stress (Porte and Woods, 1983). The abnormal glycaemic responses to adrenaline are due to the inability of these subjects to increase insulin levels (Berk et al., 1985). NIDDM is characterised by beta cell abnormalities and theoretically subjects with NIDDM might also be susceptible to stress because of a reduced ability to secrete insulin (Porte and Woods, 1983).

NIDDM is characterised by several neuroendocrine abnormalities (abnormal glucagon, growth hormone and possibly cortisol regulation) which resemble the hormonal changes seen in stress states. It has been suggested that these neuroendocrine phenomena may be due to a generalised glucoreceptor abnormality similar to that seen in the islet beta cells (Ward et al., 1984). According to this theory, a central defect in glucose detection could cause the glucose sensitive areas of the brain (or other organs) to read the prevailing glucose levels as being too low. This secondarily leads to neuroendocrine activation (i.e. the release of counterregulatory hormones) which increase blood glucose levels. Thus the hyperglycaemia of NIDDM may be analogous to stress hyperglycaemia (Porte and Woods, 1983).

Relatively few studies have examined the relationship between psychological stress and hyperglycaemia in NIDDM. The early literature on the effects of psychological stress rarely differentiated between IDDM or NIDDM and small groups of subjects or individual cases have usually been reported (Lustman et al., 1981).

Several studies have been carried out aiming to induce psychological stress in insulin dependent diabetic subjects in the laboratory. These have failed to demonstrate that psychological stress causes an increase in blood glucose levels. A recently reported study found that significant psychological stress, as judged by cardiovascular responses, was not accompanied by altered glycaemia in either well-controlled or hyperglycaemic subjects with IDDM (Kemmer et al.,1986). This type of study has not been attempted in NIDDM.

In support of a relationship between psychological stress and hyperglycaemia in NIDDM, one study demonstrated that an improvement in glucose tolerance

occurred following one week of in-patient relaxation therapy (Surwit and Feinglos, 1983). The improvement appeared to be independent of effects on insulin sensitivity or secretion and was considered to be due to effects on the liver. Interestingly, the same researchers have recently reported their findings in a group with IDDM using an identical protocol. In contrast to the NIDDM study, relaxation conferred no benefit on glucose tolerance (Feinglos et al., 1987). Thus the available evidence suggests that subjects with NIDDM may be susceptible to the effects of psychological stress, whereas there is little evidence for such a phenomenon in IDDM.

In summary, there is sufficient evidence to postulate that abnormal liver glucose production in NIDDM could be due to abnormal regulation by the sympathetic nervous system. Excess liver glucose output could be due to an increased sympathetic neural drive to the liver, or alternatively, subjects with NIDDM could be hypersensitive to the effects of sympathetic stimulation.

6.2 Aims of this chapter

The aim of these studies was to test whether hepatic glucose production and plasma glucose levels could be influenced by stimulation of the sympathetic nervous system and whether subjects with NIDDM had altered sensitivity to sympathetic stimulation.

The effects of psychological stress were examined to see whether NIDDM was characterised by an increased sensitivity to psychogenic central neural stimulation.

6.3 Sympathetic nervous stimulation

The specific aim of the following studies was to determine whether: (i) psychological stress causes hyperglycaemia in subjects with NIDDM, (ii) activation of the sympathetic nervous system promotes hepatic glucose production and causes blood glucose levels to rise, and (iii) there is altered sensitivity to a noradrenergic stimulus in NIDDM. The studies included measurements of glucose turnover and the responses of nondiabetic and diabetic subjects (NIDDM) were compared. Sympathetic activation was induced pharmacologically by oral administration of the sympathomimetic agent tyramine. Noradrenaline was administered intravenously at a dose expected to increase noradrenaline levels into the high physiological range in order to compare the sensitivity of the nondiabetic and diabetic subjects to hormonal noradrenergic stimulation. Psychological stress was induced in the laboratory using competitive computer games. Control studies were performed for comparison.

Tyramine is a naturally occurring sympathomimetic amine which is present in minute quantities in several brain regions (Tallman et al., 1976). Tyramine acts by releasing noradrenaline from sympathetic nerves and also inhibits neuronal uptake of noradrenaline (Rapoport et al., 1981). Tyramine does not alter adrenaline secretion (Scriven et al., 1983) and therefore represents a relatively pure pharmacological sympathetic neuronal stimulus. Tyramine was administered orally in these studies and it was accepted that there may be some difficulty, because of variation in absorption, in accurate determination of any differential sensitivity to tyramine between the two groups of subjects.

Noradrenaline infusion has been shown to cause metabolic as well as haemodynamic changes when infused to achieve high physiological levels (Silverberg et al., 1978). In the present studies, noradrenaline was infused at a dose likely to cause a mild increase in glycaemia in nondiabetic subjects (Schade and Eaton, 1978) to determine whether there was any difference in sensitivity to the adrenergic stimulus.

6.4 Subjects and methods

Eight male subjects with NIDDM and six male nondiabetic subjects participated in these studies. Criteria for inclusion were: absence of hypertension or antihypertensive therapy, no clinical evidence of autonomic neuropathy (normal postural blood pressure responses and absence of symptoms) and no contraindication to receiving adrenergic agents (e.g. absence of ischaemic heart disease). All subjects had normal serum creatinine levels and none had proteinuria. The diabetic subjects were tested for heart rate interval variation at rest, which has been shown to be a sensitive test of parasympathetic function (Chipps et al., 1981).

Table 6.1 includes demographic and clinical data for the subjects. The two groups were well matched for age, degree of obesity (body mass index, kg/m^2) and blood pressure; all except one diabetic subject were within 15% of ideal body weight. Subjects 3 to 7 (table 6.1) took sulphonylureas, the rest were treated with diet alone. Two diabetic subjects had clinical evidence of sensory peripheral neuropathy (subjects 1 and 4) and another two (subjects 3 and 6) had abnormally low heart rate interval variation (i.e. more than one standard deviation less than age matched controls, Chipps et al., 1981) indicating the presence of abnormal cardiac parasympathetic function. One of the nondiabetic subjects (subject 1, table 6.1) who was taking thyroxine for

hypothyroidism, had been biochemically and clinically euthyroid for 3 years before the studies.

All studies were performed in the morning after an overnight fast. Oral hypoglycaemic agents were ceased 48 hours before each study. Studies were performed at intervals of at least 2 weeks. All except one of the diabetic subjects participated in all four studies (control, stress, tyramine and noradrenaline) which were performed in random order. One diabetic subject suffered a vaso-vagal attack following his first study (psychological stress) and was willing only to return for a control study (subject 8).

The subjects were seated comfortably at a desk throughout each study. Prior to each study, a catheter was inserted into an antecubital vein for [3-^{3}H]glucose infusion for glucose turnover measurements and a second catheter was inserted into an ipsilateral dorsal hand vein for arterialised venous blood sampling. An additional venous catheter was inserted in the contralateral arm for hormone infusion before the noradrenaline infusion studies. A 90 minute period was allowed for equilibration of the [3-^{3}H]glucose infusion prior to any intervention. Blood pressure (standard mercury sphygmomanometer) and pulse rate (continuous ECG monitor) measurements were obtained throughout each study. Blood was drawn for plasma glucose and [3-^{3}H]glucose specific activity at 5-10 minute intervals and for serum insulin, C peptide, FFA, adrenaline, noradrenaline, noradrenaline metabolites, cortisol, ACTH, growth hormone, prolactin and plasma glucagon at 15-30 minute intervals throughout the studies.

Table 6.1 Clinical details of the subjects who participated in the studies.
* p<0.01 compared with diabetic subjects.

Diabetic subjects

Subject	Age yrs	Wt kg	BMI kg/m2	Duration yrs	Sys BP mmHg	Dias BP mmHg	Fasting glucose mmol/l
1	66	84.5	29.6	3	140	79	7.1
2	54	85	26	1	103	66	6.3
3	51	102	31.8	3	138	99	15.2
4	66	75	24.8	11	134	79	11.2
5	58	78	27	0.5	132	96	7.9
6	47	69	23.9	18	101	70	10.2
7	52	83	27.9	2	109	77	9.3
8	58	76	26.3	2	100	68	7.2
mean	56.5	81.6	27.2	5.1	120	79	9.3
±SEM	±2.4	±3.5	±0.9	±2.2	±6	±4	±1.0

Nondiabetic subjects

1	61	65	23.9	-	118	73	5.9
2	45	70	23	-	124	79	5.7
3	57	68	23	-	115	78	5.3
4	41	64	23.5	-	104	75	4.7
5	61	87	26.3	-	139	90	5.5
6	38	82	25	-	114	71	5.1
mean	50.5	72.7	24.1	-	119	78	5.4*
±SEM	±4.2	±3.9	±0.5		±5	±2	±0.2

Psychological stress: The subjects were required to perform challenging psychological tasks by playing competitive computer games. This has previously been shown to induce moderate degrees of psychological tension and alterations in blood pressure and pulse in nondiabetic individuals (Carroll et al., 1985). Only one (nondiabetic) subject had previously played computer games and only 3 subjects (one diabetic and 2 nondiabetic) had experience with computer terminals.

Before the study, the subjects completed both sections of the State-Trait Anxiety Inventory (Spielberger et al., 1983) which has been designed to measure both chronic (trait) and acute (state) anxiety levels. The state section (S-anxiety scale) has been used extensively to assess the level of anxiety induced by stressful experimental procedures and may be used serially (Spielberger et al., 1983). This was completed again at the end of the study to allow an estimation of the subjects' own perception of the experimental stress.

The subjects were seated in front of a computer terminal (Apple IIe, Apple Computers). They were informed that they would be required to perform a series of unspecified psychological tests, the results of which were to be compared with other previously studied subjects. After the 90 minute equilibration period, they were then asked to play a series (2 to 3) of commercially available computer games for a period of 60 minutes. Each game was briefly demonstrated to them and they were given a (difficult to achieve) target score. They were informed that the given target score was the average obtained by previously studied subjects and had to be reached within 20 minutes. After 20 minutes, they were given a new task, which could start sooner when they had reached the target score of the previous game. Their

performance was critically evaluated at intervals throughout the 60 minutes in an attempt to encourage greater effort.

Tyramine administration: Following the 90 minute equilibration period (of tritiated glucose infusion), the subjects swallowed 800 mg tyramine hydrochloride in capsules (200 mg per capsule). Blood pressure, pulse and blood samples were obtained for a further 90 minutes.

Noradrenaline administration: Noradrenaline hydrochloride solution was dissolved in 500 ml 0.9% saline and 250 mg ascorbic acid to give a concentration of 8 μg/ml. The infusion was prepared on the morning of the study and protected from light before administration. Noradrenaline solution was infused at a rate of 60 ng/(min.kg) for 60 minutes after the initial 90 minute equilibration period and blood sampling, pulse and blood pressure measurements continued for a further 90 minutes.

Catecholamine and tyramine measurments: Plasma catecholamines were measured using two methods (see chapter 2.3). Plasma levels of noradrenaline and adrenaline were measured using a radioenzymatic assay. In addition, plasma noradrenaline was measured at baseline and at 60 minutes after the commencement of each study condition using a highly specific and precise gas chromatograph/mass spectrometry (GC/MS) assay. In these samples, plasma free levels of the noradrenaline metabolite, dihydroxyphenylethylene glycol (DHPG) were also determined by GC/MS. Circulating DHPG levels may provide a better index of sympathetic nervous activity than noradrenaline levels in some circumstances (Goldstein et al., 1988).

Plasma levels of free methoxyhydroxyphenylethylene glycol (MHPG), another metabolite of noradrenaline, were also determined in these studies using GC/MS methodology. MHPG appears to be the major noradrenaline metabolite released from the brain into the blood (Kopin, 1985) and significant correlations between brain, CSF and plasma concentrations of MHPG have been observed in several species including humans (Crawley et al., 1978; Jimerson et al., 1981) It has been suggested that plasma levels of MHPG may reflect central neuronal noradrenergic activation (Maas, 1984; Elsworth et al., 1983). This is controversial, as MHPG diffuses freely into the CSF, which may explain the observed relationships (Kopin, 1985). Recently however, plasma MHPG levels have been shown to correlate directly with hypothalamic noradrenergic turnover in the rat (Grunstein et al., 1986) supporting the use of MHPG measurements as an index of central noradrenergic activation at least in that species.

In the tyramine administration studies, tyramine levels were estimated using GC/MS in the MHPG samples by reference to the internal deuterated MHPG standard. As no internal standard for tyramine was incorporated in these assays, the tyramine levels obtained were expressed in arbitrary units/ml. These estimations at best, provide a semi-quantitative estimate of the degree and timing of tyramine absorption after oral administration of the agent.

Data handling and statistics: The mean blood pressure was calculated from the systolic and diastolic pressures (1/3 difference + diastolic pressure). The data was evaluated as absolute responses and incremental responses above baseline. Baseline hormone and FFA values are the mean of two samples taken at -30 and 0 minutes before each study condition. Baseline plasma glucose and glucose turnover measurements are the mean of two samples taken at -10 and 0 minutes before the study condition. Total incremental

responses were calculated (area under the curve) using a computer program. Where any parameter altered during the control studies (e.g. plasma glucose levels fell in the diabetic subjects), total and incremental responses were subtracted from the control curve. Total incremental responses were compared between the diabetic and nondiabetic groups by unpaired Student's t test. Paired t tests were used where appropriate. Single and multiple linear regression analysis used the method of least squares.

6.5 Results

Baseline values: Mean basal values (mean of basal estimations for all study conditions, each basal value the mean of at least two pre-study estimations) for heart rate, arterial plasma glucose, glucose turnover measurements, FFA and hormone estimations are displayed in table 6.2. There were significantly increased levels of plasma glucose ($p<0.01$), insulin ($p<0.02$) and C peptide ($p<0.02$) and significantly increased rates of hepatic glucose output (R_a, $p<0.005$) and peripheral glucose disposal (R_d, $p<0.005$) in the diabetic compared with the nondiabetic subjects. Glucose clearance was non-significantly greater in the nondiabetic subjects ($p<0.1$). There was no difference in basal levels of FFA, noradrenaline, MHPG, DHPG, adrenaline, glucagon, cortisol, ACTH, prolactin, growth hormone, heart rate or blood pressure (table 6.1) between the two groups.

There was no difference in basal values for any measured parameter before each of the four study conditions in either group (ANOVA, table 6.3).

Table 6.2 Mean baseline data for the diabetic and nondiabetic subjects. These values are the mean of basal values before each study condition (i.e. 2 or 4 studies). * indicates significant difference between groups (see text).

	Plasma glucose mmol/l	R_a μmol/ kg.min	R_d μmol/ kg.min	Glucose clearance l/min	Insulin mU/l	C peptide μg/ml
NIDDM	9.3±1.0*	22.7±1.4*	23.7±1.4*	0.20±0.01	11.9±1.9*	2.7±0.4*
Nondiabetic	5.4±0.2	16.0±0.9	16.3±0.9	0.23±0.01	4.4±1.5	1.3±0.2

	FFA mmol/l	Glucagon ng/ml	Cortisol nmol/l	ACTH ng/l	GH mU/l	Prolactin mIU/l
NIDDM	0.66±0.08	33±5	165±27	9.6±1.9	0.9±0.2	121±20
Nondiabetic	0.67±0.06	41±5	192±20	12.5±2.9	3.3±1.3	122±16

	Pulse	Adren nmol/l	Noradren (radioenz) nmol/l	Noradren (GC/MS) nmol/l	MHPG nmol/l	DHPG nmol/l
NIDDM	70±4	0.4±0.1	1.2±0.2	2.5±0.2	21.1±2.4	6.5±0.8
Nondiabetic	67±5	0.4±0.1	1.3±0.3	2.5±0.3	22.4±1.4	6.8±0.4

Table 6.3 Mean baseline parameters before each study condition for the diabetic and nondiabetic subjects, units as in table 6.2.

Parameters	NIDDM subjects				Nondiabetic subjects			
	Control	Stress	Noradren	Tyramine	Control	Stress	Noradren	Tyramine
glucose	9.8±1.2	9.0±0.8	9.8±1.4	9.1±1.1	5.3±0.2	5.3±0.2	5.4±0.1	5.5±0.2
R_a	23.4±2.4	24.4±4.0	22.0±2.9	22.1±3.0	16.3±1.6	15.9±1.9	16.7±3.0	15.1±2.6
R_d	24.1±2.4	25.0±4.1	21.7±2.7	22.6±2.9	16.1±1.6	16.6±2.0	17.1±3.1	15.1±2.0
insulin	11.0±0.4	14.7±3.0	10.5±1.9	12.0±2.5	3.7±1.0	4.8±0.4	4.4±1.5	4.3±1.4
C peptide	2.8±0.6	2.7±0.4	2.2±0.3	2.2±0.3	1.2±0.2	1.3±0.2	1.6±0.4	1.1±0.2
FFA	0.64±.11	0.67±.09	0.73±.08	0.67±.10	0.60±.08	0.65±.11	0.68±.11	0.72±.10
glucagon	48±13	26±7	29±6	28±5	40±11	50±19	25±10	47±16
NA(GC/MS)	2.0±0.3	2.2±0.5	2.7±0.4	2.4±0.2	2.4±0.3	2.4±0.4	2.3±0.2	2.4±0.4
NA(radio)	1.0±0.2	1.1±0.6	1.5±0.4	1.1±0.4	1.9±0.7	1.0±0.5	1.4±0.6	1.5±0.4
adrenaline	0.4±0.1	0.5±0.2	0.4±0.1	0.5±0.1	0.4±0.2	0.7±0.4	0.4±0.2	0.4±0.1
cortisol	121±24	138±32	142±15	188±33	219±48	194±32	166±32	218±31
ACTH	8.8±2.0	7.8±1.6	13.9±4.7	11.8±2.4	12.5±4.5	17.0±3.5	11.5±2.4	13.0±3.8
prolactin	118±24	109±18	139±29	117±25	116±18	121±22	123±12	127±18
GH	0.7±0.1	0.7±0.2	0.8±0.2	1.4±0.7	3.4±2.2	3.2±1.9	3.1±1.2	1.9±1.3
systolic BP	118±6	117±8	122±7	124±6	120±4	114±4	119±7	123±5
diastolic BP	78±4	78±5	81±6	82±5	77±3	73±3	79±4	80±2
pulse	71±4	68±4	71±6	68±5	67±5	68±5	67±5	65±4

6.5.1 Basal correlations

There was a significant positive correlation between fasting plasma glucose levels and basal rates of hepatic glucose production (R_a) in the diabetic subjects ($r=0.70$, $p<0.04$) and in the total subject group ($r=0.83$, $p<0.001$) but not in the nondiabetic subjects ($r=0.14$). Basal plasma glucose levels correlated with basal DHPG ($r=0.77$, $p<0.042$) but not with basal noradrenaline or MHPG levels in the diabetic subjects (figure 6.1). There were no correlations between plasma glucose and these parameters in the nondiabetic subjects. There were significant positive correlations between basal plasma glucose levels and basal insulin ($r=0.57$, $p<0.033$) and the insulin/glucagon ratio ($r=0.89$, $p<0.001$); there was also a nonsignificant negative correlation with glucagon ($r=-0.50$, $p<0.07$, figure 6.2). In addition, R_a correlated positively with the insulin/glucagon ratio ($r=0.70$, $p<0.006$) and there was a significant negative relationship between R_a and basal glucagon ($r=-0.69$, $p<0.006$). There were also significant positive correlations between basal FFA levels and basal DHPG ($r=0.72$, $p<0.005$) and MHPG levels for all the subjects combined ($r=0.63$, $p<0.02$).

Multiple regression analysis was used to examine further the relationships between basal levels of glycaemia (as the dependent variable) and insulin, glucagon and DHPG. In these analyses, the data for the diabetic and the nondiabetic subjects were entered into the regressions separately and combined. No significant relationships were seen for the nondiabetic data alone. However, the insulin/glucagon ratio ($p<0.001$) and DHPG ($p<0.006$) independently correlated with plasma glucose in the diabetic subjects ($r=0.97$) and similar correlations were seen ($p< 0.001$ and $p<0.01$ respectively) for the combined group of subjects ($r=0.94$). Similar analysis was performed

substituting MHPG or noradrenaline for DHPG in the regression. This also revealed significant independent correlations for MHPG ($p<0.044$) and the insulin/glucagon ratio ($p<0.0001$) with basal plasma glucose levels ($r=0.93$). There were no correlations with noradrenaline.

Figure 6.1 The relationship between basal DHPG and plasma glucose levels in the diabetic (closed symbols) and nondiabetic subjects (open symbols). The correlation and the fitted regression refer to the diabetic subjects only.

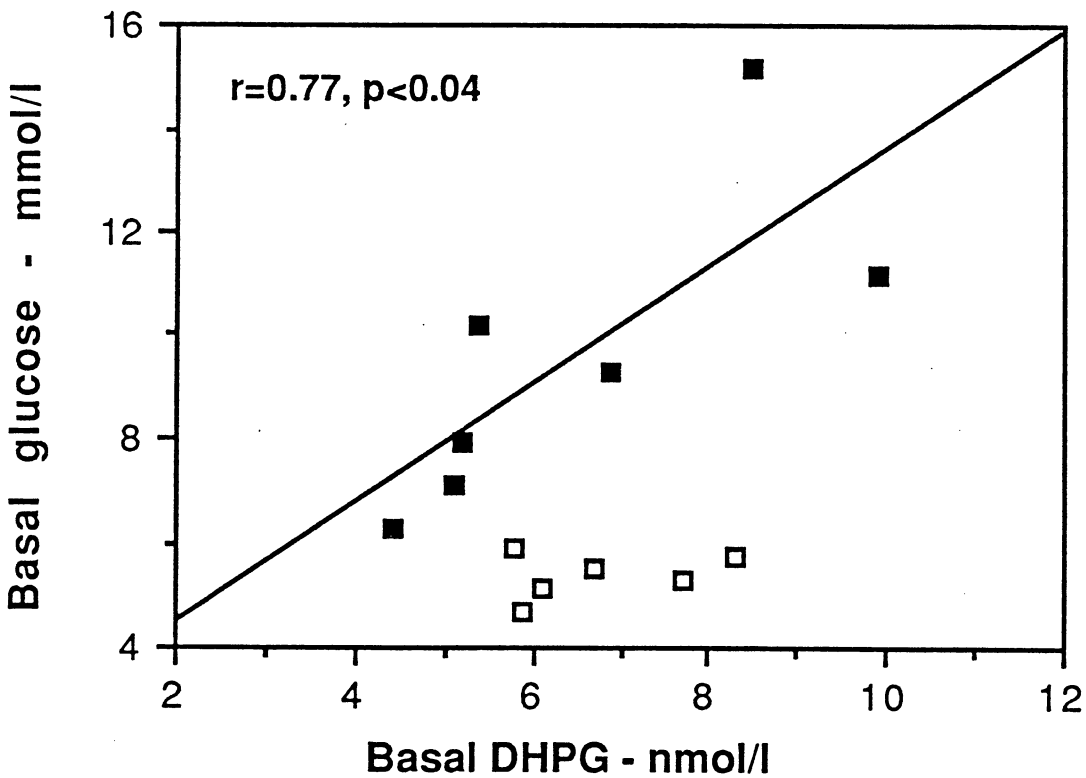
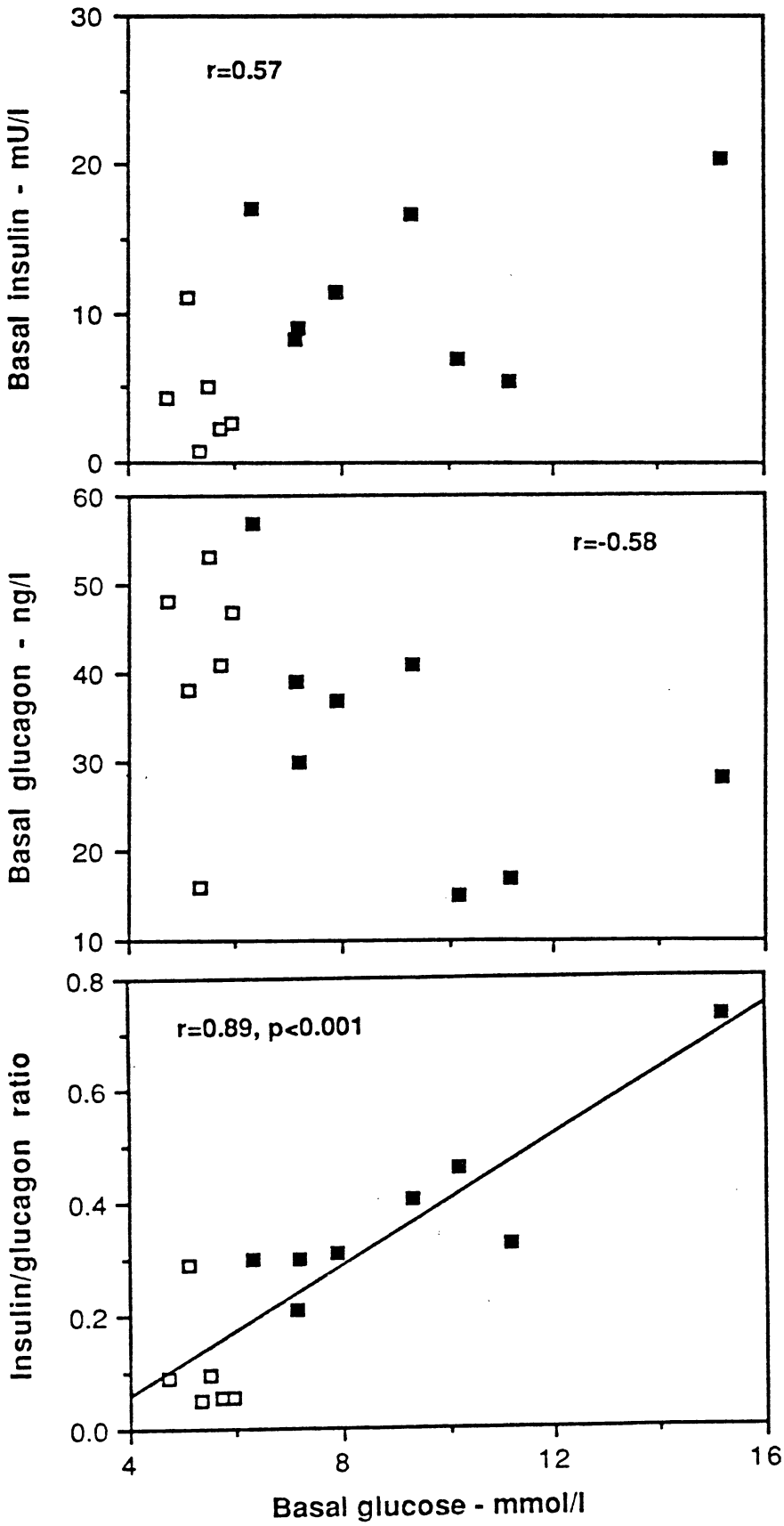


Figure 6.2 Relationships between basal plasma glucose levels and serum insulin (top panel), plasma glucagon (middle panel) and the insulin/glucagon ratio in the diabetic (closed symbols) and nondiabetic subjects (open symbols). The correlation coefficients and regression lines refer to all subjects combined.

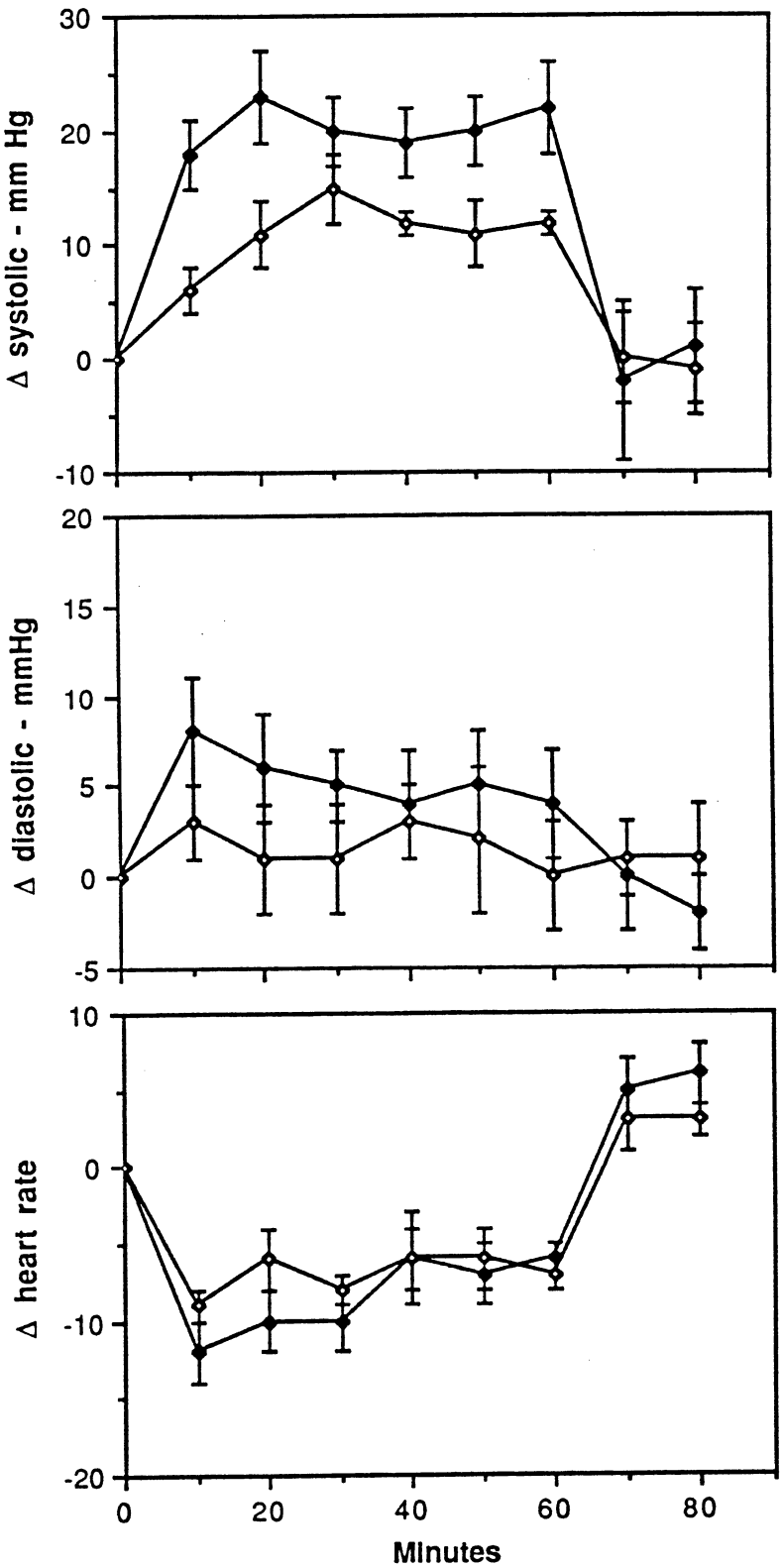


6.5.2 Noradrenaline study

Noradrenaline infusion resulted in similar increases in arterial noradrenaline levels in both groups of subjects whether measured by GC/MS or the radioenzymatic method (table 6.4). There was however, a disparity in the levels achieved by the infusion measured by the two noradrenaline assays (diabetic vs nondiabetic: final level (GC/MS assay) 14.1±1.5 vs 12.9±1.0 nmol/l; mean steady state level (radioenzymatic assay) 5.6±1.6 vs 4.2±1.9 nmol/l). Adrenaline levels did not alter during noradrenaline infusion.

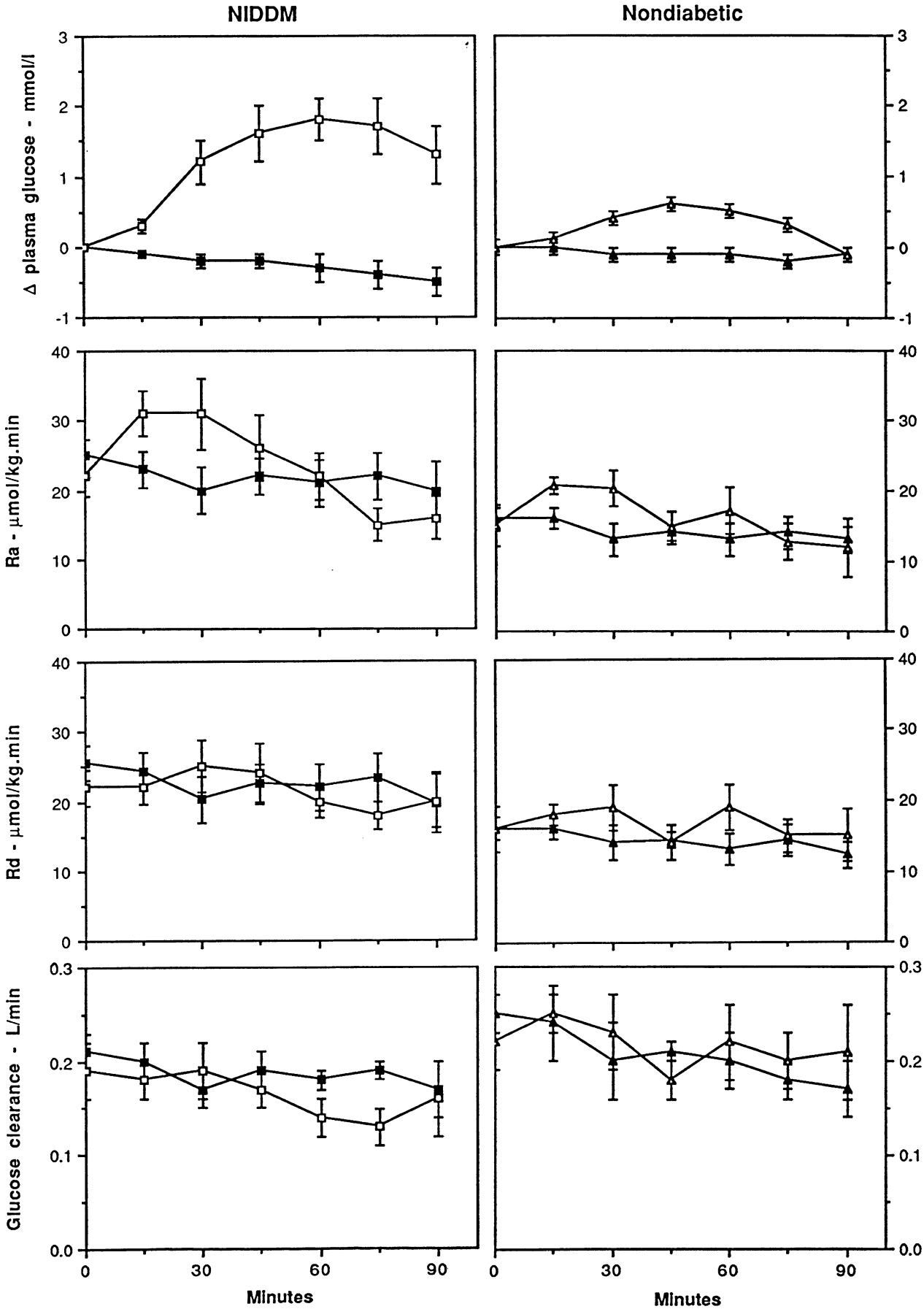
Cardiovascular responses (figure 6.3): Noradrenaline infusion caused increases in systolic blood pressure and reductions in heart rate in all subjects which rapidly normalised after cessation of the infusion. The diastolic pressure rose significantly in the diabetic ($p<0.05$) but not the nondiabetic subjects. There was a significantly greater systolic pressor response and a nonsignificantly greater diastolic pressor response in the diabetic compared with the nondiabetic subjects (integrated systolic response above baseline: 1112±149 vs 599±53 mmHg/60 mins, $p<0.02$; diastolic 289±136 vs 100±133 mmHg/60 mins, NS). In addition, the mean blood pressure response was significantly greater in the diabetic subjects ($p<0.02$). There was no difference in the heart rate responses between the two groups.

Figure 6.3 Mean (±SEM) incremental systolic pressor, diastolic pressor and heart rate responses to the noradrenaline infusion (from 0-60 minutes) in nondiabetic (open symbols) and diabetic subjects (closed symbols).



Glucose and glucose turnover responses (figure 6.4): Noradrenaline infusion resulted in a significant increase in plasma glucose levels in both groups of subjects. The diabetic subjects had significantly greater plasma glucose responses than the nondiabetic subjects with greater maximal increments (above control condition, 2.1±0.4 vs 0.6±0.2 mmol/l, $p<0.005$), greater integrated responses (71.3±13.3 vs 19.4±4.1 mmol/(60 min.l), $p<0.01$) and greater maximum increments when expressed as percent of baseline values (21±3 vs 10±3%, $p<0.05$)). R_a increased significantly in both groups of subjects during noradrenaline infusion ($p<0.01$). This increase in R_a then waned despite continued hormone infusion. The increase in R_a was significantly greater in the diabetic subjects compared with the nondiabetics (peak increment, 17.3±2.9 vs 8.1±3.0 μmol/(kg.min), $p<0.05$; integrated increment, 521.9±145.6 vs 115.9±57.6 μmol/(kg.60 min), $p<0.05$). There were no significant changes in R_d in either group. However the glycaemic response in the diabetic subjects appeared to be influenced by a decrease in peripheral glucose uptake at the end of the noradrenaline infusion as rates of glucose clearance fell and were significantly lower 60 minutes after the start of the infusion ($p<0.05$). Glucose clearance did not alter in the nondiabetic subjects.

Figure 6.4 Mean (±SEM) incremental plasma glucose responses (top panel) to the noradrenaline infusion (open symbols) compared with control (closed symbols) and the R_a, R_d and glucose clearance responses, (diabetic subjects, left panels; nondiabetic subjects, right panels).

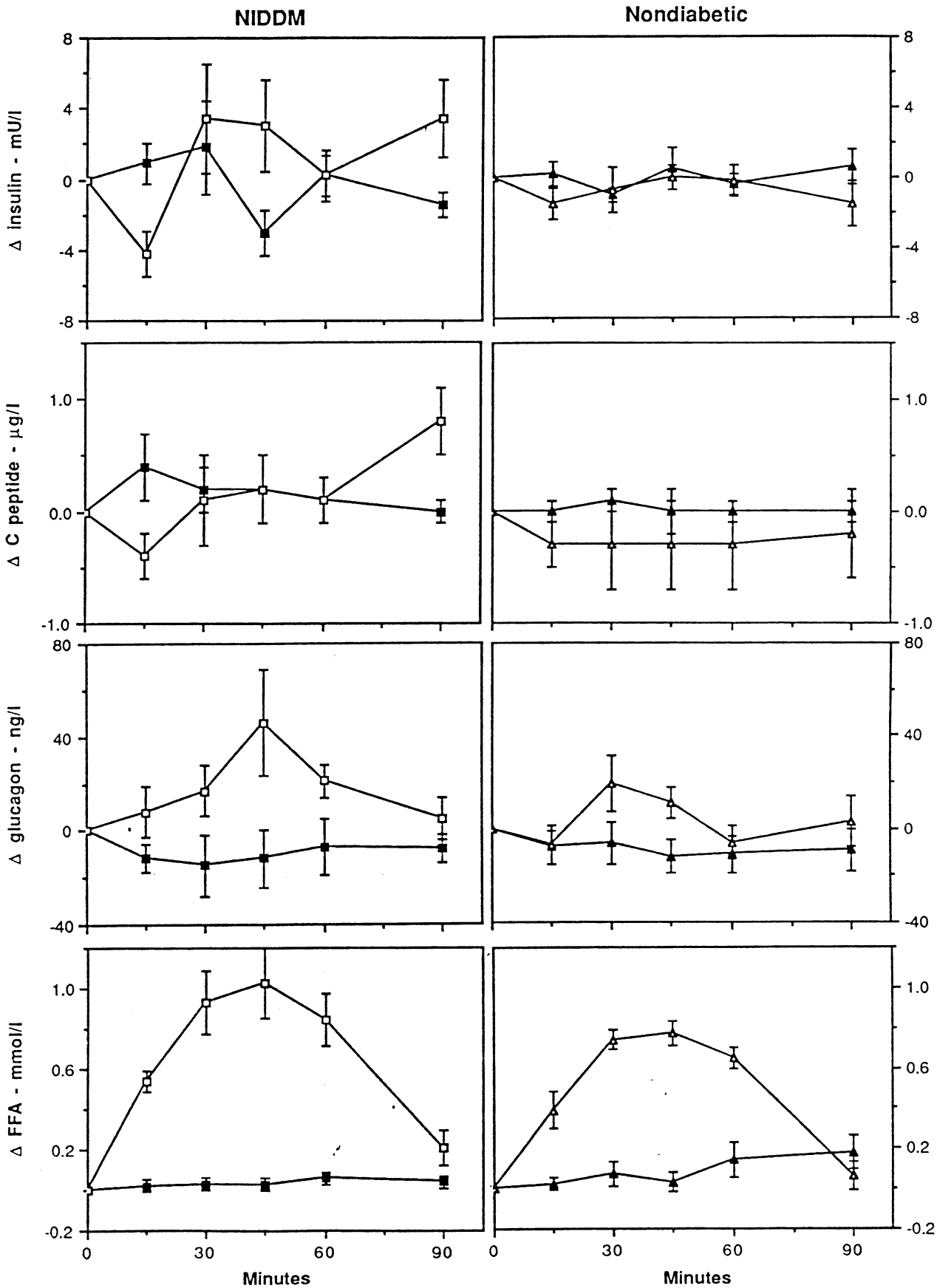


Insulin, C peptide, glucagon and FFA (figure 6.5): Arterial plasma insulin levels were not significantly altered by noradrenaline infusion in the nondiabetic subjects. In the diabetic subjects, plasma insulin levels became significantly depressed 15 minutes after the start of noradrenaline infusion (-4.5±1.7 mU/l, $p<0.05$) and subsequently rose above control levels despite continuing hormone infusion presumably due to glycaemic stimulation of the beta cells. There was no significant alteration in C peptide concentrations in the nondiabetic subjects whereas in the diabetic subjects, there was an initial transient, significant fall in C peptide concentrations 15 minutes after the start of the infusion (-0.8±0.3 μg/l, $p<0.05$). After noradrenaline was ceased, plasma C peptide levels rose nonsignificantly above control ($p<0.1$) in the diabetic subjects compatible with release of suppression of the beta cells after cessation of the hormone infusion.

Mean arterial plasma glucagon levels rose nonsignificantly during noradrenaline infusion in both the diabetic and nondiabetic subjects ($p<0.1$). The glucagon responses were nonsignificantly greater in the diabetic subjects compared with the nondiabetics (integrated responses above control condition, 1862±770 vs 743±326 ng/(l.60 min)).

Noradrenaline infusion resulted in considerable increases in plasma FFA levels in both groups of subjects. These were nonsignificantly greater in the diabetic subjects than in the nondiabetic group (integrated responses above the control condition, 42.60±5.85 vs 31.23±2.73 mmol/(l.60 min)).

Figure 6.5 Mean (±SEM) incremental insulin, C peptide, glucagon and FFA responses to the noradrenaline infusion in the diabetic (left panels) and the nondiabetic subjects. Control data is also displayed, symbols are as in fig 6.4.



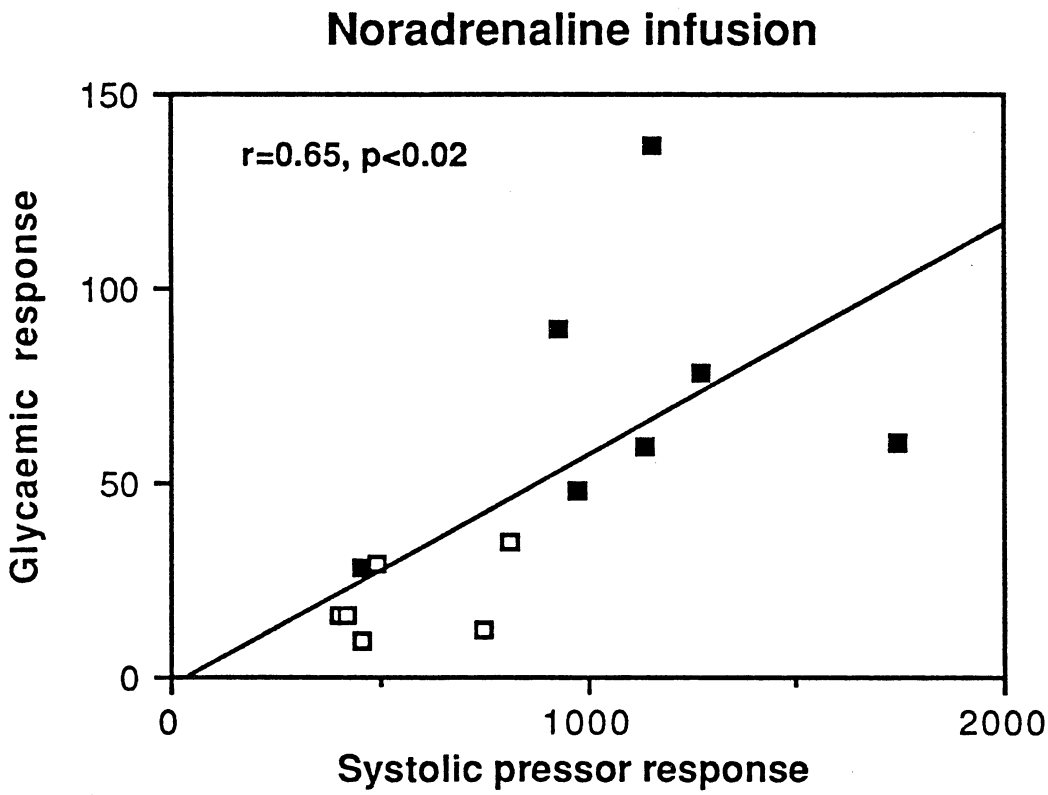
Noradrenaline metabolites (table 6.4): There was a significant transient increase in circulating levels of the noradrenaline metabolite MHPG during intravenous noradrenaline administration which was of similar magnitude in both groups of subjects. After the initial rise, MHPG levels then fell and became not significantly different from basal despite continued noradrenaline infusion. Circulating DHPG levels at the end of the noradrenaline infusion increased nonsignificantly from baseline in both groups of subjects.

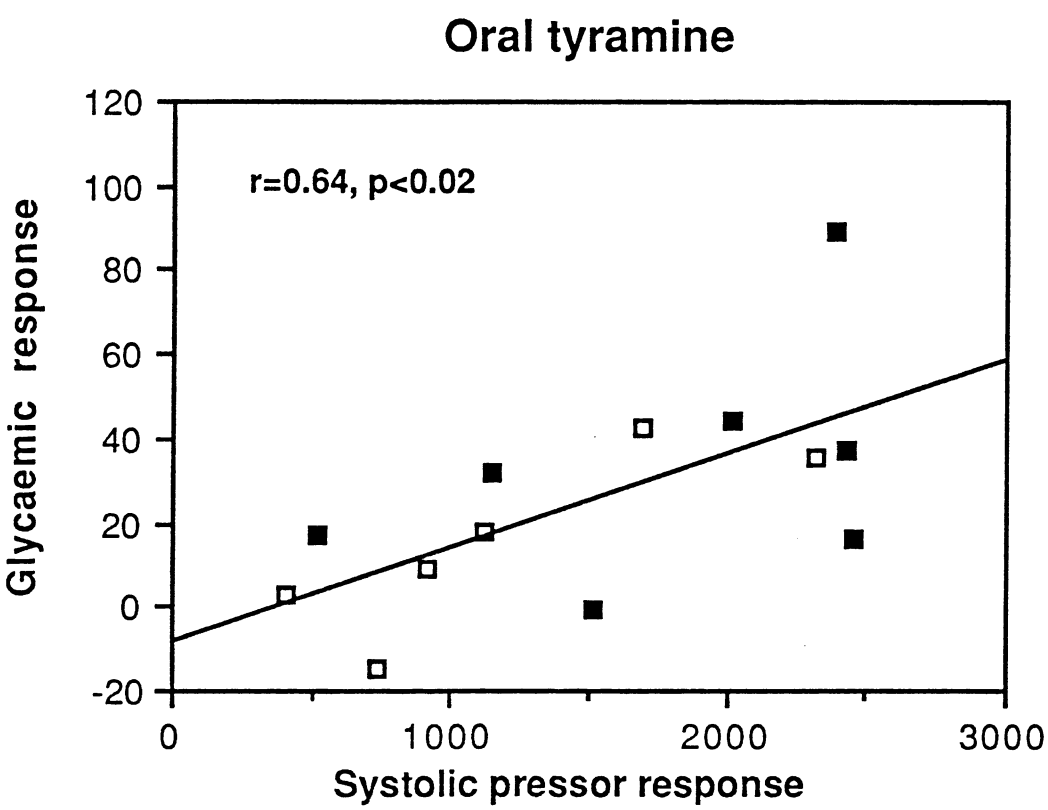
Correlations: There was a significant positive correlation between the glycaemic and the systolic pressor responses during noradrenaline infusion ($r=0.65$, $p<0.02$, figure 6.6).

Table 6.4 Mean (±SEM) MHPG, DHPG, noradrenaline (NA, measured by both GC/MS and radioenzymatic methods) and adrenaline levels (all in nmol/l) at baseline and after 30 and 60 minutes of constant noradrenaline infusion (0-60 minutes). * significant increase compared with basal.

	NIDDM			Nondiabetic		
	Basal	**30**	**60**	**Basal**	**30**	**60**
MHPG	13.7±3.0	31.1±4.9*	17.1±4.3	12.3±2.6	29.0±5.1*	15.9±1.9
DHPG	6.8±1.1	-	7.3±1.6	5.9±0.4	-	8.6±1.3
NA(GC/MS)	2.7±0.4	-	14.1±1.5*	2.3±0.2	-	12.9±1.0*
NA(radio)	1.5±0.4	5.2±1.7*	5.7±2.0*	1.4±0.6	5.0±2.0*	4.5±2.4*
Adren	0.4±0.1	0.4±0.2	0.4±0.1	0.4±0.2	0.4±0.2	0.5±0.3

Figure 6.6 Correlations between the integrated glycaemic and systolic pressure responses during noradrenaline infusion (top panel) and after oral tyramine (bottom panel) for all subjects, (diabetic subjects, closed symbols).





6.5.3 Tyramine study

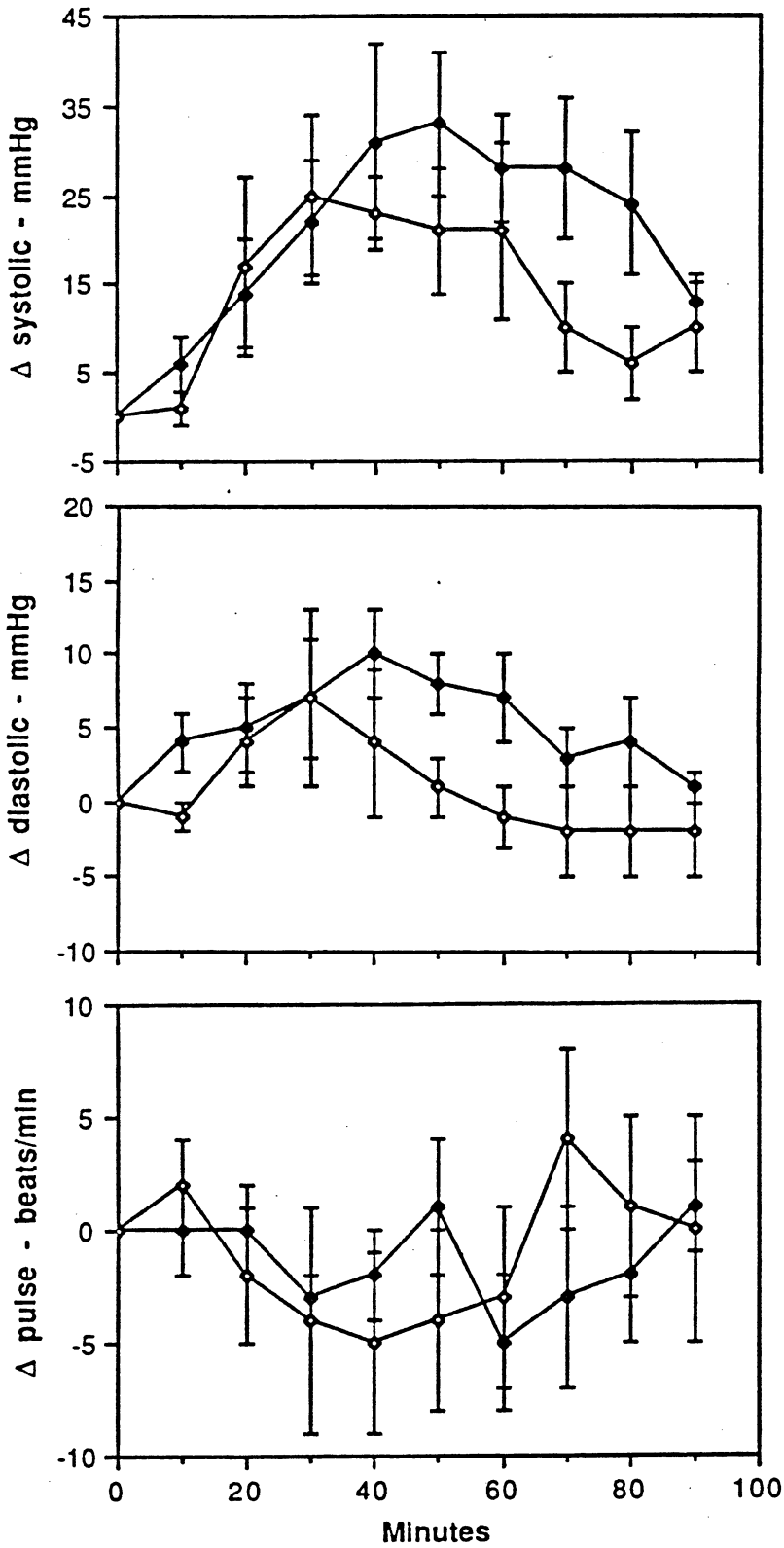
Oral tyramine administration resulted in significant increases in circulating levels of noradrenaline, MHPG and DHPG in both groups of subjects with no difference in the responses between the groups (table 6.5). Circulating tyramine levels also increased in both groups of subjects with little apparent difference in the absorption profiles of either group although there was wide variation in the estimated levels (table 6.5). There was no change in adrenaline levels.

Cardiovascular responses (figure 6.7): Tyramine administration caused a significant increase in the systolic blood pressure in both groups of subjects. This was nonsignificantly greater in the diabetic subjects compared with the nondiabetic subjects (peak systolic increment, 49±8 vs 40±7 mmHg; total integrated increment, 1926±321 vs 1293±254 mmHg/180 min, $p<0.2$). The diastolic blood pressure rose significantly in the diabetic subjects ($p<0.05$) and nonsignificantly in the nondiabetic subjects with no significant difference between the responses of the two groups. Five of the seven diabetic subjects and five of the six nondiabetic subjects experienced a fall in heart rate at the time of maximal systolic pressure response whereas the other subjects had an increase in heart rate. This variability resulted in no significant change in heart rate in the nondiabetic subjects and a significant fall in heart rate 30 minutes after tyramine in the diabetic subjects ($p<0.01$).

Table 6.5 Mean (±SEM) noradrenaline, MHPG, DHPG (determined by GC/MS and in nmol/l) and tyramine levels (arbitrary units) at baseline and at 30 and 60 minutes after oral tyramine administration. * $p<0.01$ versus basal.

	NIDDM			Nondiabetic		
	Basal	**30**	**60**	**Basal**	**30**	**60**
NA(GC/MS)	2.4±0.2	-	3.3±0.3*	2.4±0.4	-	4.6±0.8*
NA(radio)	1.1±0.4	1.3±0.5	1.2±0.4	1.5±0.4	3.0±1.0	3.2±0.9*
MHPG	23.4±4.0	26.0±4.8	34.0±6.8*	30.8±4.8	36.4±5.4	45.9±5.4*
DHPG	7.0±0.6	-	15.3±1.3*	6.1±1.1	-	13.7±2.6*
Tyramine	0	645±352	1476±444	0	598±466	840±474
Adrenaline	0.5±0.1	0.4±0.2	0.5±0.2	0.4±0.2	0.4±0.2	0.5±0.3

Figure 6.7 Mean (±SEM) incremental systolic pressor, diastolic pressor and heart rate responses from baseline to oral tyramine administration in the diabetic (closed symbols) and nondiabetic subjects (open symbols).



Glucose and glucose turnover (figure 6.8): There was a significant increase in plasma glucose after tyramine administration in both groups of subjects ($p<0.025$) with no significant difference in the glycaemic responses between the two groups (integrated responses above control: 33.7±10.9 vs 20.5±6.3 mmol/(l.90 mins)). The increase in plasma glucose following tyramine appeared to be due to an increase in R_a. This increased nonsignificantly in the diabetic ($p<0.1$) and nondiabetic subjects (integrated increment above control, 347.1±148.6 and 111.4±288.6 μmol/(kg.90 min)). When the data for all subjects were combined, there was a significant increase in R_a after tyramine ($p<0.05$). There was no significant alteration in R_d or glucose clearance after tyramine administration.

Insulin, C peptide, glucagon and FFA (figure 6.9): There were no significant changes in serum insulin or C peptide after tyramine in the diabetic and the nondiabetic subjects. There were variable (nonsignificant) increases in plasma glucagon levels in both groups of subjects with no difference seen in the responses between the diabetic and nondiabetic subjects (integrated increments, 1613±883 vs 1210±597 ng/(l.90 min)). Oral tyramine caused a significant increase in mean FFA levels in the diabetic subjects ($p<0.05$). In the nondiabetic subjects, FFA levels rose to an extent comparable to the diabetic subjects in four of the six subjects resulting in no significant difference between the FFA responses of the two groups.

Correlations: There was a significant positive correlation between the systolic blood pressure responses and the glycaemic responses after oral tyramine ($r=0.64$, $p<0.02$, figure 6.6). There was also a significant positive correlation between the glycaemic responses and the estimates of tyramine absorption (integrated tyramine areas, $r=0.65$, $p<0.032$).

Figure 6.8 Mean (±SEM) incremental plasma glucose, R_a, R_d and glucose clearance responses to oral tyramine (open symbols) compared with control (diabetic subjects, left panels; nondiabetic subjects, right panels).

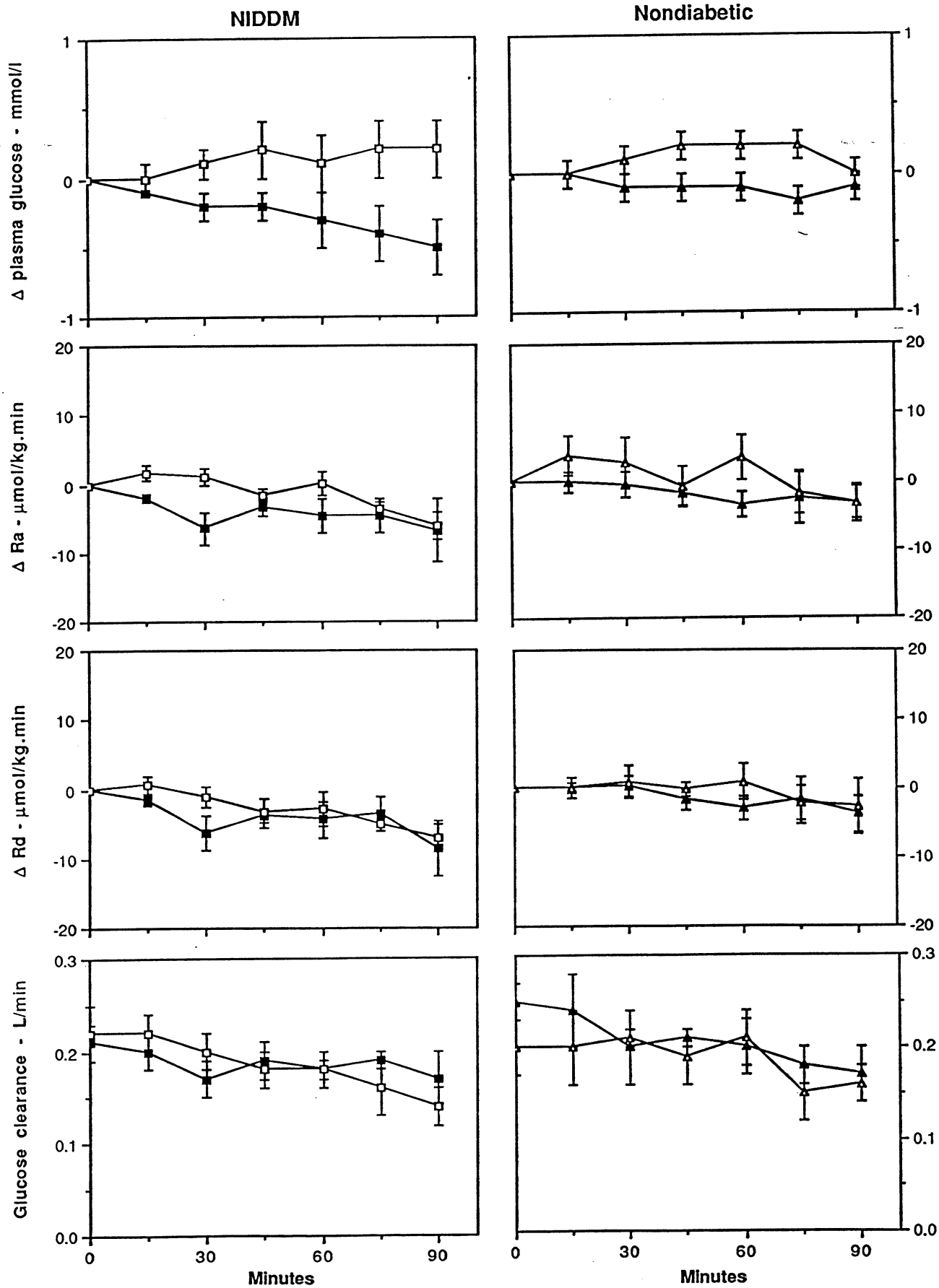
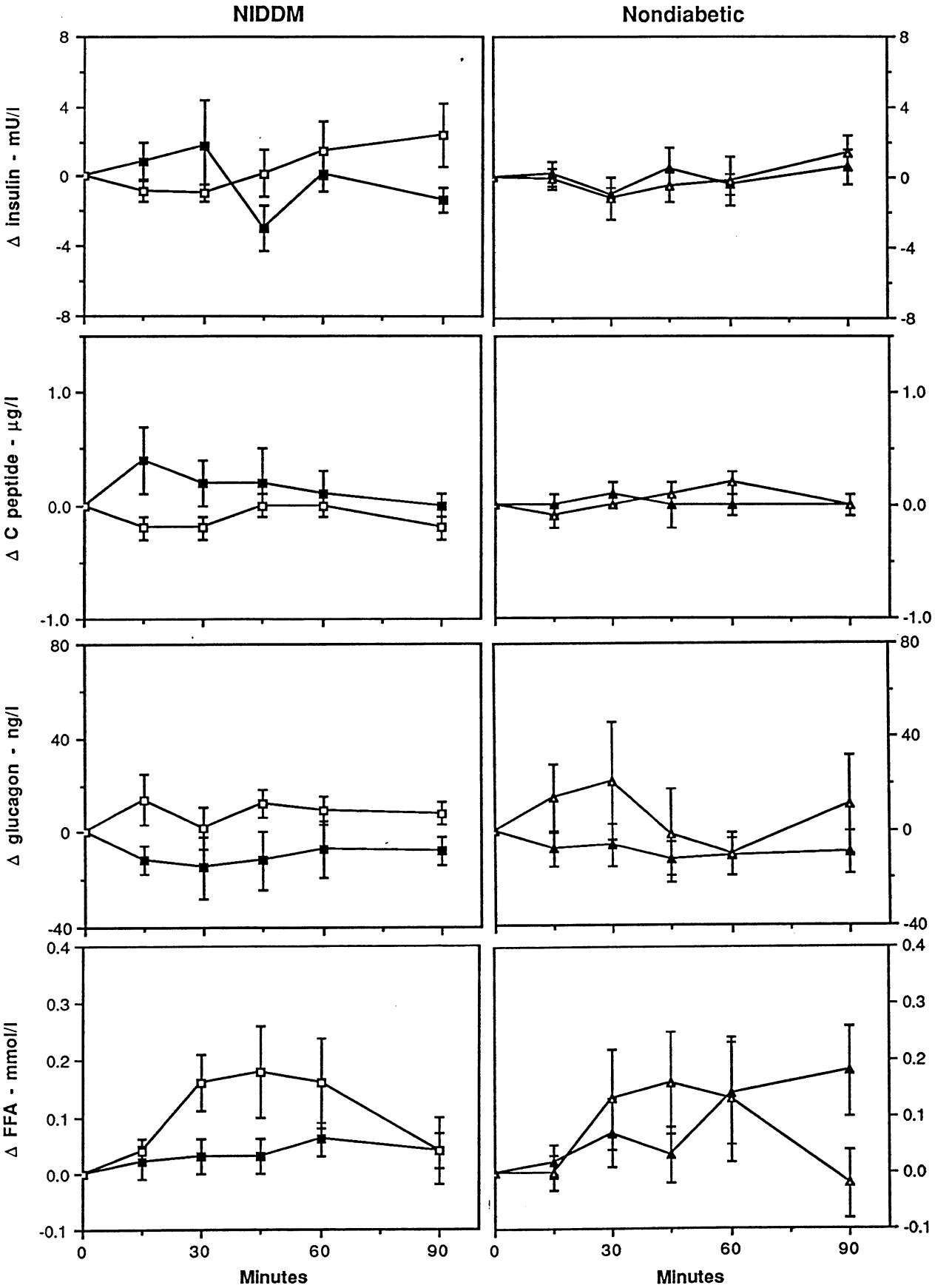


Figure 6.9 Mean (±SEM) incremental insulin, C peptide, glucagon and FFA responses to oral tyramine (open symbols) compared with control (diabetic subjects, left panels; nondiabetic subjects right panels).



6.5.4 Psychological stress

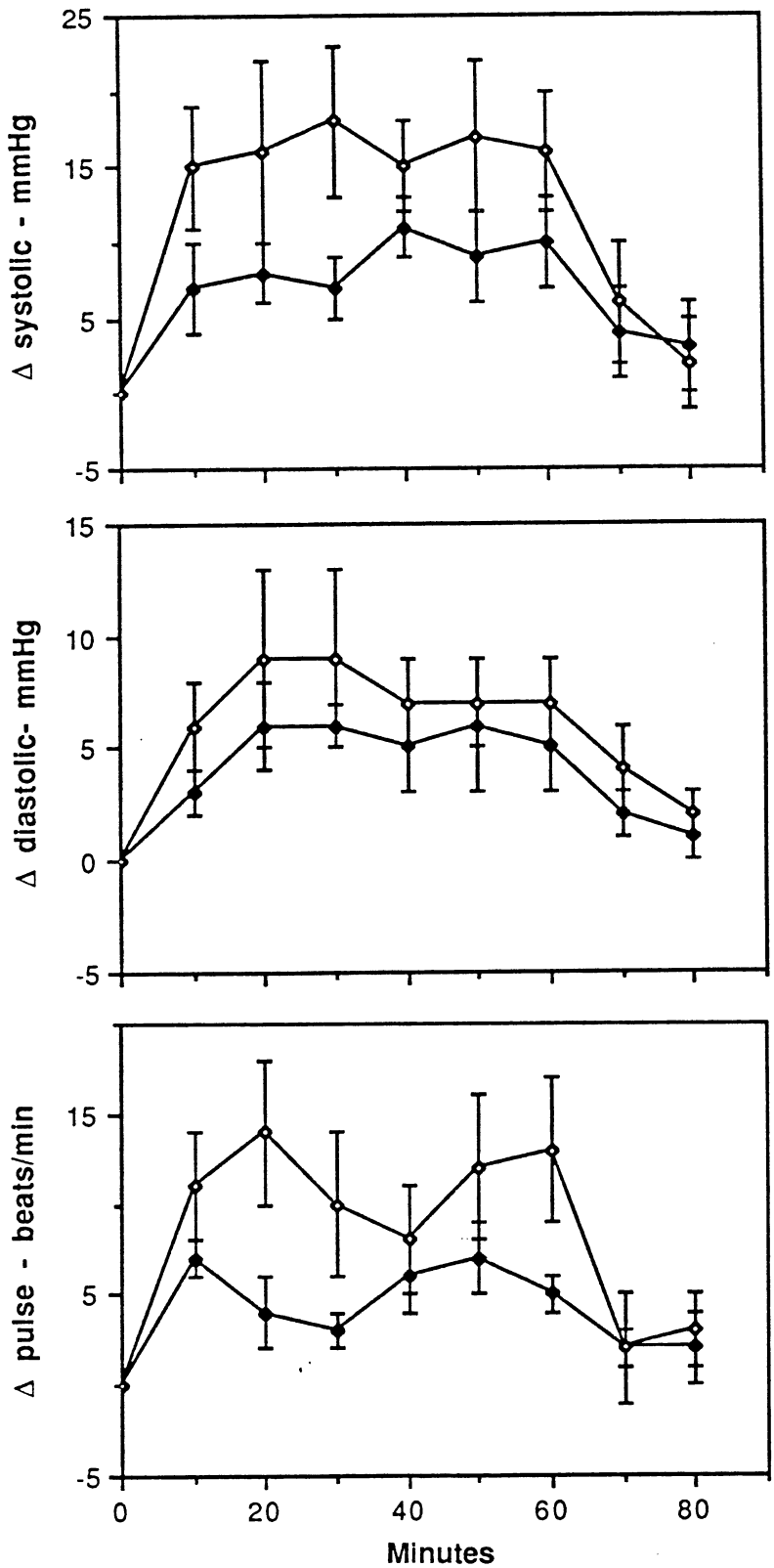
Psychological and cardiovascular responses: There was considerable variation in the responses (both psychological and physical) of the subjects to the psychological stressor. Three subjects (two diabetic and one nondiabetic) reported that they did not feel pressured or stressed; most subjects reported feelings of mild to moderate irritation, frustration or anxiety; one diabetic subject felt (and looked) severely stressed and upset during the computer games. One other diabetic subject (who reported feeling moderately frustrated by the games) suffered a vaso-vagal attack within five minutes of ceasing the task.

Five of the eight diabetic subjects and all six of the nondiabetics completed the anxiety inventory satisfactorily (one diabetic subject was not sufficiently proficient in written English, the responses of the subject who fainted were not considered appropriate and in one subject the post-stressor questionnaire was omitted in error). There was no difference between the groups in baseline scores obtained from the anxiety questionnaire (acute anxiety, 25±2 vs 26±2; chronic anxiety, 35±4 vs 34±6; possible range, 20-80 for each). All subjects scored higher state-anxiety scores after the task than at baseline ($p<0.005$). The nondiabetic subjects scored significantly higher during the games than the diabetic subjects (delta score, 19±4 vs 8±1, $p<0.05$).

All the subjects had an increase in blood pressure and all, except one, had an increase in heart rate during the computer games (figure 6.9). One diabetic subject had a bradycardic response with episodes of the Wenckebach phenomenon. This has been reported to occur in some subjects during psychological stress (Carruthers and Taggart, 1973). The heart rate responses (excluding the subject with a bradycardic response) were nonsignificantly greater in the nondiabetic subjects (maximum increase in heart rate, 16±4 vs

11±1 beats/min; integrated incremental response, 597±164 vs 346±46 beats/60 min). The nondiabetic subjects had a nonsignificantly greater systolic pressure response (integrated incremental response, 879±235 vs 458±66 mmHg/60 min, $p<0.1$; maximum increment from baseline, 21±5 vs 16±5 mmHg, NS) and a nonsignificantly greater diastolic pressure response than the diabetic subjects (integrated response, 403±147 vs 284±77 mmHg/60 min).

Figure 6.9 Mean (±SEM) incremental heart rate, systolic and diastolic pressor responses to the psychological stress in the diabetic (n=8, closed symbols) and nondiabetic subjects (n=6, open symbols).



FFA and stress hormones: There was a significant increase in FFA levels during the psychological stress in both groups of subjects ($p<0.05$, figure 6.10) which was nonsignificantly greater in the nondiabetic subjects (integrated responses above control condition, nondiabetics 6.81±2.26 vs 2.35±0.86 mmol/(l.60 min)). There was no significant change in mean circulating levels of cortisol, ACTH, adrenaline, noradrenaline, or growth hormone (table 6.6). There was a significant fall in prolactin levels compared with baseline in the diabetic subjects only (see below and fig 6.12).

Glucose, glucose turnover and pancreatic hormones: Psychological stress had no significant effect on mean blood glucose levels in either subject group (figure 6.10). Neither was there any substantial glycaemic response in any individual subject. There was also no change in R_a or R_d in either group.

Psychological stress caused nonsignificant falls in insulin and C peptide levels in the diabetic subjects (integrated difference from control: insulin, -86.9±71 mU/(l.60 min); C peptide, -21.7±13.9 μg/(l.60 min), figure 6.10). In the nondiabetic subjects, psychological stress did not alter plasma insulin levels appreciably although there was a nonsignificant fall in C peptide levels (-15.6±10.7 μg/(l.60 min)). There was no change in plasma glucagon levels during the stress condition (table 6.6).

Figure 6.10 Mean (±SEM) incremental FFA, insulin, C peptide and plasma glucose responses during psychological stress (open symbols) compared with control in the diabetic (left panels) and nondiabetic subjects (right panels).

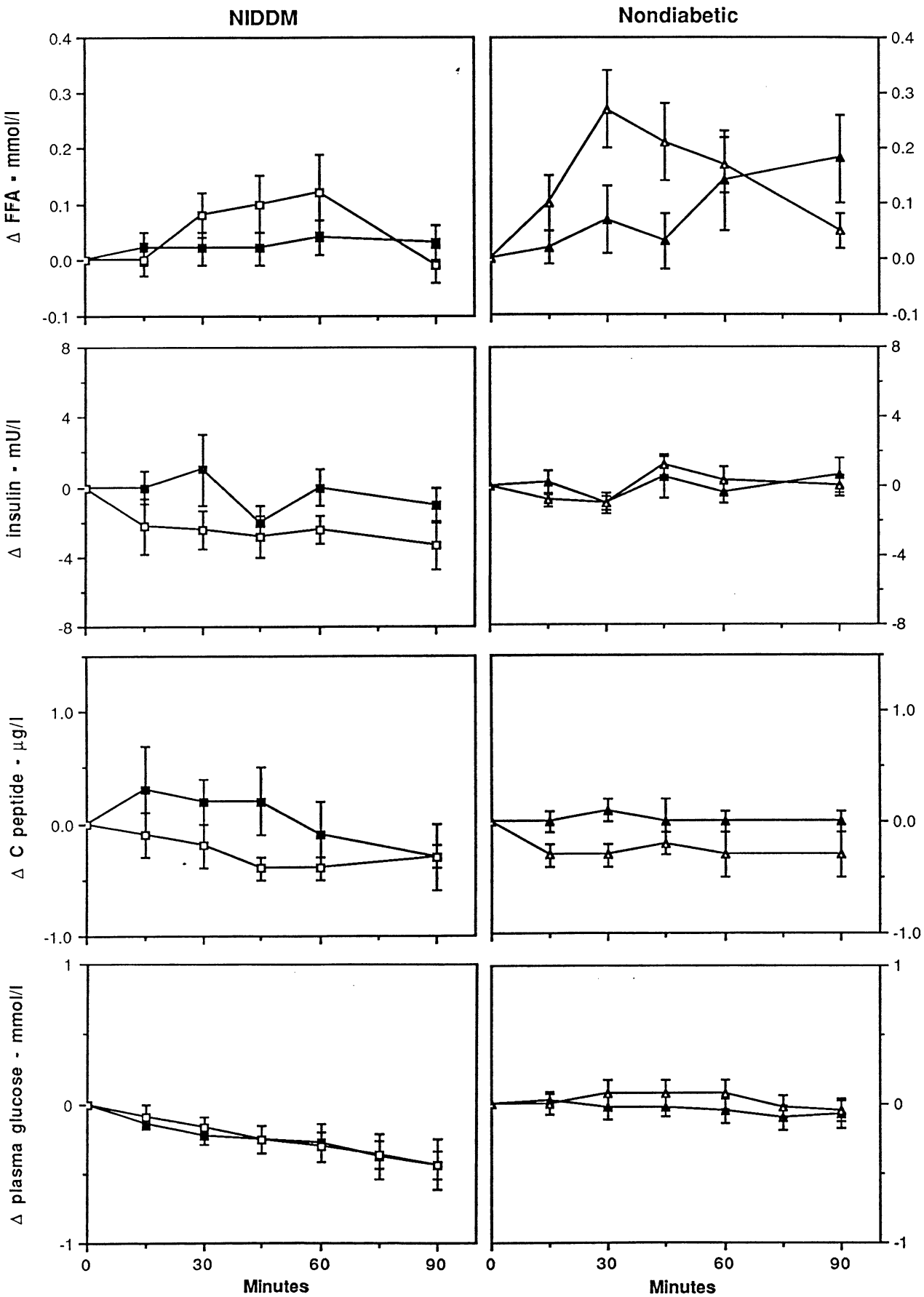


Table 6.6 Mean (±SEM) values of the major stress hormones during the control and psychological stress conditions in the diabetic (n=8) and nondiabetic subjects (n=6). Data at times 0, 30 and 60 minutes are given.

		NIDDM subjects			Nondiabetic subjects		
		0	30	60	0	30	60
Cortisol	**control**	150±52	203±53	182±37	210±50	214±50	230±58
nmol/l	**stress**	132±34	181±50	140±37	203±30	202±23	159±25
Glucagon	**control**	50±17	34±7	42±13	44±15	40±25	34±17
ng/l	**stress**	18±3	34±12	30±10	59±18	50±18	34±9
Adrenaline	**control**	0.3±0.1	0.3±0.1	0.5±0.2	0.4±0.2	0.4±0.1	0.3±0.1
nmol/l	**stress**	0.5±0.2	0.5±0.3	0.3±0.1	0.4±0.2	0.4±0.2	0.5±0.3
Noradrenaline	**control**	1.0±0.2	1.1±0.2	1.8±0.4	1.3±0.6	1.4±0.7	1.8±0.7
(radio) nmol/l	**stress**	1.1±0.6	1.3±0.4	1.0±0.5	1.0±0.5	1.2±0.4	1.6±0.4
Noradrenaline	**control**	2.0±0.3	-	2.0±0.2	2.4±0.3	-	2.6±0.4
(GC/MS) nmol/l	**stress**	2.4±0.2	-	2.6±0.4	2.4±0.4	-	3.4±0.4

Correlations: There were significant positive correlations between the delta score of the anxiety questionnaire and the FFA ($r=0.71$, $p<0.014$) and heart rate responses ($r=0.70$, $p<0.01$) during the stress condition. There was a correlation between the delta anxiety score and the adrenaline responses which approached statistical significance ($r=0.59$, $p<0.08$). There were also significant correlations between the FFA responses and the heart rate ($r=0.73$, $p<0.005$), systolic blood pressure ($r=0.63$, $p<0.02$) and diastolic blood pressure responses ($r=0.66$, $p<0.02$) during the stress. There was a significant positive correlation between the cortisol and diastolic pressure responses ($r=0.64$, $p<0.03$). Finally, there were significant negative correlations between the prolactin responses to the stress condition and the cortisol ($r=-0.72$, $p<0.008$) and diastolic pressure responses ($r=-0.66$, $p<0.019$).

6.5.5 Cortisol, ACTH, prolactin and GH responses

Cortisol and ACTH: As already described, no significant change in cortisol or ACTH levels was seen during psychological stress. Similarly, during tyramine administration, no significant changes in circulating levels of cortisol or ACTH were seen (data not shown). However, during noradrenaline infusion, there was a significant fall in cortisol levels compared with control in both the diabetic and nondiabetic subjects (integrated responses, $p<0.05$) with no significant difference in the magnitude of the responses between the groups (figure 6.11).

These changes appeared to be due to a fall in ACTH levels. There was a nonsignificant fall in the integrated ACTH responses in both the diabetic (integrated fall, $p<0.1$) and nondiabetic subjects (integrated fall, $p<0.2$) and ACTH levels were nonsignificantly lower than control 60 minutes after noradrenaline infusion in both groups ($p<0.1$). As there was no difference between the groups in either the cortisol or ACTH responses, the ACTH data was analysed together. When analysed thus, there was a significant fall in ACTH levels in the whole experimental group during noradrenaline administration ($p<0.02$).

Prolactin (figure 6.12): There was a significant fall in prolactin levels during psychological stress in the diabetic subjects (integrated response, $p<0.05$) which was not seen in the nondiabetic subjects. There were no significant changes in prolactin levels during noradrenaline infusion. Tyramine administration resulted in a nonsignificant fall in prolactin levels compared with control in both groups of subjects. When the data from the diabetic and nondiabetic subjects was analysed together, the fall in prolactin after tyramine remained statistically nonsignificant ($p<0.1$).

Growth hormone: Growth hormone levels did not alter significantly during any of the study conditions compared with control.

Figure 6.11 The cortisol and ACTH responses during noradrenaline administration in diabetic (left panels) and nondiabetic subjects (right panels). Closed symbols represent the control condition.

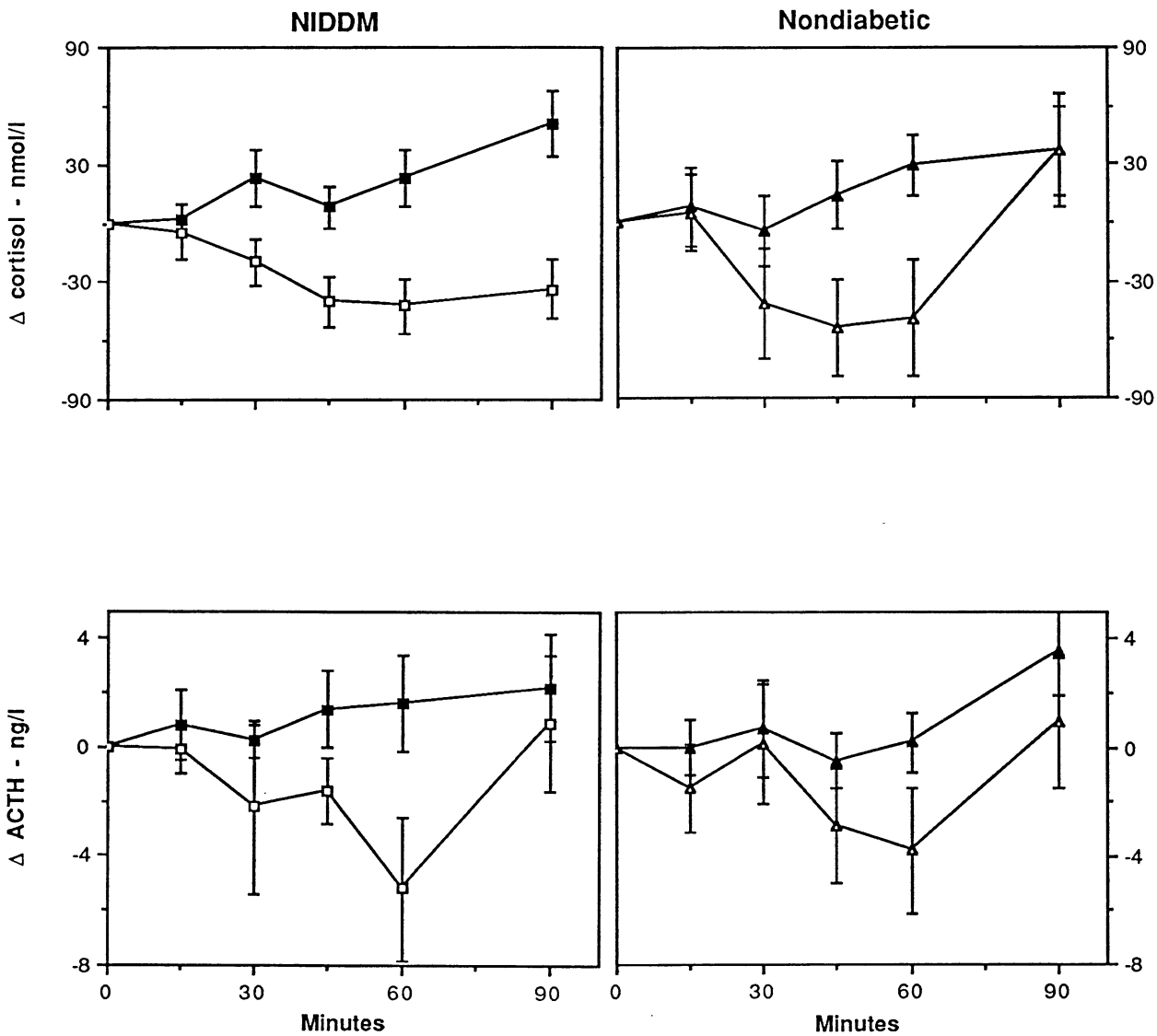
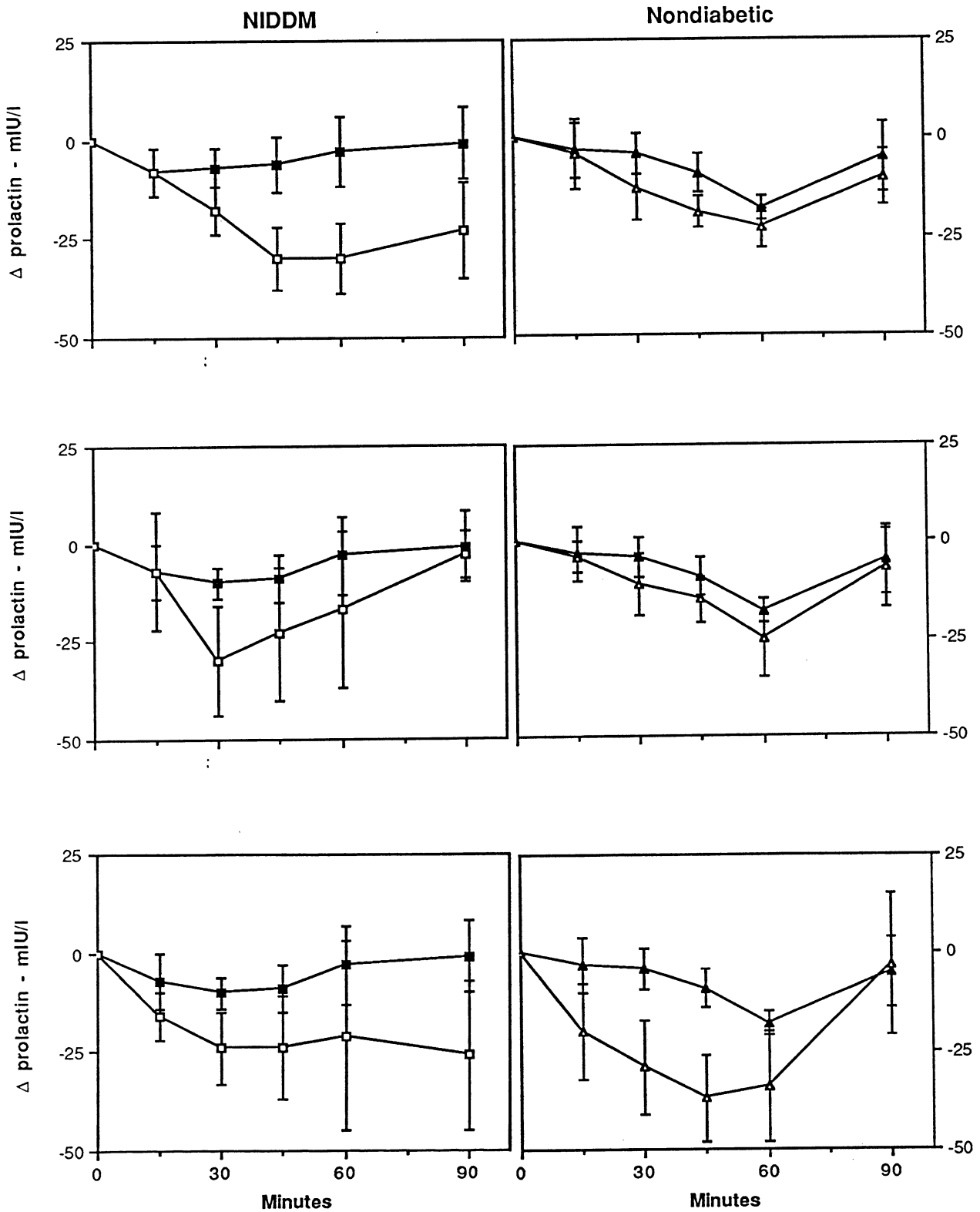


Figure 6.12 The prolactin responses during psychological stress (top), noradrenaline infusion (middle), and tyramine administration (bottom) in the diabetic (left panels) and nondiabetic subjects (right panels). Closed symbols represent the control condition.



6.6 Discussion

The main findings of these studies are as follows: (i) infusion of noradrenaline to moderately high physiological levels caused a greater pressor response and a greater hyperglycaemic effect in the subjects with NIDDM compared with nondiabetic subjects, (ii) oral tyramine administration caused a modest rise in glycaemia in both nondiabetic and diabetic subjects which was due to an increase in hepatic glucose production, (iii) acute psychological stress, sufficiently severe to cause cardiovascular responses and increased levels of FFA levels, failed to cause an increase in blood glucose levels in the nondiabetic and diabetic subjects, and (iv) basal plasma glucose levels correlated with baseline indices of sympathetic noradrenergic activity in the subjects with NIDDM.

6.6.1 Noradrenaline study

As stated above, noradrenaline infusion resulted in significantly greater pressor and glycaemic responses in the subjects with NIDDM compared with nondiabetic subjects. There was a positive correlation between the pressor and glycaemic responses indicating that the effect of noradrenaline on these different physiological systems was probably mediated by a similar mechanism.

The difference in responsiveness between the diabetic and nondiabetic subjects was not due to differences in noradrenaline metabolism as similar arterial noradrenaline levels were seen basally and similar concentrations were achieved by the infusions in both groups of subjects. This is consistent with published data showing unchanged noradrenaline clearance rates in insulin dependent diabetic subjects (Hoeldtke and Cilmi, 1984; Dejgaard et al., 1986). Noradrenaline was measured by two separate assays in these studies and the results from either method support this conclusion.

There was a disparity in the levels of noradrenaline measured by the two assays, with the radioenzymatic method estimating lower levels. The GC/MS assay is an extremely precise and accurate measure and it is likely that the radioenzymatic assay underestimated the levels basally and achieved during noradrenaline infusion. In support of this, the levels achieved during the noradrenaline infusion as ascertained by the GC/MS assay correspond closely to the threshold for biological effects (Cryer, 1980) and are consistent with the pressor and glycaemic effects produced in the nondiabetic subjects. The lower levels (as determined in the radioenzymatic assay) lie below this threshold and such concentrations would not be expected to produce effects on blood glucose levels (Cryer, 1980).

The physiological mechanisms for the hyperglycaemic effect of noradrenaline are complex. They include direct and indirect (hormone mediated) actions which cause alterations in both hepatic glucose production and peripheral glucose utilisation. These actions of noradrenaline may be mediated by different adrenergic receptor types. Noradrenaline infusion raises circulating levels of the catecholamine and therefore its effects may reflect hormonal rather than neurotransmitter actions of noradrenaline. However, high circulating levels of noradrenaline may more closely reproduce the levels of noradrenaline seen at the synapse during sympathetic nerve firing. On the other hand, circulating noradrenaline could also have effects on endogenous sympathetic nervous activity by altering presynaptic receptor activity (Langer et al., 1977) and high levels may suppress endogenous sympathetic activation (Izzo et al., 1982). Finally, the effects of circulating noradrenaline are likely to act peripherally but central neural mechanisms could also be involved.

Noradrenaline infusions at comparable rates to the present study have previously been shown to cause modest elevations in blood glucose in nondiabetic subjects (Schade and Eaton, 1979; Sacca et al., 1980) due to a transient increase in hepatic glucose production with little increase in peripheral glucose uptake (Sacca et al., 1980). A similar mechanism was operative in both the diabetic and nondiabetic subjects in the present study. However there was a greater rise in hepatic glucose output in the subjects with NIDDM which caused the excessive glycaemic response.

Peripheral glucose uptake did not alter greatly in either group but this may have been inappropriate in the diabetic subjects considering the higher levels of blood glucose. Reduced peripheral glucose uptake probably played a role in maintaining the hyperglycaemia as glucose clearance (which is a measure of the efficiency of glucose disposal (Radziuk and Lickley, 1985)) fell significantly in the diabetic subjects as glucose production declined to basal. Thus the reduction in glucose clearance explains why blood glucose levels remained elevated in the diabetic subjects after the noradrenaline infusion was ceased whereas blood glucose rapidly returned to baseline in the nondiabetic subjects.

Noradrenaline administration also caused pancreatic hormone responses. Insulin levels fell transiently in both groups of subjects but the fall was of greater magnitude and statistically significant in the diabetic subjects. Subsequently insulin concentrations rose in the diabetic subjects presumably due to glycaemic stimulation.

Catecholamines inhibit insulin secretion by an alpha-adrenergic mechanism (probably $alpha_2$) and stimulate insulin secretion by a ß-adrenergic mechanism (Skoglund et al., 1986; Samols and Weir, 1979). The initial inhibition of insulin

secretion was therefore due to an alpha-inhibitory effect and the data suggest that pancreatic beta cells in NIDDM may be abnormally sensitive to the inhibitory effect of noradrenaline. As some of the nondiabetic subjects had basal insulin levels at or below the limit of sensitivity of the assay, an inhibitory effect of noradrenaline on insulin secretion could have been missed. However the C peptide data also indicate that noradrenaline had a greater inhibitory effect in the diabetic compared with the nondiabetic subjects.

Noradrenaline infusion caused a variable increase in glucagon levels in both groups of subject. Noradrenaline has been shown to stimulate glucagon secretion in humans in some but not all studies (Schade and Eaton, 1977; 1978; Silverberg et al., 1978; Keller et al., 1984). The glucagon responses in the diabetic subjects occurred in the face of greater increases in blood glucose levels which normally would be expected to inhibit glucagon secretion (Gerich et al., 1976; Beard et al., 1983). Therefore the pancreatic alpha cells in the subjects with NIDDM may have had an increased sensitivity to noradrenaline. Adrenaline stimulates glucagon secretion (Rizza et al., 1979; Gray et al., 1980) by ß-adrenergic mechanisms (Rizza et al., 1980) although alpha-receptors may also play a role (Samols and Weir, 1979). Thus the data suggest the possibility of increased ß-adrenergic sensitivity of the islet alpha cells in NIDDM.

The effects of noradrenaline on insulin and glucagon secretion could have caused the excessive glycaemic responses in the subjects with NIDDM. The relative role of indirect (hormonal) versus direct noradrenergic effects on the liver are not known. Enhanced glycaemic responses are seen in subjects with IDDM during adrenaline administration (Shamoon et al., 1980; Berk et al., 1985) due to the inability of these subjects to mount a compensatory insulin response to the hyperglycaemia (Berk et al., 1985). A similar mechanism could

be operative in subjects with NIDDM, due to impaired beta cell function. However in contrast to adrenaline, noradrenaline administration does not cause exaggerated glycaemic responses in subjects with IDDM (Schade and Eaton, 1978). This suggests that the abnormal glycaemic responses to noradrenaline in the present study may not be due to insulin deficiency but to a direct hepatic effect.

The effect of catecholamines on glucose production is probably independent of glucagon secretion in normal (Gray et al., 1980) and in insulin dependent diabetic humans (Berk et al., 1985). In the present study, the glucagon responses, although somewhat greater in the diabetic subjects, occurred inconsistently and peak levels were reached after the increase in hepatic glucose output had occurred. Therefore glucagon may not have had a role in hepatic glucose production.

Noradrenaline has a direct action on the liver which in dogs is predominantly responsible for its hyperglycaemia producing effect (Connolly et al., 1987). The direct action of noradrenaline causes an increase in both glycogenolysis and gluconeogenesis (Connolly et al., 1987). Gluconeogenesis is responsible for much of the excessive basal hepatic glucose production in NIDDM (Consoli et al., 1987) and excessive gluconeogenesis might explain the excessive hyperglycaemic responses in the diabetic subjects after noradrenaline. However, adrenaline stimulates gluconeogenesis by causing increased delivery of gluconeogenic substrates rather than any effect within the liver (Cherrington et al., 1984) and gluconeogenesis increases slowly during adrenaline administration. This suggests that the initial transient increase in glucose production during catecholamine infusion reflects an increase in glycogenolysis rather than gluconeogenesis (Cherrington et al., 1984). Thus in

the present study, the greater transient hepatic response in the diabetic subjects may indicate an increase in glycogenolysis.

Stimulation of hepatic glucose production in nondiabetic man is mediated predominantly by ß-adrenergic mechanisms (Rizza et al., 1980; Best et al., 1984) although alpha-adrenergic stimulation also has a role (Rosen et al., 1983). Recently, it has been established that substantial numbers of both $alpha_1$-adrenergic and $ß_2$-adrenergic receptors are present in human liver (Bevilacqua et al., 1987; Kawai et al., 1986). In rats, hepatic glucose production can shift from being mediated by $alpha_1$- to $ß_2$-receptors without any modification in the number of available receptors (Kunos et al., 1984). Thus the increase in hepatic glucose output during noradrenaline administration could be mediated by hepatic alpha-receptors (which probably are normally of minor importance) or alternatively by ß-receptors (on which noradrenaline has relatively weak agonist activity). These considerations probably explain why noradrenaline is less potent than adrenaline in stimulating hyperglycaemia (Sacca et al., 1981). The cause for the greater hepatic glucose production in NIDDM could be due to greater adrenergic sensitivity of the liver to the hormone and either increased ß- or alpha-adrenergic receptor sensitivity (or both) may be invoked.

Limitation of peripheral glucose uptake during adrenaline infusion has been shown in vivo to be mediated by ß-adrenergic receptors (Rizza et al., 1980; James et al., 1986) Thus the reduction in glucose clearance in the diabetic subjects may represent an effect mediated by ß-receptors. Alternatively, glucose clearance may fall with increased hyperglycaemia in NIDDM (Best et al., 1983) and the change in glucose clearance may not be related to adrenergic effects.

Noradrenaline has a pronounced stimulatory effect on adipose tissue lipolysis (Ostman et al., 1969). The FFA response to noradrenaline has been shown to be proportional to the adiposity of individuals (Katzeff et al., 1986) which was somewhat (nonsignificantly) greater in the diabetic subjects in this study. In NIDDM there is reduced fractional clearance of FFA (Taskinen et al., 1985). Despite these considerations, there was no difference in the FFA responses between the two groups during noradrenaline infusion.

In addition to causing abnormal metabolic and endocrine responses, noradrenaline administration resulted in greater increases in blood pressure in the diabetic subjects. The pressor effect of noradrenaline is caused by peripheral vasoconstriction which affects most vascular beds and leads to increased arterial resistance (Greenway, 1982). These vasoconstrictor effects are mediated predominantly by peripheral $alpha_1$-adrenergic receptors although vasoconstrictor $alpha_2$ -adrenergic receptors which exist in vascular smooth muscle may have a role (Langer, 1981). Therefore, the cause of the abnormal pressor responses could be due to increased peripheral sensitivity of the peripheral vasoconstrictor mechanism in NIDDM. Baroreflex activity buffers increases in blood pressure by causing reflex slowing of the heart rate. This is mediated by both increased vagal activity and a lessening of sympathetic activity (Greene and Bachard, 1971). The increased pressor responses in the diabetic subjects were accompanied by a reduction in heart rate similar to that seen in the nondiabetic subjects indicating that there was less slowing of heart rate per unit pressure elevation.

Therefore, the cause of the abnormal pressor responses in the subjects with NIDDM could be due to peripheral hypersensitivity of the vasoconstrictor

adrenergic receptor-effector mechanism or alternatively subnormal baroreflex activity.

6.6.2 Tyramine study

Oral administration of the sympathomimetic agent tyramine caused an increase in plasma glucose levels in addition to the expected rise in blood pressure in both groups of subjects. As with noradrenaline, there was a positive correlation between the glycaemic and pressor responses after tyramine administration. The turnover data showed that the rise in blood glucose was due to an increase in hepatic glucose production rather than effects on peripheral glucose disposal. Thus pharmacological sympathetic neural activation causes hyperglycaemia by stimulating hepatic glucose production.

Tyramine had no significant effects on mean arterial insulin or glucagon levels suggesting that tyramine is less potent at the islet than noradrenaline. However, the mode of administration resulted in peak cardiovascular effects being reached at variable time points. This variation in the time of pharmacological action may have obscured any initial inhibition of insulin secretion, as occurred with noradrenaline, before the counteracting effects of increased glycaemic stimulation of the beta cell. Nevertheless, the absence of significant pancreatic hormonal responses suggests that the glycaemic effects of tyramine were more likely to be due to direct stimulation of the liver rather than indirect hormonal actions. These findings lend further support to the hypothesis that the hepatic sympathetic innervation plays a role in blood glucose regulation.

There was no significant difference in the magnitude of the cardiovascular or metabolic responses to tyramine between the two groups of subjects.

Therefore, in contrast to the response to exogenous noradrenaline, the diabetic subjects did not appear to be hyperresponsive to tyramine.

However, it is possible that these studies failed to uncover a difference in sensitivity which really existed due to the mode of administration of the agent. In support of this latter possibility, the diabetic subjects (as a group) had somewhat greater pressor and glycaemic responses although they received less tyramine on a per kilogram basis than the nondiabetic subjects (9.8±0.5 vs 11.2±0.6 mg tyramine/kg, $p<0.1$). This dosage difference was due to the diabetic group being heavier although matched for body mass index. However, correction for this factor by expressing the responses in terms of mg/kg of administered tyramine did not uncover any significant differences in the responses. Furthermore, the plasma tyramine responses which varied considerably in magnitude, were on average similar between the diabetic and nondiabetic subjects. The noradrenaline, MHPG and DHPG responses following oral tyramine were also very similar between the two groups and indicate that on average, the diabetic and nondiabetic subjects received a similar sympathomimetic stimulus.

It is stressed that the plasma tyramine responses should be interpreted with caution. The assay was qualitative only as no suitable internal standard was available for use in the mass spectrograph. Therefore the results provide only a guide to the timing and magnitude of the absorption of the administered tyramine. Nevertheless there was a significant positive correlation between the glycaemic responses and the measured tyramine levels supporting the use of the assay as a measure of the delivered sympathetic stimulus.

The magnitude of the cardiovascular responses to tyramine was comparable to that seen with noradrenaline infusion. However, the effect of tyramine on glycaemia was considerably less than with noradrenaline. Tyramine has been shown *in vivo* to have relatively weak noradrenaline-releasing actions in the liver compared with its effects in the heart (Garceau and Yamaguchi, 1982). The liver is particularly rich in monoamine oxidase and metabolic degradation of tyramine within the liver may explain its low potency on hepatic glucose production (Tipton, 1973). The observation that monoamine oxidase inhibiting drugs potentiate tyramine-induced noradrenaline release in the liver support this suggestion (Garceau and Yamaguchi, 1982).

Tyramine and noradrenaline increase blood pressure by different mechanisms (Scriven et al., 1983; 1984). Tyramine causes endogenous neuronal noradrenaline release and stimulates both alpha-adrenergic (vasoconstrictor) and ß-adrenergic (vasodilator) receptors in the blood vessels. These mutually antagonistic effects in the peripheral vasculature may cancel each other out and explain why the pressor effect of tyramine is predominantly due to its inotropic cardiac effect (Scriven et al., 1983). Circulating noradrenaline on the other hand, stimulates both extrasynaptic $alpha_2$- and postsynaptic $alpha_1$-adrenergic receptors (both vasoconstrictor) in vascular smooth muscle (Langer, 1981), has relatively weak ß-receptor actions, and its pressor effects are mainly due to peripheral vasoconstriction (Scriven et al., 1983). In a similar fashion, the relative difference in potency of tyramine and noradrenaline on metabolic functions could be explained by differential activation of adrenergic receptor populations.

Tyramine caused an increase in FFA levels which was of similar magnitude in both the diabetic and nondiabetic subjects consistent with the known effects of

sympathetic activation on lipolysis. Interestingly, the magnitude of the FFA responses was also considerably less than that seen with noradrenaline infusion indicating that circulating noradrenaline may be a more potent lipolytic stimulus than sympathetic nervous activation. In adipose tissue, vascular alpha-adrenergic receptors are located close to the adrenergic nerve terminal and can be stimulated by sympathetic nerve firing, whereas the vascular ß-receptors are subject to greater influence by circulating noradrenaline levels because of their different location (Rosell and Belfrage, 1975). Therefore, differential stimulation of adipose tissue adrenergic receptors may explain the different potencies of tyramine and noradrenaline on lipolysis.

6.6.3 Psychological stress

The computer games caused elevations in blood pressure, heart rate and a subjective estimate of increased anxiety in all subjects. In addition, there was a significant increase in FFA levels in both groups of subjects. There were however, no significant changes in cortisol and adrenaline secretion. Thus there was considerable evidence for sympathetic neural activation during the computer games although there was no significant release of stress hormones.

It is likely that on average, the degree of psychological stress induced by the stressor was not severe and the lack of significant stress hormone responses supports this. However, there was considerable variation in the responses and it is clear that some individuals experienced considerable degrees of psychological stress. One of the diabetic subjects suffered a vaso-vagal attack within 5 minutes of ceasing the computer games and several others reported feelings of distress. Furthermore, there were positive correlations between several independent measures which provide good evidence that some subjects experienced significant psychological tension. Thus there were positive relationships between the subjective anxiety score and the FFA, heart rate and adrenaline responses; between the FFA responses and the cardiovascular responses and between the cortisol and diastolic blood pressure responses. These relationships suggest that the subjects who experienced the greatest degrees of psychological stress also had increases in circulating cortisol and adrenaline levels during the stress condition.

Despite the evidence of significant sympathetic neural activation and the likelihood that some subjects had modest increases in stress hormone secretion, there was no significant increase in mean blood glucose levels and

no change in glucose turnover in either group. In addition, in no individual diabetic subject was any significant glycaemic response to the stressor observed. Therefore the present studies do not indicate that short-term psychological stress causes hyperglycaemia in subjects with NIDDM.

The FFA responses during stress were, on the whole, of similar magnitude to that seen following oral tyramine. In addition, the (nonsignificant) changes in pancreatic hormone levels seen in the diabetic subjects (fall in insulin and C peptide and rise in glucagon levels) were similar to that seen after oral tyramine. Despite these similarities in (a) the apparent intensity of the two stimuli as witnessed by the FFA responses and (b) the pancreatic hormonal responses in the diabetic subjects, there was no effect on glycaemia with stress but a modest increase in blood glucose levels with tyramine. This suggests that there may be a difference between isolated sympathetic nervous system stimulation and the physiological stress response, possibly due to some counteracting modulatory influence.

The lack of effect of psychological stress on glycaemia in this study does not support the hypothesis that there is increased susceptibility to psychological stress in NIDDM. The effects of severe life-stresses, which have been described in diabetic subjects, may be due to other factors such as changes in compliance with treatment or diet. On the other hand, it is possible that the stresses of real life, particularly if experienced for prolonged periods, could be more salient inducers of altered metabolism in diabetic subjects. Furthermore, the subjects who participated in these studies were not selected on psychological criteria and it is possible that subjects who are anxiety-prone or who have certain personality types (e.g. type A personality) could be susceptible to psychological stress.

Psychological stress appears to be a potent stimulus for FFA release which could have metabolic implications in NIDDM. Fatty acids may inhibit or retard cellular glucose metabolism (Randle et al., 1963) and cause a reduction in insulin sensitivity (Ferrannini et al., 1983) and glucose tolerance (Rousselle et al., 1982). Chronic psychological stress causing chronically elevated FFA levels might aggravate existing defects in glucose metabolism in subjects with NIDDM.

The nondiabetic subjects had greater blood pressure, heart rate, FFA and subjective responses to the stressor than the subjects with NIDDM. This difference in response may have occurred because of differences in the selection of subjects for these studies or may be related to the wide variation in response to the stressor compounded by the small number of subjects. Interestingly, another group have reported similar findings. Psychological stress was induced (using computer games) during oral glucose administration and significantly delayed the glucose curve in nondiabetic subjects. In contrast, subjects with NIDDM had reduced heart rate responses to the stress and no delay in glycaemia (Wing and Blair, 1987). These results combined with the present study suggest the intriguing possibility that subjects with NIDDM may be in some way resistant to the effects of psychological stress.

6.6.4 Basal relationships

There was no difference in basal arterial levels of noradrenaline or noradrenaline metabolites between the diabetic and nondiabetic subjects. This indicates that there was no increase in basal sympathetic tone in the subjects with NIDDM. However, there was a significant positive correlation between fasting plasma glucose and DHPG levels in the diabetic subjects which was not seen in the nondiabetic subjects. This relationship was also present when the data from all the studies were tested (i.e. with 4 values for each subject, n=30). There was no correlation between plasma glucose and noradrenaline nor between glucose and the noradrenaline metabolite, MHPG.

Circulating DHPG is derived mainly from sympathetic nerves and DHPG levels increase during stimulation of these nerves due to reuptake of noradrenaline into the the nerve endings and subsequent conversion to DHPG and release (Goldstein et al., 1988). It has been suggested that measurement of DHPG levels in conjunction with measurements of noradrenaline levels may provide additional information about sympathetic function (Goldstein et al., 1988). In the present studies, there were strong positive correlations between basal noradrenaline and DHPG levels and between DHPG and MHPG levels, indicating that these parameters are intimately linked.

The relationship between plasma glucose and DHPG in the diabetic subjects suggests that basal sympathetic tone may be a determinant of basal hyperglycaemia in NIDDM. One cannot determine whether such a relationship is causal and there may be other explanations. Hyperglycaemia could stimulate the sympathetic nervous system in NIDDM, followed by downward

readjustment (downregulation) to normal levels of sympathetic tone by some modulatory influence.

The relationship between DHPG and plasma glucose remained significant in a multiple regression which included insulin and glucagon (as the ratio). When substituted for DHPG, MHPG also correlated independently with plasma glucose in a multiple regression. These results emphasise the possibility that basal sympathetic tone may be an independent correlate of hyperglycaemia in NIDDM which is not mediated by pancreatic hormonal effects.

In contrast to other reports, the diabetic subjects did not have elevated glucagon, FFA or growth hormone levels. The subjects had only modest hyperglycaemia and these abnormalities may only occur with more substantial hyperglycaemia. There were negative relationships between plasma glucose and glucagon and between R_a and glucagon in the diabetic subjects. These data argue against a role for glucagon in the causation of hyperglycaemia and rather suggest that hyperglycaemia suppresses basal glucagon secretion in NIDDM.

6.6.5 Effects on cortisol, ACTH and prolactin

Changes in several hormones were observed during these studies which may not be directly related to carbohydrate metabolism. These will be discussed in the present section.

During noradrenaline infusion there was a significant fall in cortisol levels in both the diabetic and the nondiabetic subjects. There was also a fall in ACTH levels which achieved statistical significance when the data from all subjects were combined. Thus the fall in cortisol levels was most likely due to an inhibition of pituitary ACTH secretion.

The action of catecholamines on ACTH secretion is controversial. Noradrenaline could have effects within the hypothalamus or at the corticotroph (or both sites) and central as well as peripheral noradrenergic neurones must be considered.

Several studies have reported that intravenous noradrenaline has no effect on circulating cortisol or ACTH levels (Ganong, 1980; Jezovah et al., 1987). Interestingly, Schade and Eaton (1977, 1978) reported nonsignificant falls in cortisol levels compared with control in normal and insulin dependent subjects during their noradrenaline infusion studies. Noradrenaline can potentiate the effect of corticotropin-releasing hormone (CRF) on ACTH release from pituitary tissue *in vitro* (Vale et al., 1983) indicating a possible modulatory role for circulating noradrenaline which would increase not decrease ACTH release. Recently, it has been suggested that circulating catecholamines may play a role in the subsequent reduction of circulating ACTH levels which occurs during continued or prolonged stress (Kvetnansky et al., 1987). The effect on ACTH seen in the present studies could be related to this phenomenon.

Ganong (1980) concluded that centrally released catecholamines inhibited stress-induced ACTH secretion. This was shown following intraventricular noradrenaline and tyramine injection (Ganong, 1980; Van Loon et al., 1971). However several studies have concluded that central noradrenergic neurones cause an increase in CRF and/or ACTH secretion and that activation of central noradrenergic systems plays a major role in causing stress induced ACTH secretion (Smythe et al., 1983; Guillaume et al., 1987; Jezovah et al., 1987). Therefore, it is not clear from the literature whether the inhibitory effects of noradrenaline on cortisol secretion demonstrated in the present studies could be due to effects on central neurones.

Another mechanism to explain these results relates to the central neural effects of hyperglycaemia. Grunstein in his doctoral thesis (1985) reported that hyperglycaemia induced by a hyperglycaemic clamp was accompanied by a fall in ACTH and cortisol levels. Other studies from the same group indicate that central noradrenergic neurones are subject to negative feedback inhibition by blood glucose levels (Smythe et al., 1984). As ACTH (or CRF) appears to be regulated by the central noradrenergic system (Smythe et al., 1983) a rise in blood glucose levels may feed back on this system and inhibit ACTH (or CRF) secretion. Thus it is possible that the fall in ACTH and cortisol levels observed in this study was due to inhibition by the resultant hyperglycaemia rather than a modulatory effect of noradrenaline *per se.* If this hypothesis is correct, the present studies indicate that the putative relationship between circulating glucose levels and central noradrenergic neurones is operative in NIDDM although acting at a higher level of glycaemia.

Prolactin levels were also influenced during these studies. There was a significant fall in mean circulating prolactin levels during psychological stress in

the diabetic subjects which was not seen in the nondiabetic subjects. This difference is particularly striking given that the diabetic subjects exhibited lesser cardiovascular, metabolic and psychological responses than the nondiabetic subjects during the computer games.

The response of prolactin secretion to stress has not been extensively studied although increases in prolactin levels have been reported after physical and emotional stress (Corenblum and Taylor, 1981). Therefore, suppression of prolactin during psychological stress in diabetic subjects is difficult to explain. These results are consistent with an abnormal hypothalamic regulation of prolactin release and could indicate increased activity of the dopaminergic system in NIDDM.

Prolactin levels fell variably during tyramine administration although the data approached statistical significance when the groups were combined. In rats, prolactin release following a variety of stimulants has been shown to be inhibited by tyramine (Becu-Villalobos et al., 1985). The site of action of tyramine is unclear but may be within the hypothalamus where tyramine can release dopamine from nerve terminals (Van Voigtlander and Moore, 1973) or it may act directly at the pituitary level (Becu-Villalobos et al., 1987).

6.6.6 Mechanism for increased sensitivity to noradrenaline

The combination of abnormal cardiovascular, metabolic and possibly hormonal responses to noradrenaline suggests that there was a generalised hypersensitivity to the noradrenergic stimulus in the diabetic subjects and the correlation between the glycaemic and pressor responses suggests that there was a common mechanism for this hypersensitivity.

Noradrenaline was administered on a per kilogram basis and as the nondiabetic subjects were on average lighter they received a lower mean dose of noradrenaline. It is unlikely that any differences could have been related to this dosage factor for the following reasons. Firstly, the groups were matched for body mass index and therefore the subjects received a dose of noradrenaline reasonably matched for lean body mass. Secondly, circulating noradrenaline levels achieved during the infusions were virtually identical between the two groups. Thirdly, several parameters responded identically to the infusion, e.g. the FFA and MHPG responses, indicating that the noradrenergic stimulus received by the diabetic and nondiabetic subjects was similar.

It is possible that the exaggerated glycaemic responses could be due to insufficient compensatory insulin secretion, analogous to the situation where adrenaline is administered to subjects with IDDM (Berk et al., 1985). In addition, diabetes is often accompanied by alterations in sodium metabolism, abnormalities of the renin-angiotensin-aldosterone system (De Chatel et al., 1977) and vessel wall thickening secondary to diabetic vasculopathy (Folkow, 1971). Any or all of these might contribute to abnormal pressor responsiveness. Thus it is conceivable that the combination of beta cell

dysfunction and changes in blood pressure regulatory systems related to hyperglycaemia and chronic diabetes could account for the abnormal responses to noradrenaline.

However, it has been shown that noradrenaline, unlike the situation with adrenaline administration, does not cause abnormal glycaemic responses in subjects with IDDM (Schade and Eaton, 1978). Thus if noradrenaline administration does not cause exaggerated glycaemic responses despite the absence of beta cell function, it seems unlikely that impaired insulin secretion explains the abnormal glycaemic responses in subjects with NIDDM.

Similarly, noradrenaline infusion does not cause abnormal pressor responses in subjects with insulin dependent diabetes (Christlieb et al., 1976; Scobie et al., 1987). Christlieb et al (1976) found that patients with longstanding diabetes and retinopathy had mildly increased pressor responses to noradrenaline and angiotensin II compared with patients with equally longstanding but uncomplicated IDDM. However, the responses were not increased compared with nondiabetic controls. These studies suggest that abnormal pressor responsiveness does not occur in subjects with longstanding IDDM despite the probable presence of abnormalities of sodium and renin-angiotensin metabolism and vessel wall abnormalities. These studies with IDDM indicate that the findings of the present study are probably not due to nonspecific abnormalities of blood pressure regulation and impaired beta cell function.

In contrast to the studies cited above, increased pressor responses to noradrenaline have been recorded in hypertensive (Weidmann et al., 1979) and normotensive diabetic subjects (Beretta-Piccoli et al., 1981). These

researchers studied a heterogeneous population of diabetic patients and did not distinguish between IDDM and NIDDM in their reports. Most of their patients were not on insulin therapy and presumably had NIDDM. Furthermore, on inspection of their data, the normotensive younger subjects (who presumably were more likely to have IDDM) appear to have had normal pressor responses (see Figure 3, Beretta-Piccoli et al., 1981). Thus the available data, taken in conjunction with the results reported here, suggest the possibility that increased pressor responsiveness occurs in subjects with NIDDM but not in subjects with IDDM.

Some animal models of (non-insulin dependent) diabetes, e.g. the genetically obese (ob/ob) mouse and the kk mouse, have increased glycaemic responses to adrenergic stimuli compared with nondiabetic mice (Surwit et al., 1984; Fujimoto et al., 1981). The genetically obese mouse, which is characterised by hyperglycaemia, hyperinsulinaemia, insulin resistance (Bray and York, 1979) and CNS abnormalities not dissimilar from those seen in VMH lesioned rodents (Jeanrenaud, 1985), exhibits exaggerated glycaemic responses to immobilisation stress (Surwit et al., 1984) and adrenaline administration (Kuhn et al., 1987). Interestingly, the islet circulation of these obese mice is hypersensitive to intravenous noradrenaline and adrenaline which cause an inhibition of islet capillary blood flow (Rooth and Taljedal, 1987).

The cause of the apparent hypersensitivity to noradrenaline in the subjects with NIDDM cannot be determined from the present studies but there are several possibilities. For instance, an increased sensitivity of cellular adrenergic receptor mechanisms could be present in NIDDM. Denervation sensitivity occurs in various types of autonomic neuropathy and is characterised by hypersensitive pressor responses (Bannister et al., 1979). This phenomenon is

related to decreased circulating levels of noradrenaline and increased vascular alpha-receptor numbers (Davies et al., 1982). In subjects with IDDM and severe symptomatic autonomic neuropathy, increased pressor responses to noradrenaline (Scobie et al., 1987) and increased glycaemic responses to adrenaline administration (Hilsted et al., 1987) have been demonstrated. Scobie et al (1987) also found increased pressor responses to tyramine in diabetic autonomic neuropathy. Asymptomatic abnormalities of autonomic nerve function can be demonstrated, using sensitive tests, to be present in many recently diagnosed patients with either NIDDM or IDDM (Pfeiffer et al., 1984). Therefore it might be argued that the abnormal responses found in the present study may have been due to denervation supersensitivity of adrenergic receptors secondary to subclinical autonomic nerve dysfunction.

However, none of the subjects with NIDDM had symptoms or signs suggesting the presence of autonomic neuropathy. In addition, the group included subjects with recently diagnosed diabetes, or who did not have peripheral neuropathy and the majority had normal heart rate variation (a sensitive test of parasympathetic nerve function). Furthermore, denervation sensitivity is associated with subnormal basal levels of noradrenaline in diabetic and nondiabetic autonomic neuropathy (Hoeldtke and Cilmi, 1984; Dejgaard et al., 1986; Eckberg et al., 1986), excessive pressor responses to tyramine (Scobie et al., 1987) and excessive lipolysis during catecholamine administration (Hilsted et al., 1987; Engelman et al., 1964). The diabetic subjects in the present studies had normal basal noradrenaline levels, normal pressor responses to tyramine and normal lipolytic responses to noradrenaline. Thus these subjects had no evidence of having sympathetic autonomic neuropathy and although not definitely established, it appears likely that denervation

supersensitivity occurs in subjects with severe, symptomatic autonomic neuropathy rather than with subclinical autonomic dysfunction. Further support for this contention comes from the demonstration of normal pressor responses to noradrenaline in subjects with longstanding, uncomplicated IDDM (Christlieb et al., 1976; Scobie et al., 1987) most of whom would have been likely to have had subclinical abnormalities of autonomic function.

In humans with uncomplicated diabetes (IDDM and NIDDM) normal numbers of ß-adrenergic receptors (Reckless and Galton, 1976) and normal and reduced alpha-adrenergic receptor numbers (Reckless and Galton, 1976; Spalding et al., 1986) have been reported. Thus the available data do not support the existence of increased peripheral adrenergic receptor sensitivity in uncomplicated diabetes (either increased receptor number or binding) to explain the increased sensitivity to noradrenaline in the present study, although the possibility is by no means excluded.

Many other modulating influences may alter adrenergic neurotransmission at the peripheral receptor (Westfall, 1980). Inhibition by noradrenaline itself is the most firmly established of these modulatory influences and inhibition by presynaptic $alpha_2$-inhibitory adrenoreceptors acts at physiological levels of noradrenaline. Other factors may enhance peripheral adrenergic activation including presynaptic ß-adrenergic receptors and angiotensin. Theoretically an abnormality of any of these factors could be operative in NIDDM to explain the enhanced effects of noradrenaline administration.

Modulation of adrenergic activity could occur centrally as well as peripherally. Of considerable interest was the transient increase in arterial MHPG levels during noradrenaline administration. DHPG levels were only measured at

baseline and at the end of the noradrenaline infusion and therefore could also have increased transiently. However, other groups have established that DHPG levels do not alter during comparable noradrenaline infusions (Brown, 1984; Eisenhofer et al., 1987, Goldstein et al., 1988) and they increase only slightly after high dose noradrenaline administration (Goldstein et al., 1988). The lack of an increase in DHPG indicates that the major proportion of administered noradrenaline is metabolised by catechol-O-methyl-transferase (COMT) to normetanephrine and dihydroxy mandelic acid rather than by neuronal reuptake (where COMT is absent, Langer and Enero, 1974) and demethylation to DHPG and MHPG. Thus it seems unlikely that the elevated MHPG levels in this study are derived from the metabolism of the administered noradrenaline in the absence of changes in its immediate precursor molecule, DHPG.

There has been considerable debate concerning the possibility that peripheral MHPG levels may reflect CNS noradrenergic neuronal activity (Maas, 1984). MHPG is the principle metabolite of noradrenaline in the brain and can diffuse freely out of the CNS into the peripheral circulation (Maas et al., 1979). CNS production of MHPG has been estimated to account for approximately 30% of the total amount of MHPG in the peripheral circulation and the rest comes from peripheral nerves (Kopin et al., 1984). It has been suggested that there is a functional interaction between peripheral and central noradrenergic systems and that they act in concert (Maas, 1984). In support of this interaction, peripheral concentrations of MHPG have been shown to correlate closely with hypothalamic noradrenergic activity in the rat (Grunstein et al., 1986) and correlations between plasma MHPG and CSF noradrenaline and MHPG have been found in humans (Jimerson et al., 1981).

In the present studies, the increase in peripheral MHPG levels during peripheral noradrenaline administration is consistent with activation of central noradrenergic neurones with the resultant release of MHPG into the peripheral system. There is support for this conclusion in that peripheral noradrenaline administration has been shown to cause an increase in hypothalamic noradrenergic neuronal activation in rats (GA Smythe, personal communication).

These data therefore support the possibility that some of the effects of noradrenaline administration could be due to central actions. However, there was no difference in the MHPG responses between the diabetic and the nondiabetic subjects to indicate that the abnormal responses of the diabetic subjects were related to an abnormality of central noradrenergic regulation.

The beta cells in NIDDM may be subject to chronic excessive sympathetic activation which causes some of the impairment in insulin secretion (Robertson et al., 1976; Broadstone et al., 1987). Broadstone and coworkers (1987) considered that the effect of alpha-adrenergic blockade in improving insulin secretion in NIDDM (Robertson et al., 1976) was probably due to effects on the presynaptic alpha$_2$-adrenergic receptors. In the present studies, the abnormal responses to noradrenaline could be explained if the noradrenergic stimulus was added on to a background of increased sympathetic activity. However, there was no difference in basal levels of noradrenaline or its metabolites to suggest increased resting sympathetic tone. Alternatively, sympathetic tone is normally suppressed during noradrenaline infusion (Izzo, 1982), and it is conceivable that there is resistance to this suppression in NIDDM.

Increased pressor responses can be due to subnormal baroreflex buffering of blood pressure elevations, resulting in less heart rate slowing per unit elevation of blood pressure. This indicates either subnormal vagal activation or subnormal withdrawal of sympathetic activity as the arterial pressure rises. Although not formally assessed, the heart rate responses in the present studies were similar between the diabetic and nondiabetic subjects suggesting that subnormal baroreflex buffering may have been present in the diabetic subjects. This would be consistent with reduced withdrawal of sympathetic activity in the diabetic subjects.

At this point, it is worthwhile considering the hypertension literature as there may be several parallels between NIDDM and essential hypertension. There is evidence that increased alpha-adrenergic vasoconstriction may have a role in causing peripheral vascular resistance and initiating hypertension in human essential hypertension (Tuck, 1986). The evidence for this includes increased pressor responses to noradrenaline in hypertensive subjects (Philip et al., 1978; Meier et al., 1981). Various causes of this alpha-adrenergic component have been considered including increased sympathetic drive (Esler et al., 1977), increased alpha-receptor sensitivity (Amann et al., 1981), non-specifically increased responses to noradrenaline due to structural vessel wall abnormalities (Folkow, 1978) or abnormal cellular cation metabolism (Wessels and Zumkley, 1980). A recent study evaluated all the above mechanisms in a group of hypertensive subjects and concluded that increased sympathetic drive was the major reason for the increased vascular alpha-adrenergic vasoconstriction in hypertensive subjects and that vascular hyperresponsiveness was probably secondary to structural vessel wall changes (Egan et al., 1987).

Hypertension, not due to diabetic nephropathy, occurs more commonly than expected in NIDDM (Drury, 1984). Several studies have demonstrated higher than average blood pressures in individuals with impaired glucose tolerance (Jarrett, 1978; Garcia et al., 1974; Ostrander et al., 1980). In addition, increased blood pressures have been reported in normoglycaemic offspring of parents with NIDDM (Berntorpe and Lindgarde, 1986). It has been suggested that hypertension and NIDDM may share a common aetiology and that this could be increased central sympathetic activity (Drury, 1984).

Therefore the hypothesis that NIDDM is characterised by chronic excessive sympathetic neural activity may explain the increased pressor and glycaemic responses to noradrenaline in the present study and the association between NIDDM and hypertension. If this hypothesis is correct, then the present studies expand the number of tissues subject to abnormal sympathetic drive from the beta cells as suggested by others (Robertson et al., 1976; Broadstone et al., 1987) to the vasculature and the liver. The finding of a correlation between basal DHPG and blood glucose levels in the diabetic subjects is consistent with the above hypothesis, although the finding of normal circulating catecholamine levels does not support the presence of increased sympathetic activity.

The increased sensitivity to noradrenaline infusion in the diabetic subjects contrasts with the responses to tyramine administration and psychological stress. The major difference between the former and the latter stimuli which might explain this is that tyramine and stress both stimulate endogenous neuronal noradrenaline release. Endogenous versus exogenous noradrenaline release probably stimulates different populations of adrenergic receptors and results in vastly different concentrations of circulating hormone although perhaps similar concentrations at or near neuronal synapses. In

addition, sympathetic activity during stress involves activation of central neuronal systems which may be subject to modulation by other factors.

In summary, the mechanism and cause of the excessive responses to noradrenaline cannot be ascertained from these studies. In addition, it is not clear why there appears to be normal responsiveness to tyramine administration and psychological stress in the face of the abnormal responses to noradrenaline. However, these phenomena could be related to increased activity of the sympathetic nervous system in NIDDM. If this hypothesis is even only partially correct, then it opens up the potential for new therapies aimed at reducing sympathetic activation in subjects with NIDDM.

6.6.7 Clinical implications

The results of these studies may have relevance to the clinical management of patients with NIDDM.

The demonstration of excessive glycaemic responses during noradrenaline infusion is likely to be clinically relevant during states of severe stress. Noradrenaline concentrations similar to those reached in the present studies can be seen during and after certain forms of surgery, myocardial infarction, burns or sepsis (Cryer, 1980). Excessive glycaemic responses to these levels of noradrenaline are likely to be a contributory cause of the severe hyperglycaemia sometimes seen in individuals with NIDDM who are exposed to such conditions. The data in the present studies also indicate that such individuals may also be exposed to the risk of excessive hypertensive responses during such stresses. Severe hyperglycaemia in these situations can usually be managed by insulin therapy, however measures aimed at

preventing or reducing sympathetic activation in subjects with NIDDM may have therapeutic potential in the setting of severe physical stress.

As discussed above, the responses to noradrenaline administration and the correlations between baseline glycaemia and sympathetic tone suggest the possibility that hyperglycaemia in NIDDM may be related in some way to sympathetic overactivity at the liver and/or the pancreatic beta cell. These findings indicate the possibility that chronic treatment with adrenergic blocking drugs or other methods aimed at reducing sympathetic nervous system activity could be useful in the treatment of patients with NIDDM.

CHAPTER SEVEN

Conclusions

7.1 Prandial insulin secretion

The studies detailed in chapters three to five are concerned with the early phase of prandial insulin secretion. In chapter three, the existence of cephalic responses which influenced both insulin secretion and blood glucose levels was demonstrated in nondiabetic, normal weight humans. These observations clearly indicate that insulin secretion in normal nonobese subjects is under some degree of neural control and also indicate that cephalic phase insulin secretion has a role in metabolic regulation. The results suggested the possibility that the cephalic phase might have some direct effect on blood glucose levels in addition to effects secondary to insulin secretion.

The responses elicited by the sensory stimuli were fairly subtle which suggests that cephalic responses in the human may be relatively unimportant in metabolic regulation. However, studies of human cephalic responses are likely to underestimate the importance of neural regulatory mechanisms for two reasons. Firstly, the cephalic phase is defined as being complete once food reaches the upper gastrointestinal tract and the processes of digestion and nutrient absorption are underway. This arbitrary definition limits cephalic phase studies to the pure neural reflexes free from influences arising from the digestive phase or the effects of circulating nutrients. However, neural influences continue to act as long as there is any sensory input arising from food ingestion which means at least as long as eating continues (Berthoud, 1984). In humans, such stimulation will last for 10-15 minutes and often longer whereas the duration of the cephalic phase (as defined) is very much shorter.

The second reason why study of the cephalic phase may underestimate the importance of these neural reflexes is related to the experimental situation. These neural reflexes appear to be delicate and easily extinguished by stressful

circumstances (Powley and Berthoud, 1986) and it is likely that most research studies of this type in humans are accompanied by some degree of anxiety. The strong correlation between the subjective responses to the sensory stimuli and the blood glucose responses in the present studies suggest such an effect.

In summary, it seems quite probable that the neural component of insulin secretion is of greater magnitude during normal meal taking than can be elicited during experimental studies and this component may play a significant role in prandial carbohydrate regulation.

The studies in chapters four and five considered the problem of the delay in prandial insulin secretion in subjects with NIDDM. The studies in chapter four clearly indicated that the deficiency in early prandial insulin secretion is a major contributor to prandial hyperglycaemia and to a number of other metabolic abnormalities in NIDDM. These include post-meal hyperinsulinaemia, impaired suppression of free fatty acid levels and possibly the abnormal prandial increase in glucagon levels. In a further pilot study (chapter five), the impairment in meal-related thermogenesis which occurs in NIDDM was improved in all of the four subjects studied following correction of the deficiency in early prandial insulin secretion. This indicates the possibility that early prandial insulin secretion also has a role in regulating meal-related thermogenesis.

The constellation of prandial metabolic abnormalities which occur secondary to the delay in insulin secretion may worsen hyperglycaemia in subjects with NIDDM. There is considerable clinical and experimental data which indicates that hyperglycaemia itself causes impaired beta cell function and reduced insulin sensitivity (glucose toxicity). The subjects with NIDDM who participated in the present studies did not have severe fasting hyperglycaemia and yet prandial

hyperglycaemia was marked and prolonged in most of them. Prandial hyperglycaemia is likely to add considerably to the daylong hyperglycaemia experienced by these subjects and may, because of glucose toxicity, lead to a worsening of beta cell function and insulin resistance. Therefore prandial hyperglycaemia, secondary to the deficiency in early insulin secretion, could in the long term cause a worsening of basal hyperglycaemia in subjects with established NIDDM.

The other prandial metabolic abnormalities related to the the deficiency in early insulin secretion could also contribute adversely to the metabolic defects in NIDDM. Hyperinsulinaemia has been shown to cause reduced insulin binding *in vitro* (Insel et al., 1980) and causes insulin resistance *in vivo* (Rizza et al., 1985; Marangou et al., 1986). Elevated FFA levels lead to increased lipid oxidation (Thiebaud et al., 1982) which in turn may result in impaired glucose oxidation (Lillioja et al., 1985) and play a role in promoting insulin resistance (Ferrannini et al., 1983; Felber at al., 1987). Elevated FFA levels in NIDDM may also promote hepatic glucose production (Bogardus et al., 1984; Golay et al., 1987). Thus the reduced rate of suppression of FFA levels related to the deficiency in early prandial insulin secretion in NIDDM may have a role in promoting insulin resistance and excessive hepatic glucose production. Additionally, abnormally high prandial glucagon levels may also contribute to excessive hepatic glucose production (Gerich et al., 1974).

Finally, an impairment in meal-related thermogenesis means that energy storage is more efficient than normal and would contribute to increased fat deposition and obesity. A defect in the thermic effect of food could in part explain the tendency for diabetic subjects to gain weight excessively. As excess obesity causes insulin resistance, the deficiency in early insulin secretion may also indirectly contribute to insulin resistance in NIDDM due to a reduction in prandial energy expenditure.

There is some evidence in the literature which suggests that the deficiency in early prandial insulin secretion may occur before the onset of overt hyperglycaemia in subjects destined to develop NIDDM (see chapter 4.1). The loss of early prandial insulin secretion could be of importance in the pathogenesis of NIDDM. Such a defect occurring subclinically could cause prandial hyperglycaemia initially (impaired glucose tolerance), which because of the development of insulin resistance and impaired beta cell function, could contribute to eventual fasting hyperglycaemia.

The results of the studies summarised above suggest that giving a small dose of insulin with each main meal in a manner designed to correct the early prandial insulin deficiency could be therapeutically effective in subjects with NIDDM. Such therapy could potentially normalise or at least improve the prandial rise in blood glucose levels and might lead to improvements in hyperinsulinaemia and excessive glucagon levels, reduce FFA levels and improve meal-related thermogenesis. If, in the long term, improvements in insulin resistance and beta cell function followed, then a reduction in fasting hyperglycaemia might occur. Other potential advantages from this approach would be the use of relatively low doses of insulin and a reduced risk of therapy-associated weight gain.

Intranasal insulin administration was investigated to see if this form of insulin delivery could be used to supplement the early phase of prandial insulin secretion. Intranasal insulin administration was found to be an effective method of delivering physiological doses of insulin with meals. However, when given at the start of a meal, intranasal insulin was not effective in controlling prandial hyperglycaemia. The reason for this poor response was probably because the insulin was absorbed too rapidly into the systemic circulation. This conclusion further emphasises the importance of timing of prandial insulin secretion. When considered together, the

studies in chapter 4 indicate that insulin delivery systems which deliver insulin in a near-normal manner need to be developed to achieve optimal correction of prandial hyperglycaemia and related prandial metabolic abnormalities.

A pilot study was commenced (chapter five) to determine the mechanism of the action of early insulin secretion on prandial glucoregulation. Preliminary studies in a small number of subjects with NIDDM suggested that the major site of action was at the liver and that the improvement in prandial hyperglycaemia was most likely due to an earlier suppression of endogenous hepatic glucose production. This conclusion is preliminary and is subject to verification by future experiments. As increased hepatic glucose production is a major determinant of basal hyperglycaemia in NIDDM, nonsuppressibility of the liver is apparently a contributor to both prandial and basal hyperglycaemia in NIDDM. The positive correlation between the fasting blood glucose level and the prandial glycaemic response in the mixed meal studies in chapter four may reflect this mechanism.

The deficiency in early insulin secretion could influence hepatic glucose production in a number of ways. First, insulin could be acting directly on the liver where its concentration is greatest and the impaired suppression of hepatic glucose production would merely reflect an absolute deficiency of hormone at the target organ. Second, as argued in chapter three, the cephalic response may comprise a range of neural reflexes, one of which controls hepatic glucose production. The failure of early insulin secretion and the impaired suppression of hepatic glucose production could reflect a general failure of cephalic control mechanisms. Alternatively, as discussed in chapter one, insulin may be detected within the hypothalamus, causing a reduction in central noradrenergic activation and sympathetic activity with a resulting reduction in hepatic glucose output. A failure in early insulin secretion would impair this feedback mechanism.

The cause of the deficiency in early prandial insulin secretion in NIDDM was not determined in these studies. The literature suggests that the defect may be due to an abnormality of either neural regulation or insulinotropic gut hormone secretion rather than an abnormality of glucose stimulated insulin secretion (see chapter 1). Neither of these possibilities are mutually exclusive as, for instance, gut hormone secretion may itself be under neural influence. When the cephalic phase is eliminated in animals a number of metabolic abnormalities quite similar to those seen in NIDDM are produced. Thus it is possible that the early deficiency in prandial insulin secretion is due to a deficiency in the neural component of insulin secretion in NIDDM. This could be due to an abnormality in cephalic phase insulin secretion or could be part of a generalised derangement of cephalic responses. The implications of this hypothesis are that the hypothalamus and the autonomic nervous system have an important role in normal prandial metabolism and that abnormalities of this system could underlie some of the abnormalities of NIDDM.

An abnormality of neural control of insulin secretion could reside in either the afferent or efferent side of the reflex arc; could be a central (hypothalamic) or peripheral autonomic defect; or could be due to an alteration in the sensitivity of the beta cells to autonomic innervation. The studies in chapter six indicate that subjects with NIDDM may have increased sensitivity to adrenergic stimulation. This finding is consistent with several studies in the literature which suggest that beta cell secretion in NIDDM is influenced by increased adrenergic sensitivity or that the beta cells are subject to increased sympathetic tone. Therefore the delay in prandial insulin secretion in NIDDM could be due to an abnormality of the sympathetic nervous system component of beta cell regulation.

In summary, the initial phase of prandial insulin secretion is mediated at least partly by neural reflexes and appears to have considerable importance in prandial

glucoregulation, thermogenesis and a number of other metabolic processes. The early rise in insulin levels may act to initiate (or be part of) a series of metabolic events including suppression of hepatic glucose production, in order to prepare the animal for the influx of nutrients consequent on ingesting a meal. The failure of this early rise would appear to contribute significantly to the metabolic abnormalities of NIDDM. The implications are that the hypothalamus and the autonomic nervous system may have an important role in modulating normal prandial metabolism and that some of the metabolic abnormalities of NIDDM may be due to abnormalities of this system. Finally, insulin therapy which corrects the early phase of prandial insulin secretion may prove to be effective in subjects with NIDDM.

7.2 Basal glucoregulation and the sympathetic nervous system

The studies in chapter six considered the possibility that basal hyperglycaemia and excessive hepatic glucose production in NIDDM are due to either abnormal sympathetic activation of the liver or an abnormal sensitivity to such stimulation. This hypothesis was based on human and animal studies which indicated that the hepatic sympathetic nerves play an important role in the regulation of hepatic glucose production and other research which suggests the possibility of abnormal sympathetic nervous system activity in NIDDM.

The effect of psychological stress on plasma glucose levels was examined. Short-lived psychological stress failed to influence plasma glucose or hepatic glucose production in either nondiabetics or subjects with NIDDM. As a group, the stress caused relatively mild hormonal and metabolic responses however no adverse effect on blood glucose levels was seen in several subjects who experienced quite severe anxiety. These studies do not rule out the effects of more prolonged psychological stress on blood glucose and it is possible that certain anxiety-prone subjects may be especially susceptible to stress. However it may be that problems

of compliance with therapy and/or eating behaviour are responsible for the relationship between stress and hyperglycaemia in diabetes which has been reported in several epidemiological studies.

There was a marked FFA response to the psychological stress in both the diabetic and nondiabetic subjects. Elevated FFA levels are believed to have adverse effects on carbohydrate metabolism (via the glucose-fatty acid cycle, Randle et al., 1963) and chronic stress could further impair glucose metabolism in subjects with NIDDM by causing chronically elevated FFA levels.

Tyramine administration resulted in selective activation of the sympathetic nerves and caused increases in blood pressure and noradrenaline levels. Tyramine also caused an increase in hepatic glucose production and plasma glucose levels in nondiabetic and diabetic subjects. These metabolic effects probably occurred independently of pancreatic hormonal changes although an indirect action of tyramine was not definitely excluded. These findings indicate that pure sympathetic activation can promote hepatic glucose output and increase blood glucose levels and support a possible role for the sympathetic system in both normal and abnormal carbohydrate regulation.

Noradrenaline administration caused the expected hormonal, metabolic and cardiovascular effects. However the diabetic subjects had greater hyperglycaemic and pressor responses than the nondiabetic subjects to the noradrenergic stimulus.

The metabolic effects of noradrenaline may be due to direct effects on the liver or indirect effects due to changes in circulating levels of insulin and glucagon and both actions may normally be operative. The present studies do not distinguish which mechanism was responsible for the abnormal glycaemic responses in the diabetic subjects.

The underlying cause of the abnormal pressor and glycaemic responses in the diabetic subjects was not determined in these studies. It is possible that the difference in glycaemic responses was due to existing beta cell abnormalities and that the increased pressor responsiveness was due to non-specific abnormalities in the vasculature of the diabetic subjects. However there was a positive correlation between the glycaemic and pressor responses suggesting that there was a common mechanism for the hyperresponsiveness.

There could have been increased sensitivity of adrenergic receptors in the diabetic subjects secondary to autonomic neuropathy, although the patients studied did not have clinical evidence of that condition. There is some evidence for increased sympathetic modulation of the beta cells in subjects with NIDDM (Robertson et al., 1976; Broadstone et al., 1987). Thus it is possible that the noradrenergic stimulus was added on to a background of augmented peripheral sympathetic activation to cause apparently increased metabolic and pressor responses.

Alternatively, noradrenaline administration normally suppresses endogenous sympathetic tone (Izzo, 1982) and there could be a resistance to the suppressive effect in the diabetic subjects. This mechanism may underlie reduced baroreflex buffering of pressor responses in some hypertensive subjects. Baroreceptor function was not formally assessed in the present studies, but there was some suggestion of reduced baroreceptor activity in the diabetic subjects during noradrenaline infusion, i.e. heart rate responses were equal to nondiabetic responses despite greater pressor responses.

There were positive correlations between basal hyperglycaemia and measures of basal sympathetic activity in the diabetic subjects suggesting that there may be altered sensitivity to sympathetic activation in the basal unstimulated state in

NIDDM. These relationships are consistent with the possibility that excess basal hepatic glucose production in NIDDM (the main determinant of basal hyperglycaemia) may be due to abnormal regulation by the sympathetic nervous system.

The results of the studies in chapter 6 provide support for the hypothesis that the sympathetic nervous system plays a role in the production or maintenance of basal hyperglycaemia in NIDDM. This may occur as a primary abnormality in NIDDM, may develop secondary to the diabetic state or be related to diabetic autonomic neuropathy. In addition, abnormal sympathetic function could explain the deficiency in early insulin secretion during meals considered above. An increased sensitivity to the normal prandial increase in sympathetic activity could cause an inhibition of prandial insulin release in subjects with NIDDM.

These studies indicate that sympathetic activation probably contributes to the stress hormone response which causes hyperglycaemia during severe physical stress. Subjects with NIDDM are likely to be more susceptible than nondiabetic subjects to such physical stress and new therapeutic agents or modalities aimed at reducing sympathetic activation could be useful in this situation.

Further research is required to define the mechanism for the increased sensitivity to the noradrenergic stimulus and explore the role of sympathetic activation in contributing to basal hyperglycaemia in NIDDM. Should the mechanism be related to a chronic increase in sympathetic activity, then therapies aimed at reducing sympathetic tone could have value as chronic hypoglycaemic treatment in NIDDM.

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