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REGULATION OF DIET INDUCED BROWN ADIPOSE TISSUE THERMOGENESIS IN THE GENETICALLY OBESE (fa/fa) ZUCKER RAT.

A thesis presented for the degree of
Doctor of Philosophy
by
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ABSTRACT

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REGULATION OF DIET INDUCED BROWN ADIPOSE TISSUE THERMOGENESIS IN THE GENETICALLY OBESE (fa/fa) ZUCKER RAT.

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The obesity of the fa/fa rat is thought to result from an inability to activate the sympathetic drive to brown adipose tissue (BAT) in response to dietary signals, which may be related to an imbalance in the autonomic nervous system and a hypersensitivity to corticosterone. The role of central glucose metabolism in the control of diet induced BAT thermogenesis (as assessed by measurement of [^{3}H]GDP binding to BAT mitochondria) was investigated in lean and obese Zucker rats by use of 2 deoxy-D-glucose (2DG), an inhibitor of glucose metabolism.

2DG inhibited BAT GDP binding in lean, but not obese, Zucker rats. 2DG had no effect on GDP binding in animals of either phenotype exposed to cold, but decreased BAT function in lean overfed rats, suggesting that 2DG was preferentially inhibiting diet-induced thermogenesis. Adrenalectomy restored the ability of fa/fa rats to respond to 2DG with an inhibition of BAT GDP binding. This restoration was abolished by corticosterone replacement and lean animals treated with corticosterone were unable to respond to 2DG. BAT denervation, but not subdiaphragmatic vagotomy abolished the response of BAT to 2DG. Intracarotid infusion of 2DG depressed BAT and rectal temperatures and tended to reduce BAT GDP binding effects not seen after intrahepatic portal infusion of 2DG. It is suggested that 2DG acts centrally to inhibit BAT mitochondrial GDP binding through depression of efferent nerve activity to BAT, and the effects of 2DG on BAT are inhibited by corticosterone.

Adrenalectomy was shown to be equally effective at restoring energy balance and BAT function in obese rats fed either a high fat or high carbohydrate diet and normalised 32K protein levels in BAT mitochondria of obese animals. 32K protein concentration in BAT mitochondria was unaffected by diet but the molar binding ratio of GDP:32K protein was consistently higher on the high carbohydrate diet.

The role of brain opioids in the hyperphagia of obese rats was examined by opiate blockade by naloxone. Acute naloxone injection depressed food intake in young obese, but not lean rats,and increased BAT GDP binding in lean but not obese animals. Adrenalectomy abolished this increase in GDP binding in lean rats. Chronic naloxone treatment of adult Zucker rats had no effects on food intake, body weight gain or BAT function in rats of either phenotype.

The hypothesis that the obesity of the fa/fa rat arises from an imbalance in the autonomic nervous system due to glucocorticoid hypersensitivity in the hypothalamic glucose sensing areas is discussed.

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ABREVIATIONS.

ABTS	2.2'-azino-di-3[ethyl-benzthiazolin-sulfonate (6)] disodium salt
ACTH	adrenocorticotropic hormone (corticotropin)
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BAT	brown adipose tissue
BMR	basal metabolic rate
BSA	bovine serum albumin (fraction V)
cAMP	adenosine 3',5'-cyclic monophosphate
CCK	cholecystokinin
CoA	coenzyme A
CoQ	coenzyme Q
CRF	corticotropin releasing factor
CSF	cerebrospinal fluid
2DG	2-deoxy-D-glucose
DIT	diet induced thermogenesis
DMH	dorsomedial nucleus of the hypothalamus
DMN	dorsal motor nucleus of the vagus
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetracetic acid
FAD/H_2	flavin adenine dinucleotide/reduced form
FMN/H_2	flavin mononucleotide/reduced form
GABA	gamma amino butyric acid
GDP	guanosine diphosphate
GTG	gold thioglucose
HC	high carbohydrate diet
HEPES	2-(N-2-hydroxyethylpiperazine-N'-2)ethane sulphonic acid
HF	high fat diet
I.D.	internal diameter
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
32K	32000 Da uncoupling protein
LH	lateral hypothalamus
MOPS	3-(N-morphalino) propane-sulphonic acid
mRNA	messenger ribonucleic acid

NAD/H	nicotine adenine dinucleotide/reduced form
NCP	nitrocellulose paper
NST	non shivering thermogenesis
O.D.	outer diameter
PRD	purina rat diet
PNS	parasympathetic nervous system
PVN	paraventricular nucleus
RIA	radioimmunoassay
s.c.	subcutaneous
SDS	sodium dodecyl sulphate
SNS	sympathetic nervous system
T_3	triiodothyronine
T_4	thyroxine
TAC	2-thiazoylazo-p-cresol
TEMED	N N N' N' - tetramethylethylene diamine
TRH	thyrotrophic releasing hormone
TRIS	tris (hydroxymethyl) aminomethane
TSH	thyroid stimulating hormone
VMH	ventromedial hypothalamus
WAT	white adipose tissue

CONTENTS.

CHAPTER I. INTRODUCTION.

1.1 Obesity.

In Britain 32-39% of the adult population are classified as 'overweight', that is they weigh in excess of 15% more than their ideal body weight for height. (James,1983). The consequences of obesity in man are increased risks of developing coronary artery disease, hypertension, gall bladder disease and diabetes mellitus. The 1983 Royal College of Physicians report on obesity indicated that even mild obesity increases the incidence of morbidity and mortality, especially in people with familial predisposition to any of the above diseases.

Obesity occurs when positive energy balance is achieved, that is, where energy intake exceeds energy expenditure. Energy consumed as food in excess of expenditure will be stored in the body in the most economical form - fat. Positive energy balance could result from an increase in energy intake, a decrease in expenditure or a combination of both.

1.2.1 Control of Energy Intake.

Energy intake is determined by food intake which is dependent upon food availability and the type of diet. The central control of food intake is integrated mainly in the hypothalamus, particularly the ventromedial (VMH) and lateral (LH) hypothalamic areas and the paraventricular nucleus (PVN). Destruction of the VMH leads to hyperphagia and obesity (Brooks et al.,1946) whereas electrical stimulation of this area produces anorexia (Wyrwicka and Dobrzecka,1960). Conversely, lesions of the LH give rise to anorexia (Anand and Brobeck,1951) and electrical stimulation, to hyperphagia (Delgado and Anand,1953) which can cause reversible obesity (Steinbaum and Miller,1965). These observations led to the formulation of the dual centre hypothesis for the control of food intake (Anand and Brobeck, 1951; Stellar,1954). This hypothesis postulated that the LH acted as a feeding centre, the VMH as a satiety centre

and the balance between the two centres regulated food intake and feeding behaviour. Albert et al (1971) showed that there was interdependence between the LH and VMH. Knife cuts between the two centres caused overeating consistent with a tonic LH ('feeding centre') inhibition of the VMH ('satiety centre'). However, recent studies using retrograde tracing techniques to trace the path of hypothalamic projections have shown that there are no direct connections between the LH and VMH (Luiten et al 1986) so balance appears not to be maintained by direct communication between the two centres. It may be that the PVN is involved in regulating the balance between these two centres, as knife cuts between the PVN and LH also result in hyperphagia (Gold 1970).

Electrical stimulation of the VMH leads to an inhibition of ongoing feeding (Hoebel and Teitelbaum 1962) and an activation of catabolic responses such as increased glucose and fatty acid mobilisation, increased glucagon release and a depression of gastric acid secretion (Powley and Laughton 1981). LH stimulation on the other hand initiates feeding activity, can increase gastric acid secretion (Carmona and Slagen 1973) and stimulates anabolic responses (Shimazu and Ogasawara 1975, Powley and Laughton 1981). The responses elicited by VMH and LH stimulation are caused by opposing effects on the autonomic nervous system. The LH is effective primarily through activation of the parasympathetic nervous system (PNS) and the VMH, through the sympathetic nervous system (SNS) (see Bray and York, 1979; and Section 1.4.1).

The dual centre hypothesis for the control of food intake proved to be an over-simplification, as it was shown that damage to certain nerve fibre tracts close to the VMH and LH could produce similar effects to lesions of the two nuclei. The ventral noradrenergic bundle, a catecholaminergic tract arising in the preoptic and anterior hypothalamic areas, passes close to the VMH. Knife cuts in this region produced obesity in the absence of damage to the VMH itself (Gold 1973). Similarly, the dopaminergic nigrostriatal bundle

is in close proximity to the LH and damage to this tract causes similar effects to LH lesions (Marshall et al.,1974). It had also been demonstrated by this time that hyperphagia was not essential for the development of obesity caused by VMH lesions in weanling (Han and Liu,1966) or hypophysectomised (Han,1968) rats.

As previously mentioned, lesions of the PVN also cause hyperphagia and obesity in rats (Leibowitz et al.,1981). However, whereas development of the VMH obesity syndrome is not dependent on increased food intake but a parasympathetically mediated enhancement of anabolic responses, including hyperinsulinaemia (Powley and Opsahl,1974; Powley and Laughton,1981), PVN obesity is dependent upon hyperphagia and the development of hyperinsulinaemia depends mainly upon increased food intake (Leibowitz et al 1981, Tokunaga et al 1986b). The PVN is the seat of an α_2-noradrenergic mediated feeding rhythm which correlates with the diurnal pattern of circulating corticosterone (Bhakthavatsalam and Leibowitz,1986). The activation of feeding in sated animals by noradrenaline injected into the PVN is dependent upon the presence of corticosterone and is abolished by hypophysectomy or adrenalectomy (Leibowitz et al.,1984). Noradrenaline injected into the PVN in the presence of corticosterone is thought to disinhibit hypothalamic feeding centres normally held under tonic inhibition by the PVN (Leibowitz et al.,1981). The PVN thus acts as a 'satiety centre' and lesions in this area lead to hyperphagia and obesity.

The VMH and LH are thought to regulate food intake through a system of insulin sensitive glucoreceptor and glucosensitive cells. The LH and VMH contain glucosensitive and glucoreceptor neurones respectively (Oomura,1976). Activity of the LH glucosensitive cells is inhibited by local application of glucose and enhanced by insulin, free fatty acids and glucose antimetabolites. VMH glucoreceptors are stimulated by

electrophoretically applied glucose, an effect that is enhanced by simultaneous insulin administration. These VMH neurones are inhibited by **insulin** alone, free fatty acids and glucose antimetabolites (Oomura,1978). In fed animals, high levels of glucose and insulin and low serum concentrations of free fatty acids would be present, conditions which would stimulate VMH glucoreceptor neurones but inhibit LH glucosensitive cells,resulting in an inhibition of ongoing feeding activity. Conversely, starved animals would have low glucose and insulin levels and high free fatty acid concentrations, resulting in an inhibition of the VMH glucoreceptors and a stimulation of LH glucosensitive cells, resulting in the initiation of feeding. Glucagon has recently been demonstrated to act on the LH glucosensitive cells in a manner consistent with its suggested role as a satiety factor (Martin and Novin,1977; Geary and Smith,1983; Weick and Ritter,1986). LH glucose sensitive neurone activity is inhibited by electrophoretically applied glucagon at a dose which elicits no responses in VMH glucoreceptors (Inokuchi et al.,1986).

VMH glucoreceptor neurones are thought to have glucose receptor sites which trigger neuronal firing when glucose binds or is taken up. Insulin alone inhibits the activity of the neurones but activity is enhanced when glucose and insulin are applied simultaneously. Free fatty acids are thought to prevent glucose binding to its receptor, thus preventing the cell from firing (Oomura,1976). The glucose sensitive cells of the LH have insulin receptor sites which probably hyperpolarise the cell membrane (Oomura et al.,1974). Glucose inhibits and free fatty acids enhance firing rates by accelerating and slowing the ouabain-sensitive sodium pump respectively (Oomura,1976). The effects of glucagon are ouabain-sensitive, demonstrating that its effects are also mediated through sodium pump activity (Inokuchi et al., 1986).

The peripheral inputs contributing to the information assimilated in the central areas controlling food intake are

manifold. The gastrointestinal tract plays an important role in the modification of feeding behaviour. Some signals are generated via the vagus nerve from from the stomach, regulating gastric emptying (Hunt,1980), gastric satiety signals (Deutsh,1978), intestinal stretch receptors and duodenal 'nutrient receptors' (see Sullivan et al.,1981). Non-vagal signals generated by the gastrointestinal tract are hormonal, namely cholecystokinin (CCK), bombesin and serotonin (5-hydroxytryptophan, 5-HT). CCK is thought to be a satiety factor that has physiological actions at the peripheral (Niijima,1981; Collins et al.,1985) and central (Morley and Levine,1983) levels. Gut secretion of this peptide modifies hepatic and pancreatic vagal afferent activity (Niijima,1981),whereas central administration (Della-Fera and Baile,1979) significantly reduces food intake. Bombesin (Gibbs et al.,1979) and serotonin (Niijima, 1981) also appear to be peripheral satiety factors. Injections of bombesin in to the LH reduce food intake (Stuckey and Gibbs,1982). Serotonin is an important regulator of the VMH 'satiety' centre and intraventricular injections produce anorexia (Goldman et al.,1971). Other peptides and neuropeptides involved in feeding are thyrotropin releasing factor, calcitonin, neurotensin and corticotropin releasing factor. Three recent reviews including sections on the neuroendocrine control of feeding behaviour are by Morley (1980), Morley and Levine (1983) and Bray (1985). All these peptides are present in the brain at various hypothalamic nuclei (see Morley and Levine,1983).

Another class of neuropeptides involved in the central regulation of food intake are the endogenous opioids. Unlike the other neuropeptides mentioned, the dynorphins, enkephalins and endorphins increase food intake. Central injection of β-endorphin stimulates food intake (Grandison and Guidotti,1977) and injection of naloxone, a specific opiate receptor antagonist, suppresses food intake (Frenk and Rogers,1979; King et al.,1979). Peripheral injections of naloxone also suppress food and water intake and it has been suggested that this is a vagal effect (Jones and Richter,1981).

Naloxone is also known to vary macronutrient selection in rats by selectively depressing fat intake (Marks-Kaufman and Kanarek,1981). Both genetically obese (Margules et al., 1978) and VMH-obese (Levine et al.,1981) rodents show enhanced responsiveness to the naloxone-induced reduction of food intake, suggesting that increased levels of β-endorphin are present during hyperphagic situations. High levels of pituitary and plasma β-endorphin have indeed been discovered in genetically obese rodents (Margules et al., 1978). Indirect evidence for the involvement of the endogenous opioids in appetite regulation is that food deprivation provokes sufficient endogenous opiate release in the rat to effect a significant analgesic state (McGivern et al.,1979). Analgesia is perhaps the most widely known function of opiates. Circadian increases in β-endorphin levels coincide with nocturnal feeding phases, providing further evidence for the involvement of opioids in feeding (Frederickson et al.,1978). The role of the endogenous opioids in the regulation of appetite has been recently reviewed by Morley and Levine (1982).

Russek in 1963 first postulated a role for the liver in the control of food intake. Based on observations of latency to eat after glucose injection, he hypothesised the existence of a hepatic receptor system feeding information to the central nervous system regarding the difference in arterio-portal glucose, or the hepatic concentration of a related metabolite. This information subsequently affected the satiety of the animal and modified feeding behaviour.

Many studies on hepatic portal infusion of glucose, insulin, glucagon and glucose antimetabolites (primarily 2-deoxy-D-glucose) have shown that the brain monitors hepatic glucose metabolism via the vagus nerve and alters feeding behaviour accordingly (Novin and VanderWeele,1977; Niijima, 1981, 1984; VanderWeele,1985). Injections of glucose, CCK and serotonin caused a decrease in the afferent firing rate of the hepatic branch of the vagus nerve (Niijima,1981, 1983).

2-deoxy-D-glucose (2DG) when injected into the hepatic portal vein causes an increase in feeding (Novin et al.,1973) which is attenuated by subdiaphragmatic vagotomy. Hepatic portal glucose infusion decreases food intake (Russek, 1970 ; VanderWeele et al.,1976) in food-deprived animals, an effect which is again abolished by subdiaphragmatic vagotomy (see Novin and VanderWeele,1977).

Duodenal infusions of carbohydrate, protein, fat and fat metabolites will all suppress food intake in rats and rabbits, but after vagotomy only glucose infusion fails to elicit a suppression in feeding (Rezek and Novin,1976), implying that the hepatic vagal output is concerned primarily with carbohydrate metabolism.

Having discussed the role of hepatic glucoreceptors in the control of food intake, it should be mentioned that lipostatic and aminostatic systems have been postulated for the peripheral control of food intake as well as the glucostatic theory. The lipostatic theory is based on the premise that food intake is regulated in order to maintain body lipid stores (see LeMagnen,1976, 1983). The 'aminostat' monitors the proportions of amino acids in the diet and the protein content and alters food intake to maintain the intake of essential amino acids (Harper,1976). These two mechanisms would have a longer term control over food intake, whereas glucostatic effects have been reported as occurring within 10 minutes of glucoprivation (see VanderWeele,1985).

It is still unclear how the body monitors its energy stores as a regulatory factor for the control of food intake and energy balance. There have been suggestions that the fat depot status of an animal can be monitored by the hypothalamus either by sensitivity to hypothalamic lipid content (Van Itallie et al.,1977) or by a steroidal (Hervey , 1969) or prostaglandin (Baile et al.,1973) messenger quantitatively signalling the body's fat depot status to the hypothalamus. There is also some evidence to indicate that liver glycogen status is communicated to the hypothalamus,possibly via

vagal impulses (Sullivan and Triscari,1976 ; Novin and VanderWeele,1977).

The control of energy intake is precisely regulated. Normal animals will adjust their gross intake of a calorically diluted diet in order to maintain their energy intake (Adolph,1947; Carlisle and Stellar,1969). The observations that hyperphagia is not necessary to the development of VMH or genetic obesity (Han,1968; Goldman et al., 1974; Boulange et al.,1979) and that increased energy intake does not necessarily cause obesity (Rothwell and Stock, 1982),have led to the suggestion that the regulation of energy expenditure may be more important than the control of food intake in the development of obesity.

1.2.2 Energy Expenditure.

Energy Balance Equation.

Energy Storage = Energy Input - Energy Expenditure.

- Basal Metabolic Rate (BMR)
- Physical Work
- Thermogenesis (adaptive)
- Heat

As can be seen from the above equation, energy expenditure can be divided into several components. Resting metabolic rate (waking BMR) normally represents 65-70% of total daily energy expenditure (Danforth,1985; Owen et al.,1986) and is the metabolic cost of keeping the body alive. The resting metabolic rate of obese subjects has been reported as similar or increased in some studies (Ravussin et al.,1982; Schutz et al.,1984) and decreased in others (Miller and Parsonage,1975). Work (exercise) accounts for 15-20% of daily expenditure in individuals not engaged in heavy labour (Danforth,1985; Owen et al.,1986) and is another factor which has been variously reported as either reduced (Bullen et al.,1964) or unaffected (Stunkard and Postka,1962) in obese humans. It has also been suggested that the energy cost of activity is greater in obese people (Miller and Parsonage,1975).

Thermogenesis can be divided into cold-induced and diet-induced components. Cold induced thermogenesis can be further subdivided into shivering thermogenesis and non-shivering thermogenesis (NST). During exposure to an environment below thermoneutrality ($28^{o}C$ for man) heat is produced in acute situations by rapid asynchronous muscular contractions (shivering thermogenesis) and in longer term exposure this is gradually replaced by adaptive NST. Obese subjects seem less able to increase their metabolic rate (Quaade,1963) or maintain their body temperature (Andrews and Jackson,1978) in response to a drop in environmental temperature, suggesting a blunted thermoregulatory response.

Diet-induced thermogenesis can also be further subdivided into obligatory and adaptive components. Obligatory thermogenesis is associated with the energy released during the breakdown and assimilation of nutrients after a meal and depends on the energy composition of that meal. The obligatory component of thermogenesis can be calculated from the composition of a meal, and it has been demonstrated that the actual thermic effect of feeding is greater than predicted by this method (see Danforth,1985). This non-obligatory or adaptive component of thermogenesis, as assessed by the measurement of the thermic effect of a standard meal, has been observed to be depressed in obese humans (Schutz et al.,1984; Owen et al.,1986) and rodents (Rothwell et al.,1982), and is increased in lean, large eaters (Morgan et al.,1982) or growing hyperphagic rats (Rothwell and Stock,1979). Metabolic rate increases with long term overfeeding and decreases with starvation (see Garrow,1974). Genetic obesity in rodents is generally characterised by a depressed BMR, a lowered core temperature and sometimes a low level of spontaneous activity (see Bray and York, 1979; Bray,1984). These animals also exhibit a reduction in cold tolerance and their capacity for adaptive diet-induced thermogenesis(DIT) (Trayhurn and James,1978; Sclafani,1984). Energy expenditure in genetically obese rodents is discussed at greater length in Section 1.5.

Variations in food intake in experimental animals can cause large variations in energy expenditure without necessarily increasing body weight. This has been demonstrated in pigs (Gurr et al.,1980) and rats (Rothwell and Stock,1979 ,1982; Tulp,1981).

Increased energy intake is not essential to the development of obesity in man (Miller and Parsonage,1975) or rats (Han,1968; Boulange et al.,1979) and an impairment in NST and the adaptive component of diet-induced thermogenesis seems to exist in both man and some experimental animals when predisposed to obesity. Investigations into the development and maintenance of obesity have, therefore, concentrated on the mechanism and regulation of NST and DIT

1.3 Brown Adipose Tissue.

Smith, in 1961,first defined brown adipose tissue (BAT) as a thermogenic effector tissue. More recently, blood flow studies have established BAT as the main site of NST and DIT in the rat (Foster and Frydman,1978, 1979; Rothwell and Stock,1979, 1980a, 1981c). BAT occurs in many mammals and is most common in smaller animals (rodents), neonates (including humans - Aherne and Hull,1966, harp seals - Grav et al.,1974), hibernators (bats, hedgehogs, squirrels) and cold-dwelling non-hibernators (wild rats and mice) (see Smith and Horwitz,1969). The tissue is located in discrete depots around the body, such as the cervical, subscapular and interscapular depots. Deposits are also found along the aorta, around the carotid artery and jugular veins, and around the kidneys and heart. BAT can account for up to 5% of body weight, depending on the species (see Smith and Horwitz,1969). The largest and most easily accessible depots are found in the interscapular and dorso-cervical regions, therefore, most work has been carried out on BAT from these sites.

1.3.1 Morphology of Brown Adipose Tissue.

BAT is visually identifiable as a brown coloured mass,

the colour being attributed to the high proportion of haems present, from blood haemoglobin, haem porphyrins (mostly cytochromes) and from flavin compounds. Incursion of white adipocytes turns the colour towards beige (see Smith and Horwitz,1969). Electron micrographs of BAT reveal cells with multilocular lipid droplets within the cytoplasm, which is itself densely packed with heavily invaginated mitochondria. This is in stark contrast to white adipose tissue (WAT) where there is one large lipid droplet occupying most of the cell and few mitochondria (see Girardier,1983). The nucleus of a BAT cell is round and centrally located, again in contrast to WAT, where the nucleus tends to get squashed against the cell membrane by the lipid droplets (Smith and Horwitz,1969). Approximately 80% of the tissue volume consists of brown adipocytes (Barnard,1977).

1.3.2 Sympathetic Innervation of Brown Adipose Tissue.

BAT is innervated predominantly with fibres of the sympathetic nervous system. The interscapular BAT depot consists of two bilateral lobes, each having its own discrete nerve supply. Entering each lobe are five nerve fibre bundles containing morphologically heterogenous fibres, which arise through the intercostal muscles beneath the pad (Foster et al.,1982). It is thought that the cervical and interscapular depots are innervated by the spinal nerves C_1 to C_5 plus the first five branches of the thoracic sympathetic chain (Smith and Horwitz,1969). The heterogeneity of the fibres is probably due to their functional dissimilarity - innervation of adipocytes, blood vessels within the fat pad, and of overlying skin (Flaim et al.,1976). The nerve fibres within a BAT pad innervate both adipocytes and arterioles. Fluoresence histochemistry has revealed that BAT adipocytes are covered with an intricate web of adrenergic fibres (Cottle and Cottle,1970). There is no functional crossover between the sympathetic innervation of each lobe of interscapular BAT, despite an apparent 25% cross innervation and 15% non-intercostal innervation of the tissue (Foster et al.,1982).

1.3.3 The Vascular Supply of Brown Adipose Tissue.

The blood supply of BAT arises from the left and right thoracodorsal arteries, and drainage occurs through their complementary veins and the unpaired Sulzer's vein. The venous drainage system of BAT is often collectively termed 'Sulzer's Vein' (Smith and Roberts,1964). Sulzer's Vein drains into the precaval vein and thence directly into the vena cava and the heart, thus ensuring rapid distribution of warmed blood around the body. A dense capillary network covers up to a third of each BAT adipocyte's surface within a BAT pad (Aherne and Hull,1966). Some of the most convincing evidence for the importance of BAT in NST came from the blood flow studies of Foster and Frydman (1978). Using radiolabelled microspheres, they measured the flow through interscapular BAT in cold acclimated rats after intravenous noradrenaline injection. A 25 fold increase in blood flow through the tissue occured, accounting for 33% of the total cardiac output.

1.3.4 Mechanism of Heat Production in Brown Adipose Tissue.

The dense invagination of BAT mitochondria is indicative of a high respitory capacity (Flatmark and Pederson,1975). Respiration in normal mitochondria is usually tightly coupled to the rate of ATP synthesis (see Nicholls,1982). The rate of ATP synthesis in BAT mitochondria is abnormally low and this is partially explained by a low activity (Lindberg et al.,1967) and concentration (Cannon and Vogel,1977) of ATP synthetase in the tissue. However, the rate of ATP synthesis which does occur is too low to account for the observed respiration rates (Bulychev et al.,1972) indicating that some unusual form of respiratory control is present (Flatmark and Pederson,1975; Nicholls,1976b, 1979). There exists in brown fat an unique proton conductance pathway which provides a means of re-entry of protons into the mitochondrion without the synthesis of ATP. The presence of purine nucleotides inhibits the uncoupling of ATP synthesis (Nicholls,1976b).

The site of uncoupling was identified by photoaffinity labelling with a purine nucleotide analogue, as a protein of 32000 Daltons molecular weight (32K protein) situated in the inner mitochondrial membrane (Heaton et al.,1978). The nucleotide with the greatest affinity for this protein was found to be GDP, although ATP is probably the physiological regulator. The GDP binding property has been exploited to give a method of measuring the thermogenic capacity of BAT by measurement of the specific binding of radiolabelled GDP to BAT mitochondria (Nicholls,1976a). The level of GDP binding has been found to correspond to the thermogenic state of the animals (Heaton et al.,1978, Desautels et al 1978), as has the mitochondrial concentration of 32K protein (Ashwell et al.,1983; Ricquier et al.,1984; Ashwell et al.,1985). The 32K protein has been isolated (Lin and Klingenberg,1982) and there is evidence to suggest that it may be dimeric (Lin et al.,1980) and possess two nucleotide binding sites of high and low affinity (Bryant et al.,1983; French et al.,1985). 32K protein has been found to occur exclusively in BAT (Cannon et al.,1982; Lean et al., 1983) and to be present in all thermogenically active BAT from the species investigated to this time.

The initial stimulus for BAT thermogenesis is the release of noradrenaline from sympathetic nerve endings within the tissue. Neural stimulation of BAT is followed by a small decrease in temperature which is thought to be due to an α-mediated vasoconstriction. This is followed by a β-induced vasodilation (Flaim et al.,1977). However, Foster and Depocas (1981) found that blood flow in thermogenically active BAT was related to arterial oxygen tension rather than noradrenaline concentration and suggested that the production of a local vasodilator could be linked to the intracellular oxygen tension. It has since been suggested that histamine acting on H_2 receptors could perform such a function (Rothwell et al., 1984a).

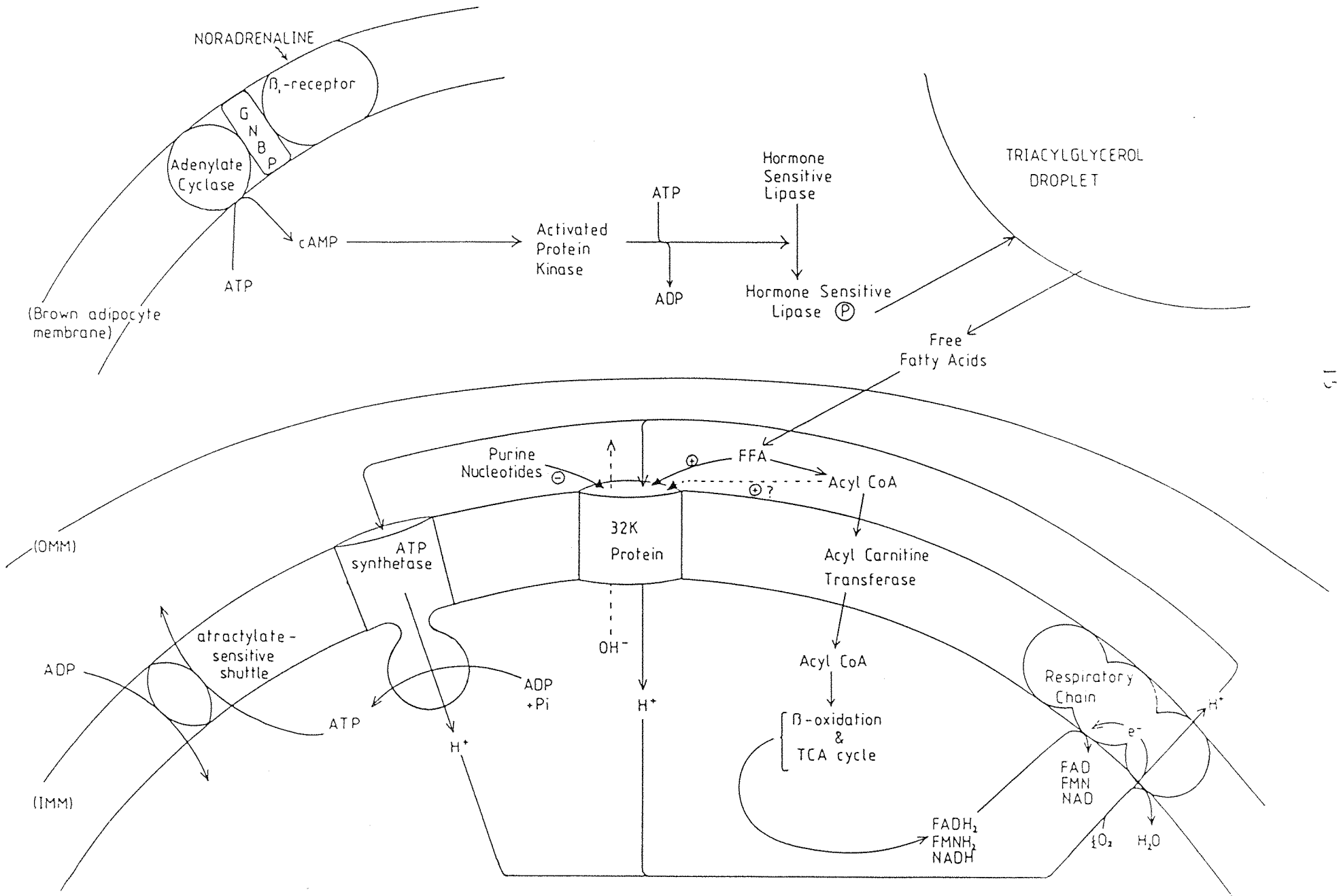
The thermogenic activation of BAT by noradrenaline seems to occur mainly through β-receptors. Tritiated dihydroalprenolol binding studies characterised the β receptors in BAT as mainly β_1 (Bukowiecki et al.,1980) but additional studies using selective and non-selective β-antagonists suggest a 60:40 β_1:β_2 mixed receptor population (Rothwell et al.,1985). Recent work by Arch et al (1984a)using novel β-agonists suggests that the BAT β-receptor may be of an atypical subtype. Mohell et al.(1983) found that 20% of the noradrenaline induced increase in BAT adipocyte respiration can be attributed to α_1-adrenoreceptor mediated effects. However, for the time being at least, it seems that the major thermogenic effects of noradrenaline are mediated through β_1 receptors (Mohell et al.,1983; Skala,1984; Harris et al.,1986; Levin and Sullivan,1986).

Upon noradrenaline binding to the β_1 receptor intracellular cyclic AMP (cAMP) is produced through the activation of adenylate cyclase (Pettersson and Vallin, 1976) (see Figure 1.1). cAMP dependent protein kinases are then activated within the cell (Knight and Skala,1977) one of which activates hormone sensitive triacylglycerol lipase by phosphorylation (Skala and Knight,1977). The action of hormone sensitive triacylglycerol lipase is to hydrolyse triacylglycerol to diacylglycerol and free fatty acids. Other lipases hydrolyse diacylglycerol to free fatty acids and glycerol. Free fatty acids are transported into the mitochondrion after activation into fatty acyl CoA,across the acyl carnitine shuttle. Fatty acyl CoA then undergoes β-oxidation yielding NADH, $FADH_2$ and acetyl CoA . Acetyl CoA enters the tricarboxylic acid cycle and produces further reducing equivalents in the form of NADH. Oxidation of NADH and $FADH_2$ by the electron transport chain results in the translocation of protons out of the mitochondria to form a proton gradient (see Nicholls,1982). In normal mitochondria the proton gradient thus generated would be used to drive the energetically unfavourable formation of ATP. In stimulated

Figure 1.1 The Mechanisms of Thermogenesis in Brown Adipose Tissue.

Noradrenaline binds to the β_1 receptor and activates adenylate cyclase through dissociation of the guanine nucleotide binding protein. Intracellular cAMP concentration rises and activates cAMP dependent protein kinases, one of which activates hormone sensitive lipase by phosphorylation. Free fatty acids are released into the cytoplasm by the actions of hormone sensitive lipase and provide the substrate for mitochondrial β-oxidation. Free fatty acids may also act to disinhibit purine nucleotide inhibition of the 32K uniport, dissipating the proton gradient formed by mitochondrial respiration and uncoupling respiration from ATP synthesis.

ADP	adenosine diphosphate
ATP	adenosine triphosphate
cAMP	adenosine 3',5'-cyclic monophosphate
CoA	coenzyme A
FAD/H_2	flavin adenine dinucleotide/reduced form
FFA	free fatty acids
FMN/H_2	flavin mononucleotide/reduced form
GNBP	guanine nucleotide binding protein
IMM	inner mitochondrial membrane
NAD/H	nicotine adenine dinucleotide/reduced form
OMM	outer mitochondrial membrane
Ⓟ	phosphorylated protein
P_i	inorganic phosphate
TCA	tricarboxylic acid cycle


NORADRENALINE
β1-receptor
G
N
B
P
Adenylate Cyclase
cAMP
ATP
(Brown adipocyte membrane)
Activated Protein Kinase
ATP
ADP
Hormone Sensitive Lipase
Hormone Sensitive Lipase (P)
TRIACYLGLYCEROL DROPLET
Free Fatty Acids
Purine Nucleotides
FFA
Acyl CoA
(OMM)
ATP synthetase
32K Protein
Acyl Carnitine Transferase
atractylate-sensitive shuttle
ADP
ATP
ADP +Pi
OH⁻
H⁺
Acyl CoA
β-oxidation & TCA cycle
Respiratory Chain
e⁻
FAD
FMN
NAD
FADH₂
FMNH₂
NADH
½O₂
H₂O
(IMM)

BAT mitochondria, the 32K protein allows the protons to leak back into the mitochondrion and dissipate the gradient (Nicholls,1976b). The nature of the 'proton leak' appears to be the simplest proton transporter yet known. Studies on ion fluxes through the protein reconstituted into phospholipid vesicles suggest that it is not a hydroxyl uniport, and that proton influx is mediated via a translocating, rather than a channel, mechanism (Klingenberg and Winkler,1985). Oxidation of substrates continues, and the rate of proton pumping is limited only by substrate supply. The enthalpy released as heat during substrate oxidation is conducted away from the tissue by the increased blood flow.

The intracellular mechanism controlling the state of the 32K protein uniport is not yet fully understood. Upon acute stimulation of BAT by cold exposure or noradrenaline injection, isolated BAT mitochondria exhibit a large increase in GDP binding capacity. The increase in binding has been associated with an unmasking of binding sites already present in the membrane (Desautels and Himms-Hagen,1979; Gribskov et al.,1986). This means that GDP binding gives a measure of the amount of unmasked protein in the membrane, rather than its concentration. The unmasking of binding sites is thought to be accomplished by a noradrenaline stimulated intracellular messenger. Freshly isolated BAT mitochondria are usually uncoupled (Nicholls and Lindberg,1973). Respiratory control can be restored by the addition of purine dinucleotides (Nicholls, 1976a) and removed by the addition of fatty acids (Heaton and Nicholls,1976). The concentration of adenine nucleotides in brown fat cytosol is in the millimolar range (Grav,1970) but micromolar additions to isolated mitochondria are sufficient to inhibit proton conductance (Nicholls,1976). ATP is probably the physiological agent by which the pathway is closed (see Rothwell and Stock, 1984c). Fatty acids can stimulate BAT adipocyte respiration in the presence of millimolar concentrations of adenine nucleotides, and the concentration of free

fatty acids required (0.2μM) is within the expected physiological range of concentration in the cytosol (Locke et al.,1982). Fatty acyl CoA's are also known to inhibit purine nucleotide binding in isolated mitochondria (Cannon et al.,1977). It is still unclear whether free fatty acids (Locke et al.,1982; Cunningham and Nicholls, 1985) or fatty acyl CoA (Cannon et al.,1977; Strieleman and Shrago, 1985) have a physiological role in the unmasking of GDP binding sites which leads to the uncoupling of BAT mitochondria.

The primary substrate for BAT thermogenesis is thought to be fatty acids. BAT has a high capacity for fatty acid synthesis from carbohydrate which is associated with the in vivo stimulation by insulin of glucose transport, pyruvate dehydrogenase activity and acetyl CoA carboxylase activity (McCormack and Denton,1977; Agius and Williamson, 1981). Ketone bodies may also provide an important substrate for lipogenesis, since the rate of incorporation of hydroxybutyrate into lipid is much greater in BAT than in WAT (Agius and Williamson,1981). Trayhurn (1980) reported that 50% of the lipids incorporated into BAT during cold acclimation are synthesised elsewhere in the body. The activity of BAT lipoprotein lipase is tissue-specifically induced by cold exposure (Radomski and Orme, 1971) through β-mediated mechanisms (Carneheim et al.,1984).

There is some recent evidence that glucose may be an important substrate for BAT metabolism. The activities of pyruvate dehydrogenase (McCormack and Denton,1977), hexokinase (Cooney and Newsholme,1982; Young et al.,1984), phosphofructokinase (Cooney and Newsholme,1984; Young et al., 1984) and pyruvate kinase (see Gibbins et al.,1985) are elevated in the cold exposed rat. Gibbins et al.(1985) report a 70% decrease in fatty acid synthesis in BAT of cold adapted rats after noradrenaline injection accompanied by a similar decrease in acetyl CoA carboxylase activity. At the same time, the proportion of active (non-phosphorylated) pyruvate dehydrogenase is increased. So, fatty acid synthesis is reduced and glycolysis is increased, leading

to increased acetyl CoA metabolism through the tricarboxylic acid cycle, the activity of which is reported as being increased in BAT (Cannon and Johansson,1980). In this way, reducing equivalents are produced through glucose metabolism and are oxidised by the respiratory chain, contributing to the net outflow of protons.

1.3.5 Evidence for Brown Adipose Tissue as an effector of NST.

BAT blood flow measurements in cold adapted rats using radiolabelled microspheres demonstrated that the tissue could account for 60% of the increase in oxygen consumption observed after noradrenaline injection (Foster and Frydman, 1978). The remaining 40% would be accounted for by the increased work rate of the heart and intercostal muscles and by the increase in general metabolism induced by the rise in temperature. The oxygen tension of blood leaving BAT is very nearly zero, so the tissue has the capacity to clear the oxygen from the blood in the face of a 25 fold increase in flow (Foster and Frydman,1978).

The thermogenic capacity of BAT is increased on cold exposure as demonstrated by measurements of BAT mitochondrial GDP binding (Desautels et al.,1978; Himms-Hagen, 1979), 32K protein concentration (Ashwell et al.,1983, Hansen et al.,1986), heat production (Hansen et al.,1986) and 32K mRNA concentration (Bouillaud et al.,1984; Ricquier et al.,1984). Tissue wet weight and mitochondrial content also increase on adaptation to cold (see Smith and Horwitz, 1969).

The surgical removal of BAT from newborn rabbits practically abolished the animals' capacity to increase its metabolic rate in response to cold (Dawkins and Hull, 1965). It was estimated that brown fat was responsible for 80% of the increased body heat produced by the cold exposed neonatal rabbit.

It has recently been shown that GDP binding in BAT mitochondria is reduced during hibernation in hamsters (Horwitz et al.,1985) and that BAT thermogenesis contributes

toward the increase in temperature required for the arousal from hibernation (Nedergaard and Cannon,1984).

1.3.6 Evidence for Brown Adipose Tissue as an effector of DIT.

NST and DIT have synergistic effects. Rats exhibiting DIT induced by cafeteria feeding (see Section 1.4.2) adapt more quickly to cold exposure and show a larger metabolic response to noradrenaline under the combined stimulus of cold and overfeeding (Rothwell and Stock,1980a). Similarly, cold adapted rats show a more rapid increase in metabolic rate in response to cafeteria feeding (Rothwell et al.,1982b). Cafeteria feeding increases BAT blood flow (Rothwell and Stock,1981c), tissue wet weight (Rothwell and Stock,1979), GDP binding (Brooks et al.,1980), 32K protein concentration (Nedergaard et al.,1984; Falcou et al.,1985) and 32K mRNA (Falcou et al.,1985). It has recently been shown that GDP binding is increased after a single meal (Lupien et al.,1985).

The surgical removal of BAT from animals maintained on a cafeteria diet would be expected to result in an increased adiposity due to an inability to effect DIT. Such an experiment has been carried out by Stephens et al. (1981), with the result that no difference in weight gain was seen between adult or weanling cafeteria fed rats and those which had had their interscapular BAT depot removed. As interscapular BAT represents 25% of the total body BAT content the finding that its removal had no effect upon weight gain could be taken to suggest that BAT has not as important a role in DIT as has been ascribed to it. However, both cafeteria feeding (Stephens et al.,1981) and mild cold acclimation (Horwitz et al.,1985) of BAT lipectomised rats results in the hypertrophy of remaining depots which may compensate for the BAT removed. It has also been reported that the denervation of interscapular and subscapular BAT depots had no effect on the development of obesity in adult Sprague-Dawley rats maintained on a cafeteria diet (Cox and Lorden,1986). It has, however, been previously demonstrated that in animals of this age and strain the

activation of BAT thermogenesis in response to cafeteria feeding is reduced (Rothwell et al.,1984b) which perhaps accounts for the failure of BAT denervation to affect dietary obesity in this case.

It should be noted that diet induced changes in BAT function are not as great as those induced by cold.

1.3.7 Evidence for the Sympathetic Regulation of BAT.

BAT possesses a rich sympathetic innervation as described in Section 1.3.2. A generally accepted method for the estimation of sympathetic nervous system (SNS) activity is the measurement of the rate of noradrenaline turnover within a tissue. This is accomplished by the measurement of the rate of disappearance of an intravenously injected tracer dose of tritiated noradrenaline which equilibrates with noradrenaline in the nerve terminals in the tissue (see York et al.,1985b). The activation of BAT in NST and DIT is prevented by the administration of antisympathetic drugs. Propranolol, a β-adrenoreceptor antagonist, prevents the increase in metabolic rate normally seen during cold exposure and overfeeding (Rothwell and Stock,1980a). Ganglionic blockade with hexamethonium prevented the rise in metabolic rate due to cold exposure (Hsieh et al.,1957). Ganglionic blockade with chlorisondamine caused greater retention of tritiated noradrenaline in BAT of overfed rats than in controls (Young et al.,1982, Marchington,1985). Both NST and DIT are accompanied by a tissue specific rise in BAT noradrenaline turnover, that is to say sympathetic activity (NST - Cottle et al.,1967; Young et al.,1982; DIT - Young et al.,1982; Schwartz et al.,1983; Kevonian et al., 1984).

Circulating noradrenaline released from the adrenal medulla is probably not important in BAT regulation, as Seydoux and Girardier (1977) have shown that the apparent concentration of noradrenaline required at the synapse for half maximal stimulation of BAT (calculated from electrical and chemical dose response curves) is 30-150 times greater than circulating levels (6×10^{-10}M, Depocas and Behrens, 1977). Also, adrenal demedullation has little effect on

NST although there may be a role for circulating catecholamines in the acute response to severe cold (Himms-Hagen,1975).

Sympathetic denervation of BAT causes a slight increase in tissue weight, mainly due to an increase in triacylglyceride content. Fatty acid synthesis is decreased in denervated BAT, and the tissue takes on the morphological characteristics of WAT as seen under the light microscope (Minokoshi et al.,1986). Noradrenaline content drops by 90% and the protein and DNA content of the tissue is reduced (Granneman and Campbell,1984). The normal effects of cold acclimation and cafeteria feeding (increases in tissue wet weight, protein content and GDP binding) are abolished by sympathetic denervation, but denervated BAT still responds to subcutaneously injected noradrenaline by increasing GDP binding (Rothwell and Stock,1984b). Emery et al.(1985) reported similar increases in tissue wet weight and protein synthesis rate in intact and denervated BAT of cafeteria fed animals. However, the weight increase in denervated BAT was due only to lipid gain, and they proposed that the increase in protein synthesis rate was due to hormonal influences, but the rate of protein breakdown was determined by SNS activity allowing a net increase in the amount of metabolically active proteins. Increases in BAT lipogenesis induced by sucrose overfeeding are reduced by 75% in denervated animals (Granneman and Campbell, 1984).

These findings indicate that the effects of cold adaptation and overfeeding depend on an intact sympathetic innervation of BAT.

1.3.8 The Adaptive Response of BAT.

Prolonged stimulation of BAT by either cold or hyperphagia provokes many changes in the tissue. Interscapular BAT weight can increase 5 fold during chronic cold exposure and 2-3 fold during prolonged cafeteria feeding (Bukowiecki et al.,1982). The number of cells, total cytochrome oxidase activity, total tissue protein content and the calorigenic response to noradrenaline double in interscapular BAT

after two weeks or more of cafeteria feeding (Himms-Hagen et al.,1981; Bukowiecki et al.,1982). Cold adaptation doubles the calorigenic response to noradrenaline and increases 6 fold total cytochrome oxidase activity and cell number (Bukowiecki et al.,1982). GDP binding is increased 7 fold in cold adapted rats and 2-3 fold in cafeteria fed rats (Desautels and Himms-Hagen,1979; Himms-Hagen et al.,1981). These changes in binding are paralleled by increases in 32K protein content in both NST (Ashwell et al.,1983; Hansen et al.,1986) and DIT (Ashwell et al.,1984; Nedergaard et al.,1984; Falcon et al.,1985). The growth of BAT during prolonged stimulation is accompanied by an increase in vascularisation and innervation (Bukowiecki et al.,1982; Barnard et al.,1980). The increase in SNS activity in BAT seen on acute cold exposure and sucrose feeding is maintained during chronic cold adaptation and prolonged overfeeding (Young et al.,1982; Marchington,1985). Chronic administration of catecholamines to warm acclimated rats mimic the effects of cold acclimation on BAT (Barnard et al.,1980), decrease the rate of weight gain relative to control animals at the same level of food intake and increase body temperature (Racotta et al.,1986). Such infusions also increase the concentration of 32K protein in BAT mitochondria (Mory et al.,1984). α and β -receptor populations in BAT change on chronic stimulation . α_1 receptor number is increased and β_1 receptors decreased during cold acclimation or cafeteria feeding (Bukowiecki et al.,1978; Mohell,1984; Raasmaja et al.,1984). It has recently been demonstrated that in cold acclimated rats α_1-agonists potentiate the effects of β_1-mediated events in BAT. The thermogenic response of BAT to sub-optimal doses of noradrenaline were enhanced in the presence of α_1-agonists, although the α_1-agonists had no independent effects on BAT (Foster,1986). It could be that the increase in α_1 receptors that occurs on chronic BAT stimulation is responsible for an enhanced sensitivity of the tissue to neurally released noradrenaline, which suggests that α_1 receptors may be important in the chronic regulation of BAT (Mohell,1984; Foster,1986).

1.3.9 The Central Control of BAT Thermogenesis.

The hypothalamus is known to have an important role in the control of food intake (see Section 1.2.1) and of thermoregulation (Jansky,1973). The VMH is closely involved with the activation of BAT thermogenesis. Electrical stimulation of the VMH gives rise to an increase in BAT temperature which can be abolished by the administration of propranolol or by severing the sympathetic nerves to BAT (Perkins et al.,1981; Holt et al.,1986). Electrical stimulation of this area causes an increase in metabolic rate (Atrens et al.,1985; Morimoto et al.,1986) in tissue specific fatty acid synthesis (Shimazu and Takashi,1980), BAT lipolysis (Kumon et al.,1976) and other 'cold defence responses' such as shivering, reduction of skin temperature, and reduction of surface area by huddling (Morimoto et al., 1986). The physiological responses to VMH stimulation described by Morimoto et al (1986) are blockable with propranolol but are unaffected by α-blockade. Metabolic activity, as assessed by $[^{14}C]$-deoxyglucose uptake, is elevated by cold exposure in several specific brain sites, especially the VMH (Morimoto and Murakami,1985). The tonic firing rate of the sympathetic nerves to BAT is increased by cold stimulation, an effect which is abolished by VMH lesion (Niijima et al.,1984). VMH lesions decrease BAT mitochondrial GDP binding (Seydoux et al.,1982), BAT noradrenaline turnover (Vander Tuig et al.,1982) and leads to degeneration of the tissue (Saito et al.,1985). VMH lesioned rats still respond normally to cold (Seydoux et al.,1982; Hogan et al.,1982; Luboshitsky et al.,1984), but not to overfeeding (Hogan et al.,1982; Seydoux et al.,1982) implying that the sites within the VMH controlling DIT and NST are distinct.

Stimulation of the LH increases oxygen consumption in rats, an effect which is partially blocked by propranolol (Atrens et al.,1985; Corbett et al.,1982) but no functional connection between the LH and BAT has been demonstrated (Holt et al.,1986). Blatteis and Banet (1986) have demonstrated that the preoptic area is not essential for the autonomic regulation of thermogenesis. Stimulation of the supraoptic nucleus elicits an increase in BAT temperature

possibly through stimulation of the 'supraoptic decussations' which are known to project into the VMH (Holt et al.,1986).

1.3.10 The Endocrine Control of BAT Thermogenesis.

The sympathetic nervous system is the primary activator of BAT, but hormones can play a modulating role in the regulation of the tissue.

a. Insulin and Glucagon.

Insulin has been found to oppose many of the effects of catecholamines, such as the noradrenaline induced increase in intracellular cAMP, adrenaline stimulated fatty acid and glycerol release and noradrenaline stimulated oxygen consumption (see Begin-Heick and Heick,1984). Insulin promotes the uptake of glucose and the rate of lipogenesis in brown fat (McCormack and Denton,1977; Agius and Williamson,1980), and can stimulate metabolic rate in fasted and cafeteria fed rats - an effect that is blockable with propranolol but not 2DG,implying that this effect is due to insulin stimulation of SNS activity rather than promotion of glucose metabolism (Rothwell and Stock,1983b). Insulin seems to have a permissive role in thermogenesis, as diabetic rats fail to exhibit either NST or DIT (Rothwell and Stock,1981b). It is possible that insulin acts centrally to activate BAT thermogenesis as injections of insulin into the VMH enhance the glucoreceptor neuronal firing rate (Oomura et al.,1978), and it is known that VMH excitation stimulates BAT efferent nerve activity (Niijima et al.,1984) and elevates BAT temperature (Holt et al.,1986).

Heim and Hull in 1966 and more recently Kuroshima et al.(1977) have demonstrated that glucagon also has a role in the regulation of BAT thermogenesis. The hormone stimulates metabolic rate, BAT blood flow, temperature and lipolysis. Glucagon also depresses the activity of gluco-sensitive neurones when electrophoretically applied to the LH (Inokuchi et al.,1986) which may reduce reciprocal inhibition of the VMH and increase efferent activity to BAT. Propranolol does not affect the actions of peripherally injected glucagon (Heim and Hull,1966; Kuroshima et al.,1977) implying that the SNS does not control these responses.

b. Thyroid Hormones.

The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are permissive to BAT thermogenesis. Thyroidectomised rats do not respond to cold exposure with an increase in BAT mitochondrial GDP binding, but low replacement doses of T_3 to these rats normalises their thermogenic response (Triandafillou et al.,1982). T_3 and T_4 levels in the blood are increased in rats exhibiting NST and DIT (Scammel et al., 1981 ; Tulp et al.,1982) and are depressed in heat acclimated or 40-hour starved animals (Arieli and Chinet,1986; Glick et al.,1985). These observations suggest that thyroid hormone levels are increased and reduced in parallel to the activity of BAT. Thyroid hormones are known to have a stimulating effect on BMR (Ismail-Beigi and Edelman,1970). The increased metabolic rate in hyperthyroid rats seems to reduce the requirement for BAT thermogenesis since basal GDP binding levels are depressed and are not stimulated by mild cold exposure (Sundin,1981) or cafeteria feeding (Rothwell et al.,1983c), but more severe cold exposure (5^{o}C) does increase GDP binding (Sundin,1981). Hyperthyroid rats exhibit a potentiated response to cafeteria feeding (increased oxygen consumption) in the absence of any increase in GDP binding and it is thought that this is due to activiation of the ouabain-sensitive sodium-potassium dependent ATPase (Rothwell et al.,1982e, 1983c). A ouabain-sensitive component of BAT thermogenesis does exist (Horwitz,1973) but direct microcalorimetric measurements on BAT from normal rats housed at 23^{o}C revealed that the energy cost of sodium pumping represented only 5-6% of the total metabolism of the brown fat cell (Chinet et al.,1977), so its contribution toward BAT thermogenesis is normally discounted. It has been suggested that while thyroid hormones may be permissive to NST, they play a more direct role in DIT (Rothwell et al., 1982a; Rothwell and Stock 1984c).

T_4 is converted to the more potent T_3 by the action of 5'-monodeiodinase (Schimmel and Utiger.,1977) an enzyme which is under the control of the α_1 component of the SNS Therefore, in sympathetically activated BAT the activity of the enzyme is high leading to high local production of

T_3 (Silva and Larsen,1983). Thyroid Hormones potentiate the thermogenic effects of noradrenaline (Leblanc and Villemaire,1970), and the enhanced sympathetic activity in BAT during cold or diet stimulation leads to an increase in α_1 receptor number (Mohell,1984; Raasmaja et al.,1984) which would further increase T_3 production. Thus thyroid hormones and the SNS act synergistically to amplify the thermogenic response to endogenous noradrenaline release.

c. Pituitary-Adrenal Hormones.

Like thyroid hormones, glucocorticoids are permissive to the response of BAT to cold exposure (Deavers and Mussachia,1979; Fellenz et al.,1982). However, chronic corticosterone treatment depresses the thermogenic response to overfeeding, but not to cold, in mice (Galpin et al.,1983) and rats (Holt et al.,1983). Adrenalectomised rats cannot tolerate cold exposure but respond normally to cafeteria feeding with an increase in metabolic rate, BAT GDP binding and noradrenaline turnover (Rothwell et al.,1984a, b; Marchington,1985; Marchington et al.,1986). The enhanced thermic effect of feeding and BAT thermogenesis seen in adult adrenalectomised cafeteria-fed rats can be prevented by replacement corticosterone injections at physiological concentrations and by sympathetic denervation of BAT. Sympathetic denervation has similar effects on BAT function as high doses of corticosterone (Rothwell et al.,1984a). The effects of adrenalectomy on improving the response of BAT to cafeteria feeding require an intact sympathetic innervation and, therefore, may be mediated at a central level. The differential responses of NST and DIT to glucocorticoids suggest that the hormones' effects are not mediated at a tissue level (Rothwell and Stock,1984a; York et al.,1985a) despite the fact that BAT possesses functional glucocorticoid receptors (Feldman,1978).

ACTH has an independent stimulatory effect on BAT thermogenesis separate from its control of glucocorticoid secretion. Chronic treatment with ACTH (10 days) causes a 20% rise in BMR and enhances the thermogenic and lipolytic effects of noradrenaline in warm acclimated rats without increasing plasma corticosterone levels (Laury and

Portet,1977, 1980). ACTH treatment stimulates BAT blood flow (Kuroshima,1968). Increases in metabolic rate and BAT temperature caused by ACTH treatment are not blocked by propranolol (Heim and Hull,1966) and are not associated with an increase in BAT noradrenaline turnover (Marchington, 1985) indicating that ACTH may exert a direct effect on BAT. The mechanism of this effect is as yet unclear. ACTH may independently activate the adrenyl cyclase cascade system in BAT (Bertin and Portet,1976). Alternatively, it might increase α_1 receptor number in BAT, potentiating the effect of the SNS without increasing tissue noradrenaline turnover, or may act directly on BAT through similar mechanisms to α_1 receptors to potentiate SNS effects. The thermogenic effects of exogenous noradrenaline are attenuated by chronic ACTH treatment in cold acclimated rats (Laury and Portet,1977) which suggests that a physiological role for ACTH in the response to cold is unlikely. Hypophysectomised rats can gradually adapt to mild cold exposure (15^{o}C) but show a reduced calorigenic response to noradrenaline, although the response was significantly greater at 15^{o}C than at 28^{o}C (Laury et al.,1984). It is thought that the thermoneutral zone shifts upwards in hypophysectomised rats, hence 28^{o}C is effectively still mild cold exposure (Laury et al.,1984). For survival of more severe cold exposure (4^{o}C) corticosterone replacement is required (Fellenz et al.,1982).

Hypophysectomy increases the thermic response to a meal, BAT mass, BAT protein content and thermogenic activity (Rothwell and Stock,1985 a, c). These effects are restored to normal (thermic effects of feeding) or markedly reduced (BAT thermogenesis) by ACTH replacement, indicating that the stimulatory effects of hypophysectomy on BAT are probably due to decreased adrenal steroid release. There remains, however, a small stimulatory component after ACTH replacement to hypophysectomised rats which could be due to a direct effect of ACTH on BAT (Rothwell and Stock,1985a) or to the effects of another pituitary hormone, possibly corticotropin releasing factor (CRF), as recent experiments suggest that CRF stimulates BAT mitochondrial GDP binding and depresses food intake (Arase, York and Bray, unpublished observation).

1.3.11 Brown Adipose Tissue in Man.

Although BAT can account for 2-5% of body weight in the human neonate (Aherne and Hull,1966) there is little evidence for a significant role for BAT in adult man. BAT in man atrophies with age, a change which parallels the decline of the capacity for NST (Trayhurn and James,1983). In patients with phaeochromocytoma BAT has been identified histologically. GDP binding and GDP-sensitive mitochondrial respiration have been demonstrated in isolated human perirenal BAT (Ricquier et al.,1982, Cunningham et al.,1985), as well as the presence of uncoupling protein (Bouillaud et al.,1983; Lean and James,1983; Lean et al.,1986). BAT has also been histologically identified in larger quantities in individuals exposed to the cold (Huttenhen et al.,1981) but no differences have been demonstrated as yet in either the amount or activity of BAT in lean and obese humans. Increases in skin temperature in the neck and suprascapular regions have been demonstrated in adult man after ephedrine administration (Rothwell and Stock, 1979), although these changes in skin temperature may be due to changes in subcutaneous blood flow (Astrup et al.,1980). It has recently been calculated that only 25% of the thermic effect of ephedrine in man can be attributed to BAT, therefore, it is thought that BAT has no physiological role in DIT in adult man and that muscle is the main site of ephedrine stimulated thermogenesis (Astrup et al.,1985). Regardless of this lack of data on the activity of BAT in human obesity, much interest is currently centred on the investigation of pharmacological agents which may stimulate BAT activity (Arch et al.,1984b). Due to the impracticalities of working with human subjects most work has centred on the use of animal models of obesity.

1.4 Animal Models of Obesity.

Sclafani, in his review of animal models of obesity in 1984, listed 50 or so experimental models available to the researcher. None of these animal models are precisely representative of human obesity, but taken together they

can help to elucidate the causes, consequences and potential cures of the obese condition. The types of experimental obesity best characterised in animals are those produced by hypothalamic injury, dietary manipulation or genetic inheritance.

1.4.1 Hypothalamic Obesity.

Damage to the VMH by electrolytic lesion or gold-thioglucose (GTG) injection produces hyper-insulinaemia and hyperphagia in rats. Electrolytic lesions of the paraventricular nucleus (PVN) also produce hyperphagia and obesity (Leibowitz et al.,1981) and induce hyperinsulinaemia only on a low-fat, high carbohydrate diet (Aravich and Sclafani,1984). Parasaggital knife cuts or electrolytic lesions which do not destroy the ventromedial nucleus but interrupt the ventral noradrenegic bundle give rise to a simple model of obesity where hyperinsulinaemia develops only in the presence of hyperphagia (Bray et al.,1982). If the ventromedial nucleus is also destroyed, the result is severe hypoactivity, retarded growth, finickiness, disruption of ovarian function, increased meal size and frequency of hyperinsulinaemia which is independent of the increase in food intake (Bray and York,1979). Other methods of producing hypothalamic lesions are radiofrequency lesions, or injections of gold-thioglucose, monosodium glutamate, bipiperidyl mustard and 5,7 dihydroxytryptamine (see Sclafani,1984). Injections of 6-hydroxydopamine disrupt ventral noradrenergic bundle function and result in an obesity which is dependent upon the presence of an intact hypophysis (Ahlskog et al.,1977). This suggests that obesity arising from such damage may be dependent upon activation (disinhibition) of the α_2 noradrenergic corticosterone dependent feeding drive seated in the PVN.

VMH-lesioned rats have a lowered metabolic rate (Hustvedt et al.,1984), and spontaneous activity is reduced (Gladfelter and Brobeck, 1962) both contributing to a reduction in energy expenditure. Noradrenaline turnover in BAT is reduced in VMH-lesioned animals (Vander Tuig et al.,1982)

as is BAT mitochondrial GDP binding (Seydoux et al.,1982), implying that the sympathetic activation of the tissue is depressed in hypothalamic obesity. Noradrenaline turnover is also depressed in the heart, liver and pancreas of VMH-obese rats (Vander Tuig et al.,1982). Lipogenesis and fatty acid synthesis are both increased in BAT of VMH-obese rats (Luboshitsky et al.,1983), brown adipocyte size is increased and these cells become unilocular in appearance (Saito et al.,1985), changes consistent with an involution of the tissue. Hypothalamic obesity can be prevented by subdiaphragmatic vagotomy prior to lesioning (Powley and Opsahl,1974; Inoue and Bray,1977), which abolishes the hyperinsulinaemia that normally develops in these animals. The development of VMH-obesity requires active innervation of the pancreas, as diabetic rats with islet transplants do not develop hyperphagia or obesity after lesioning (Inoue et al.,1978). Increased food intake and hyperinsulinaemia in VMH-obese rats accompanied by reduced BMR and reduced capacity for DIT lead to increased lipid synthesis and storage, and a hypertrophy of WAT. It is currently thought that VMH lesion depresses efferent sympathetic activity and removes the tonic inhibition of vagal tone and the LH feeding centre normally exerted by the VMH, resulting in increased PNS and decreased SNS activities. Increased food intake and anabolic (parasympathetic) activity coupled with reduced catabolic (sympathetic) activity result in gross obesity which is insulin dependent in the VMH-lesioned rat. Adrenalectomy reverses VMH-obesity, but the obese state can be restored by peripheral or central administration of glucocorticoids (Bruce et al.,1982; Debons et al.,1982, 1986). The formation of gold-thioglucose lesions in the VMH of mice can be prevented by pretreatment with either glucose analogues (Likuski et al.,1967) or glucocorticoids (Brown,1986) which suggests that there is a hypothalamic site at which both glucose and glucocorticoids act, which has a role in the development of VMH-obesity. PVN-lesioned obese rats also exhibit enhanced PNS activity although hyperinsulinaemia develops only as a result of the increased food intake (Leibowitz et al.,1981; Tokunaga et al.,1986b). Under normal

conditions it is thought that the PVN holds the dorsal motor nucleus (DMN) of the vagus under tonic inhibition, so PVN lesion may remove the inhibition and increase vagal activity (Tokunaga et al.,1986a). PVN lesions which also damage the DMN result in an animal with no apparent hyperphagia or obesity, implying that damage to the DMN overrides PVN lesion induced hyperphagia (Tokunaga et al., 1986a).

Obesity associated with the VMH and PVN can also be produced by chronic infusion of noradrenaline into either of these hypothalamic sites (Lichtenstein et al.,1985, Shimazu et al.,1985). Disinhibition of the α_2-noradrenergic feeding drive is thought to be the mechanism responsible for PVN noradrenaline-induced obesity (Lichtenstein et al., 1985). Continuous infusion of noradrenaline into the VMH is thought to cause, either desensitisation of VMH neurones, leading to local inactivity, or inhibition of neuronal activity (Shimazu et al.,1985).Obesity induced by noradrenaline infusion into the VMH is still characterised by hyperinsulinaemia and hyperphagia (increased PNS activity), and by degeneration of BAT (decreased SNS output) (Shimazu et al., 1985). It thus seems possible that noradrenaline induced VMH-obesity is due to a decreased energy expenditure and an increased energy intake in a similar manner to VMH-lesion obesity.

BAT of VMH-lesioned rats responds normally to cold stimulus (Seydoux et al.,1982; Luboshitsky et al.,1984) but shows a reduced thermogenic response to overfeeding (Seydoux et al.,1982; Hogan et al.,1985), suggesting that the hypothalamic areas controlling diet-induced activation of BAT are anatomically distinct from those controlling temperature (Rothwell and Stock.,1984c).

1.4.2 Diet Induced Obesity.

Obesity can be induced in experimental animals by enticing them to overeat an energy dense (e.g. high fat) or highly palatable ('cafeteria' or 'supermarket') diet. High fat diets increase weight gain and lipid deposition as long as protein intake is maintained (Miller,1979).

Cafeteria diets offer a choice of palatable human food items in addition to laboratory chow and is a very effective way of increasing the voluntary energy intake of the rat (Sclafani and Springer, 1976 ; Rothwell and Stock, 1982). On this feeding regime, rats fail to regulate their dietary intake and obesity results, due to a combination of a higher food intake (by weight) and the increased energy density of the food eaten. Over 90% of the excess weight gain on this diet is thought to be due to fat deposition Rothwell and Stock, 1979). Another method of increasing caloric intake in rats is the provision of additional sucrose in the diet, either mixed with chow or as a drinking solution (Kanarek and Hirsch, 1977). Energy intake is increased on this diet, and although body weight may not necessarily increase there is a significant increase in body fat in rats on a supplementary sucrose diet (Kanarek and Marks-Kaufman, 1979). Lipogenesis is enhanced by sucrose feeding due to increased fructose metabolism in the liver, making more acetyl CoA available for fatty acid synthesis, accompanied by elevated intestinal glucose and fructose transport rates. Insulin secretion is also increased, possibly due to the elevated glucose and fructose transport rates, leading to a further induction of lipogenic enzyme activity (see York, 1983). Sucrose feeding tends to enhance hepatic, rather than WAT, lipogenesis (Kornacker and Lowenstein, 1965) leading to increased hepatic lipid levels, especially in young rats (Waddell and Fallon, 1973).

High fat diets are another well established method of producing dietary obesity. Mickleson et al. in 1955 observed an excessive weight gain in rats fed a high fat diet (60% by energy) which was amongst the first observations of such an effect. The weight gained by high fat fed animals is mainly due to fat deposition, although a small increase in body protein may also occur. Although rats find high fat diets highly palatable, they tend to decrease the weight of food eaten on these diets. This reduction in food intake is not, however, sufficient to compensate for the higher energy density of the diet, so there is an increase in daily energy intake (Schemmel et al., 1970). VMH-lesioned rats do not reduce their food intake on a high fat diet

and so maintain an even higher level of energy intake (Bray and York,1972). This observation suggests that the normal reduction in food intake is a hypothalamically mediated response. When the energy intake of high fat fed rats is restricted to normal levels by pair-feeding the high fat rats still become obese, which indicates that there is an increase in metabolic efficiency associated with such diets (Lemmonier,1972).

The effectiveness of dietary methods to produce obesity varies with the age and strain of the rat used. High fat diets are more effective at inducing obesity when animals are weaned directly onto them (Peckham et al.,1962). Osborne-Mendel rats are particularly susceptible and the S 5B/PI strain (derived from a Sprague-Dawley/Black NIH cross) is very resistant to obesity induced by high fat feeding from weaning (Schemmel et al.,1970). Cafeteria feeding is more effective in older animals, and again shows a difference in effectiveness in different strains of rat (Rothwell and Stock,1982; Rothwell et al.,1982c). Young (up to about 10 weeks of age) rats exhibit virtually no increase in body weight despite a doubling of energy intake on a cafeteria diet (Rothwell and Stock,1980c, 1982). This resistance to obesity in young cafeteria fed animals is thought to be due to the activation of BAT leading to increased energy expenditure and a reduction in metabolic efficiency (Rothwell and Stock,1980c, 1982, 1983a). Adrenalectomy of older rats increases their capacity for DIT which results in a reduced weight gain, in comparison to intact rats of the same age, when fed a cafeteria diet (Rothwell et al.,1984b).

Returning high fat fed or cafeteria fed rats to a normal chow diet results in hypophagia until normal body weight is regained (Peckham et.al.,1962; Rothwell and Stock, 1979; Rogers,1985), although one report maintains that the obesity due to cafeteria feeding persists after transfer to chow feeding (Rolls et al 1980).

High fat, cafeteria and sucrose-supplemented diets all induce an increase in general SNS activity (Young and

Landsberg,1977; Young et al.,1982; Schwartz et al.,1983; Kaufman et al.,1986). This increase in SNS activity is thought to be responsible for the activation of BAT thermogenesis in overfed animals, but in animals which develop dietary obesity it is thought that the increased energy expenditure, caused by SNS induction of DIT, is not sufficient to overcome the increase in energy intake or the reduced energy cost of fat deposition. The development of chronic diet-induced obesity is associated with a return to normal sympathetic activity in BAT (Levin et al.,1983a, c, 1985).

1.4.3 Genetic Obesity.

There exist several types of obese animals which inherit their obesity as a genetic trait. More than one type of genetic model exists. Polygenic inbred strains such as the New Zealand obese mouse (NZO) and the Japanese KK mouse are spontaneously obese. Obesity prone polygenic hybrids such as the Osborne-Mendel rat and the spiny mouse (Acomys cahirinus) also provide useful models. These polygenic strains probably provide a better model of the complex human condition. Single gene dominant mutants include the Yellow mouse ($A^{y}a$) and the adipose mouse (Ad), but most research has utilised the single gene recessive mutants such as the obese (ob/ob) and diabetic (db/db) mouse and the Zucker fatty (fa/fa) rat.

1.5 The Zucker Fatty (fa/fa) Rat.

The Zucker fatty rat was reported by Zucker and Zucker in 1961 as a spontaneous mutation in the 13M strain of rat. Obesity is inherited in these mutants as a single autosomal recessive gene designated 'fa' for the obese allele and 'Fa' for the wild type. Hetrozygotes are phenotypically lean, so obese animals arise in a 1 in 4 frequency from heterozygote matings, indicating simple Mendelian inheritance (Zucker and Zucker,1963).

Obese female Zucker rats are infertile (Saiduddin et al., 1973) and obese males only fertile when their food intake is restricted to 50% of their ad lib. intake (Yen et al.,1977,

Edmonds and Withyachumnarnkel,1980), so breeding normally takes place from heterozygote parents.

1.5.1 Energy Balance in the Zucker (fa/fa) Rat.

Obese Zucker rats eat 40% more than their lean littermates (Haberey et al 1980), although when food intake is calculated on a body weight basis hyperphagia only persists from 3-10 weeks of age (Dilettuso and Wangsness,1977). Hyperphagia is not present before weaning (Stern and Johnson,1977; Boulange et al.,1979). Many abnormalities are present in the control of food intake in the fa/fa rat. The normal diurnal feeding pattern is lost in obese rats although the major proportion of their daily food intake is still consumed at night (Haberey et al.,1980). Obese rats tend to eat larger, fewer meals (Becker and Grinker 1977) and nibble constantly (Castonguay et al.,1982b). 50% dilution of the diet with cellulose causes lean rats to increase their food intake to maintain their caloric intake, but obese rats appear to be less sensitive and only adjust their food intake at a lower dilution (20%)(Bray and York, 1972). Lean Zucker rats will also increase food intake in response to a low protein diet in order to maintain dietary protein intake (Young et al.,1980),an adaptive response that is lacking in the fatty rat. Normal rats tend to select diets for protein and energy intake (Musten et al.,1974) whereas obese (fa/fa) rats will self-select a diet high in fat when given the opportunity (Castonguay et al.,1982a). Lean and obese Zucker rats show differing responses to a variety of feeding stimuli. Central administration of 2DG normally causes hyperglycaemia and hyperphagia in lean rats but not in obese where only hyperglycaemia is observed (Ikeda et al.,1980). Peripherally administered insulin exerts a greater stimulus to increase food intake in obese rats (Ikeda et al.,1980) and centrally infused insulin does not reduce food intake in fa/fa rats as it does in lean (Ikeda et al.,1983). These data imply that the obese rat has an impaired insulin mediated glucosensitive site regulating food intake (Ikeda et al.,1980). Obese Zucker rats decrease food intake less than lean controls after peripheral injections of CCK (McLaughlin

and Baile,1980) but have elevated levels of this putative satiety peptide in the hypothalamus (McLaughlin et al.,1985), therefore, it seems unlikely that increased food intake in obese rats is due to the effects of CCK. β-endorphin is an endogenous opioid the action of which, when centrally administered, is to increase food intake (see Section 1.2.1). Pituitary and hypothalamic concentrations of opioids in general (Recant et al.,1983) and β-endorphin in particular (Margules et al.,1978) are found to be increased in obese rats. Naloxone, an opiate antagonist, suppresses feeding in obese rats when given peripherally (Margules et al.,1978) or centrally (Thornhill et al.,1982). In contrast, auto-immunisation against β-endorphin increases food intake in obese rats more than in lean, and it has been suggested that β-endorphin acts peripherally as a satiety signal and centrally as a hunger signal (McLaughlin and Baile,1985). This would suggest that the effect of peripherally injected naloxone on food intake, seen by Margules et al.(1978), was a centrally mediated effect of the drug. The evidence overall suggests a role for β-endorphins in the control of food intake, and it seems likely that the altered central concentration and increased responsiveness of the fa/fa rat to this opioid may be involved in the development or maintenance of hyperphagia in these animals (Thornhill et al.,1982; McLaughlin and Baile,1985).

Hyperphagia is contributary towards, but not essential for, the development of obesity in the Zucker fatty rat. Pair feeding obese rats to the ad lib. intake of their lean littermates results in a decrease in weight gain in the obese animal of 40%. However, protein deposition drops under these conditions, and fat storage is increased (Pullar and Webster,1974; Deb and Martin,1975; Zucker,1975). Even when restricted to two thirds of the caloric intake of lean rats, body composition remains unaltered in obese rats (Bray et al.,1983). Abnormalities in body composition are not, therefore, solely due to food intake. Muscle protein deposition is reduced in the obese rat over the last stages of suckling (days 16-21; Reeds et al.,1982), but recovers as hyperphagia develops after weaning. By 34 days of age, the abnormal partitioning of energy into fat and protein

stores in the obese rat is apparent (Radcliffe and Webster, 1978). Hyperphagic obese rats deposit protein at the same rate as age matched lean rats, and most of the difference in body weight can be ascribed to fat deposition . The percentage of protein per carcass is reduced in the obese rat, but the weight of protein in the fat-free mass is similar for lean and obese siblings of the same age (Bell and Stern,1977). These observations led Radcliffe and Webster (1978) to suggest that the hyperphagia of the obese rat was geared towards the maintenance of lean body mass. It seems unlikely, however, that defence of lean body mass is a stimulus for the hyperphagia of the obese rat, as these animals do not adjust their dietary intake on a low protein diet to maintain dietary protein levels (Young et al., 1980) and will self-select a diet high in fat when given free choice of macronutrients (Castonguay et al.,1982a).

Obese rats are more energetically efficient than their lean littermates. The amount of energy stored for a given energy intake is greater in the obese mutant (Deb et al., 1976; Pullar and Webster,1979). The ability to store a greater proportion of the dietary intake as fat has been attributed to a reduced energy expenditure in the fatty rat. The energy requirement for the maintenance of body weight of the obese rat has been variously reported as lower than (Haberey et al.,1980), or similar to (Deb et al., 1976) lean controls, and metabolic rate is similar or slightly lower (Rothwell et al.,1981; Rothwell and Stock, 1983). Spontaneous activity is reduced in the adult obese rat, but the decrease in activity occurs after weaning and follows the onset of hyperphagia and obesity (Stern and Johnson,1977). Thus, there seems to be no significant decrease in physical energy expenditure until after obesity has begun to develop. Forced exercise delays, but does not prevent, the obesity of the fa/fa^{rat}_{λ} (Deb and Martin,1975; Walberg et al.,1982). The effect of exercise in obese animals seems to be to reduce WAT cell number (Walberg et al.,1983; Seelbach et al., 1985), but the decreased WAT adipocyte population persists after the cessation of exercise only in obese animals that are pair-fed to the ad lib. food intake of age matched lean animals (Walberg et al.,1983.)

The reduced energy expenditure of the obese Zucker rat appears to be due to a defect in adaptive thermogenesis. Oxygen consumption has been shown to be reduced in preobese pups as early as 2-7 days of age. (Planche et al.,1983; Moore et al.,1985). As muscle oxygen consumption is normal in 6 week old obese animals, it seems likely that other tissues are responsible for the increased metabolic efficiency of the young obese rat (Wardlaw and Kaplan,1984). BAT thermogenic capacity, as measured by GDP binding, is decreased in the fa/fa rat by 50% (Holt and York,1982). This depression in BAT thermogenesis is detectable at 2 days of age and persists throughout life (Bazin et al.,1984, York et al.,1984; Ashwell et al.,1985). The depressed BAT mitochondrial GDP binding is due to a decreased number of binding sites (Holt and York,1982; Bazin et al.,1984; French et al.,1985), rather than a lower amount of 32K protein. The levels of this protein in the obese rat are still comparable to lean levels until 5 weeks of age and have been shown to be depressed at 12 weeks (Ashwell et al.,1985). The low BAT mitochondrial GDP binding capacity of obese rats in the presence of normal 32K protein levels suggests that there is a greater proportion of masked GDP binding sites in these animals. A lower population of exposed GDP binding sites would be consistent with the reduced sympathetic tone to BAT that has been observed in obese Zucker rats (see Section 1.5.2, York et al.,1985b), as unmasking of BAT mitochondrial GDP binding sites is thought to be accomplished through SNS activated mechanisms (see Section 1.3.4).

Unlike the obese mouse, young obese rats can respond normally to cold exposure and noradrenaline administration with increases in mitochondrial GDP binding (Levin et al.,1980; Triandafillou and Himms-Hagen,1983; Holt et al.,1983) and noradrenaline turnover (Marchington,1985 ; York et al.,1985b) in BAT. The ability to withstand cold exposure decreases with age in the obese animal, and cold-induced increases in GDP binding, oxygen consumption and BAT blood flow are not as great in older fa/fa rats (Wickler et al.,1982; Holt et al.,1983; Levin et al.,1984). The reduced capacity of BAT to respond to cold may be a reflection of the reduced amount of 32K protein in older animals (Ashwell et al.,1985).

Although young obese rats are able to respond normally to cold exposure, they do not increase BAT thermogenesis in response to dietary stimuli. Neither a single meal (Rothwell et al.,1983a; Marchington et al.,1983), nor prolonged overfeeding (Holt et al.,1983; Triandafillou and Himms-Hagen, 1983; York et al.,1985b) evoke the acute increases in oxygen consumption or long term changes in GDP binding and noradrenaline turnover in BAT of obese rats that are seen in their lean siblings.

Adrenalectomy of obese rats restores basal BAT mitochondrial GDP binding and noradrenaline turnover to normal, lean levels (Holt and York,1982; York et al.,1985b). Many other defects of the obese rat are also alleviated by adrenalectomy. Diurnal feeding rhythm is improved (Freedman et al.,1985), and there is a large decrease in energy intake that is not accompanied by a change in energy expenditure which results in a large overall decrease in energetic efficiency towards a level seen in lean rats (Marchington et al.,1983). The increase in oxygen consumption seen in response to a single meal in lean animals is also restored in adrenalectomised obese Zucker rats (Marchington et al., 1983) and they are able to respond to sucrose feeding with an increase in BAT mitochondrial GDP binding (Holt et al., 1983). It is possible that adrenal glucorticoids may be suppressing DIT in the obese rat. Glucocorticoids are normally permissive to NST (Fellenz et al.,1982), so it seems unlikely that the hormone is suppressing BAT thermogenesis by a direct effect on the tissue, even though glucocorticoid receptors are present in BAT (Feldman,1978). Corticosterone administration is known to inhibit DIT, but not NST (Galpin et al.,1983). Knowing that young obese rats respond normally to cold acclimation and noradrenaline injections, but only respond to dietary stimuli after adrenalectomy, it seems likely that the regulation of DIT in the obese animal is defective rather than BAT itself and that the expression of the defect requires the presence of adrenal glucocorticoids (Holt et al.,1983; York et al., 1985a; Freedman et al.,1986a).

1.5.2 The Sympathetic Regulation of BAT in the Obese (fa/fa) Rat.

As described in Section 1.3.7, the SNS is thought to be the principal regulator of BAT thermogenesis. Noradrenaline turnover, an index of sympathetic activity within a tissue, is very low in BAT of young obese rats housed at normal temperature (24^{o}C) and fed laboratory chow, despite a normal turnover rate in heart (York et al., 1985b). Cold acclimation of 5-6 week old obese rats increases noradrenaline turnover in BAT to an equivalent rate to that seen in cold-stimulated lean animals of the same age. 7-day sucrose overfeeding has no such stimulatory effect on noradrenaline turnover in BAT of fa/fa rats, although lean rat BAT noradrenaline turnover rates are increased by 2½ fold after the same stimulus (York et al., 1985b). The noradrenaline turnover rate in heart in both lean and obese Zucker rats is not affected by cold and is stimulated by sucrose feeding in obese, but not lean rats (York et al., 1985b). Sprague-Dawley rats show an increase in cardiac noradrenaline turnover after sucrose or cafeteria feeding (Young et al., 1982; Marchington et al., 1986) and also after cold acclimation (Young et al., 1979, 1982). It is possible that these different responses of cardiac noradrenaline turnover in the Zucker rat may reflect a strain difference.

The reduced noradrenaline turnover rate in BAT of obese rats is indicative of reduced nerve stimulated noradrenaline release (Landsberg and Young, 1978). This is associated with reduced neuronal synthesis of noradrenaline from tyrosine (Marchington, 1985). Activity of tyrosine hydroxylase, the rate limiting enzyme of noradrenaline synthesis, is normal in BAT of young obese rats (Marchington, 1985) but activity of dopamine β -hydroxylase is reduced (Levin et al., 1983) so dopamine β -hydroxylase may be the rate limiting enzyme in BAT of fa/fa rats. Although noradrenaline synthesis is reduced, extraneuronal uptake and the activity of catechol-O-methyl transferase, the enzyme primarily responsible for degradation of neuronally released noradrenaline, are normal. Thus reduced turnover must result from severely limited tonic and nerve stimulated noradrenaline release. The synthesis rates of

noradrenaline in heart, brain and adrenal glands were found to be normal in the obese rat (Marchington, 1985) suggesting that the reduced noradrenaline metabolism is BAT specific.

Cold exposure and sucrose feeding both stimulate noradrenaline turnover in BAT of the lean rat , but only cold exposure does so in obese animals. Sucrose feeding fails to elicit increases in noradrenaline turnover in BAT of the obese rat (Marchington, 1985). These data suggest that although the basal level of sympathetic activity is low in BAT of the obese rat, these animals are capable of sympathetically activating BAT in response to cold, but not to dietary stimuli such as sucrose feeding.

Adrenalectomy normalises the rates of noradrenaline turnover in BAT of obese rats (York et al., 1985a). However, although the sucrose feeding of both lean and obese adrenalectomised rats increased BAT mitochondrial GDP binding (Holt et al., 1983), no further increase in BAT noradrenaline turnover was seen (York et al., 1985b). This implies the existence of a further regulatory mechanism by which BAT can be activated in the absence of an increase in sympathetic stimulation.

Abnormally low levels of β-adrenergic receptors have been reported in heart (Bass and Ritter, 1985) and BAT (Levin et al., 1982a) of older genetically obese rats, but the affinity for agonist and the concentration of agonist required for half-maximal stimulation of lipolysis in BAT of lean and obese rats are idential (Levin et al., 1982a). Normally, decreased sympathetic activity would be expected to be associated with an increase in receptor number and receptor hypersensitivity (Sporn et al., 1976). The similarity in receptor number between obese rat BAT and WAT in animals of the same age (5-6 months old) led Levin et al., (1982a) to suggest that excessive infiltration by white adipocytes of BAT depots occurs, and that this is at least partially responsible for the reduction in overall thermogenic capacity of BAT from obese Zucker rats of this age. Recent observations in young (5 week old) obese rats suggest that the β-receptor population is normal in BAT adipocytes but that

α_1-receptor number is depressed (Raasmaja and York - unpublished observations). The reduced α_1-receptor number is corrected after adrenalectomy which suggests that the increased sympathetic tone to BAT may cause an increase in the number of α_1-receptors. As α_1-mediated effects are known to potentiate the thermogenic effects of noradrenaline working through β-receptors in BAT (Foster, 1986) it is possible that adrenalectomised-sucrose fed animals can increase their capacity for GDP binding through an α_1 potentiation of β-mediated effects with no further increase in sympathetic tone.

1.5.3 The Endocrine Status of the Obese (fa/fa) Rat.

a. Insulin and Glucagon.

Hyperinsulinaemia is characteristic of many forms of obesity, including that of the Zucker rat (see Sclafani, 1984). Although adult obese Zucker rats are markedly hyperinsulinaemic (Zucker and Antoniades, 1972; York et al., 1972b; Bazin and Lavau, 1982) the condition is probably not instrumental in the onset of obesity as the obese state begins to develop before hyperinsulinaemia is apparent (Turkenkopf et al., 1982). Abnormal glucose responses are apparent before hyperinsulinaemia develops, as an exaggerated insulin response to a glucose load is exhibited by fa/fa rats at 18 days of age (York et al., 1981; Blonz et al., 1985; Fletcher et al., 1986). Intolerance to an oral glucose load is present in 6 week old obese rats (after hyperinsulinaemia has developed) and the condition worsens with age (Ionescu et al., 1985). Severe food restriction or starvation will reduce adult obese insulin levels to normal (Zucker and Antoniades, 1972) and adrenalectomy will reduce (Holt, 1984) or abolish (Freedman et al., 1986b) hyperinsulinaemia and the exaggerated insulin response to a glucose load (Marchington, 1985) in young obese rats. Alloxan diabetic or streptozotocin diabetic lean and obese rats on similar levels of insulin replacement gain the same amount of weight, eat similar amounts of food on a body weight basis, and deposit similar amounts of protein. However, hepatic lipogenesis and fat deposition are still elevated in these animals (Chan et al., 1982; Stolz and Martin, 1982).

This demonstrates that the enhanced lipid deposition of the fa/fa rat is not entirely dependent on insulin and food intake.

The insulin secretory β cells of the pancreas are under the neural control of the parasympathetic nervous system (Mayhew et al.,1969) The isolated pancreata of obese rats secrete elevated amounts of insulin compared to lean animals at normoglycaemic and hypoglycaemic levels, suggesting that the obese Zucker rat pancreas is extremely responsive to glucose (Curry and Stern,1985). Obese rats are not hyperglycaemic (Bray and York,1979) so an increased vagal stimulation of the pancreas, combined with a hyperreactivity to glucose, may account for the hyperinsulinaemia. There is considerable evidence to suggest that enhanced PNS activity may be a major factor in the hyperinsulinaemia and obesity of the fa/fa rat. The treatment of obese Zucker rats with atropine restores the increase in oxygen consumption seen in response to a 40kJ meal, and increases the response normally seen in lean rats (Rothwell et al., 1982d). This suggests that the PNS may normally inhibit the thermic effect of feeding, and that the inhibitory effect is much larger in the obese rat. Atropine injection also prevents the exaggerated insulin secretory response to a glucose load in fa/fa rats (Rohner-Jeanrenaud et al.,1983; Rohner-Jeanrenaud and Jeanrenaud,1985a, b). Priming of the vagus nerve by acute electrical stimulation potentiates glucose stimulated insulin secretion to a greater extent in obese Zucker rats than lean (Rohner-Jeanrenaud and Jeanrenaud,1985a). Subdiaphragmatic vagotomy reduces the hyperinsulinaemia of older rats (Rohner-Jeanrenaud et al., 1983) but does not abolish their obesity (Opsahl and Powley, 1974), as it does in VMH-lesioned rats (Powley and Opsahl, 1974). These observations are suggestive of an enhanced parasympathatic activity to the pancreas in obese animals. The vagus nerve also stimulates pancreatic α cells to secrete glucagon, the actions of which would be opposite to those of insulin. Preobese Zucker pups show an elevated glucagon secretory response to arginine and glucose (Rohner-Jeanrenaud and Jeanrenaud,1985a,b), however, serum levels

of glucagon in the obese rat are normal (Eaton et al.,1976). Older rats show a depressed secretory response to arginine and hypothalamic stimulation (Bryce et al.,1970; Eaton et al.,1976).

As mentioned in Section 1.5, Zucker fa/fa rats have a reduced brain insulin content (Baskin et al.,1985; Figlewicz et al.,1985), and elevated CSF insulin (Stein et al.,1983). It has been suggested that a central defect in insulin binding may contribute towards inadequate perception of a central insulin feedback signal and to the hyperphagia observed in the obese rat (Figlewicz et al.,1985). In contrast, Ikeda et al.(1983) found no differences in brain insulin levels between lean and obese rats, but found that intracerebroventricular infusion of insulin reduced food intake and body weight gain of lean but not obese rats. The suggestion from this work was that the obese rat has a reduced central nervous system sensitivity to insulin and that the phenomenon may participate in the development of hyperphagia and obesity, possibly due to a decreased sensitivity of the hypothalamic glucoreceptor system.

b. Pituitary-Thyroid Hormones.

The reduced metabolic rate seen in obese rats (Bray, 1969) initially led to the investigation of the pituitary-thyroid system in these animals as a possible cause for reduced energy expenditure. The uptake and release of I^{131} iodine by the thyroid gland, the concentration of serum protein-bound iodine and of thyroxine (T_4) are all depressed in the obese rat (Bray and York,1971; Autissier et al.,1980; Young et al.,1985). Normal (Martin et al.,1978; Autissier et al.,1980) or depressed (Young et al.,1985) serum levels of T_3 are seen in older obese rats. Young obese rats have also been reported as having similar (Fletcher et al.,1986) or depressed (Holt et al.,1983) serum T_3 levels compared to lean animals. As the thermogenic effects of noradrenaline are reduced in hypothyroid animals (Fregly,1979) it was suggested that the depressed thyroid function in young obese rats may be an important factor in the impairment of BAT thermogenesis (Holt et al.,1983). Serum and pituitary levels

of thyroid stimulating hormone (TSH) are normal in obese rats, as is the thyroid response to TSH injection. Injections of thyrotropin releasing hormone (TRH) produce a normal increase in circulating levels of TSH in fa/fa rats (Bray and York,1971; York et al.,1972a). However, propylthiouracil treated obese rats do not show the large increases in TSH seen in lean rats treated the same way suggesting that there is a hypothalamic defect in the regulation of TSH secretion, possibly due to an impairment in the formation or release of TRH (York et al.,1972).

When obese rats are given thyroid powder in their food, excess weight gain is prevented (Levin et al.,1982b). Similarly, adrenalectomy (which also corrects the obesity) of young rats increases the low serum T_3 levels to normal (Holt et al.,1983). Adrenalectomy also normalises the α_1-receptor population of BAT (Raasmaja and York, unpublished observations), an effect which would increase the activity of 5'-monodeiodinase - the enzyme which is responsible for the conversion of T_4 to the more potent T_3 (Schimmel and Utiger,1977). The activity of this enzyme is increased by α_1-mediated mechanisms in BAT (Silva and Larsen,1983), so it is possible the elevated BAT sympathetic activity in obese rats, in combination with the increase in α_1-receptor number in BAT, could result in a significant increase in serum T_3 levels. Adrenal glucocorticoids are known to inhibit thyroid function (Chopra et al.,1975; Pamenter and Hedge, 1980), so it is possible that glucocorticoid inhibition of thyroid hormones has a role in the development of obesity in the fa/fa rat and is one of the defects corrected by adrenalectomy.

c. Pituitary-Adrenal Hormones.

Adrenal glucocorticoids seem to be intimately involved in the obesity of the Zucker rat. Serum levels of corticosterone in the obese rat are mainly found to be similar to lean (Yukimura et al.,1978; Holt et al.,1983; York and Al-Baker 1984), although two groups have found an increased level (Martin et al.,1978; Fletcher et al.,1986) and the diurnal rhythm is abnormal (Fletcher et al.,1986). It is thought

that obese rats may be more sensitive to the effects of corticosterone than their lean littermates, as the same replacement dose of the steroid to adrenalectomised lean and obese rats causes greater increases in food intake in the obese animals (Yukimura et al.,1978; Freedman et al.,1986a). Adrenalectomy restores body weight, food intake and meal patterns, lipogenesis, fat deposition and insulin levels to normal in the obese rat (Yukimura et al.,1978; York and Godbole,1979; Freedman et al.,1985). The decreases in food intake seen in obese rats after adrenalectomy consists mainly of a fall in fat intake when macronutrient choice is offered (Castonguay and Stern,1983). Energy balance in obese rats and BAT thermogenic defects are also corrected by adrenalectomy. BAT mitchondrial GDP binding and the thermic response to a meal are restored to lean levels (Holt and York,1982; Marchington et al.,1983), as is the decreased sympathetic drive to BAT (Marchington,1985; York et al.,1985a). Treatment of adrenalectomised fatty rats with corticosterone restores the obesity, hyperphagia, deranged meal patterns, defective BAT thermogenesis and noradrenaline turnover rate to normal obese levels (Yukimura et al.,1978; Holt et al.,1983; Freedman et al.,1985, 1986a; York et al.,1985a, b). These observations suggest that the presence of corticosterone is required for the expression of the defect in DIT in these animals.

The endocrine consequences of adrenalectomy are the removal of glucocorticoids and mineralocorticoids from the circulation, an increase in the secretion of corticotropin (ACTH) from the pituitary and corticotropin releasing factor (CRF) from the hypothalamus, due to the removal of negative feedback by corticosterone on ACTH secretion. The capacity for ACTH secretion seems to be normal in the obese rat and there is a normal change in secretion in response to adrenalectomy and corticosterone replacement (Yukimura et al 1978). Treatment of obese rats with ACTH mimics the effects of adrenalectomy on BAT thermogenesis and food intake in the short term, but chronic ACTH injection eventually increases serum corticosterone levels and BAT thermogenesis (measured by BAT mitochondrial GDP binding)

returns to pretreatment levels, although food intake remains depressed (York and Al-Baker, 1984). Corticosterone replaced adrenalectomised animals have decreased ACTH levels (Yukimura et al.,1978) and ACTH injection can acutely counteract the detrimental effects of steroid replacement on the improved BAT thermogenesis normally seen in obese adrenalectomised animals (York and Al-Baker, 1984). Hypophysectomy, which results in the removal of ACTH and, therefore, the suppression of glucocorticoid secretion, also prevents the excessive weight gain of the obese Zucker rat (Powley and Morton,1976). Although much work has recently been centred on the involvement of the hypophysis in the control of BAT thermogenesis (Rothwell and Stock,1985 a, b, c), these investigations have not yet been undertaken in the Zucker rat, so the significance of the pituitary-adrenal system in the obesity of these animals remains unclear .

1.5.4 The Development of Obesity in the Zucker Rat.

Obese Zucker rats can be visually distinguished from their lean littermates at 3-4 weeks of age (Bray,1977), however, differences between lean and preobese pups have been detected before this. 25% of 21 day old foetuses of hetrozygote parents have increased serum and decreased pancreatic insulin levels (Turkenkopf et al.,1982). Hyperinsulinaemia is not apparent during suckling when the animals are on an effectively high fat diet, but reappears on weaning on to the high carbohydrate chow diet (Turkenkopf et al.,1982). The earliest detectable post-natal defects to date in the preobese pup are reduced oxygen consumption at thermoneutral (33^{o}) or cool ($26^{o}C$) temperatures (Moore et al.,1985) and depressed BAT mitochondrial GDP binding (Bazin et al.,1984) at 2 days of age. 10 day and older animals have also been demonstrated to have depressed BAT mitochondrial GDP binding levels (York et al., 1984; Ashwell et al.,1985). This depressed GDP binding is associated with a reduced number of binding sites (Bazin et al.,1984) rather than a reduction in the mitochondrial concentration of 32K protein. The earliest investigated age with depressed 32K protein levels is 12 weeks (Ashwell et al.,1985).

7 day old obese pups exhibit reduced oxygen consumption, lower core temperature, increased inguinal fat pad weight and cell size, and high lipoprotein lipase activity (Boulange et al.,1979; Planche et al.,1983). Fatty acid synthetase activity is also increased from this age (Bazin and Lavau, 1982; Lavau et al.,1985), and taken together with the high lipoprotein lipase activity (Boulange et al.,1979; Greenwood et al.,1981) it seems that preobese Zucker rats have a high capacity for lipid synthesis and storage. In fact, high lipoprotein lipase activity persists in the obese rat throughout life. Animals up to 20 weeks of age still maintain higher lipoprotein lipase activity compared to their lean littermates and the increase in activity in fa/fa rats is due to a greater lipoprotein lipase activity per cell and so is not wholely attributable to increased WAT cell number (Gruen et al.,1978). Increased inguinal fat cell size at 7 days, and reduced core temperature at 14-16 days, are commonly used criteria for the identification of preobese pups (Boulange et al.,1979; Godbole et al.,1978, respectively). 17-18 day old preobese pups show an exaggerated insulin response to a glucose load in the absence of basal hyperinsulinaemia (York et al.,1981; Rohner-Jenrenaud and Jeanrenaud,1985a). With the onset of weaning at about 17 days, pups begin to eat the high carbohydrate laboratory chow and several more of the defects of the obese rat become apparent. Hyperinsulinaemia reappears and is associated with the hypertrophy and hyperplasia of the β-cells of the pancreas (York et al 1972, Bazin and Lavau 1982). Hyperphagia, which is absent during suckling, develops with the availability of solid food (Boulange et al 1979). Lipogenesis is increased in white adipose tissue and liver and the activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase and acetyl CoA carboxylase are stimulated (York et al.,1971; Godbole et al.,1978). Delaying weaning (usually day 21) prevents the rise in hepatic lipogenesis and glucose-6-phosphate dehydrogenase activity, and in serum insulin (Godbole et al.,1978) and early access to laboratory chow induces earlier increases in serum insulin, WAT lipogenesis and food intake (York et al.,1981; Bazin and Lavau,1982). At weaning WAT hypertrophy

increases, a process which is accompanied by a proliferation of white adipocytes which continues in the fa/fa rat past the normal age of 14 weeks at which lean rat WAT cell numbers plateau (Johnson et al.,1971). It appears that the increased capacity for fat synthesis and deposition, accompanied by the defective DIT in brown fat, leads to increased adiposity and obesity in the fatty rat. It has been suggested that the hyperinsulinaemia that occurs at weaning causes an overstimulation of target organs (Godbole and York, 1978), followed by the development of peripheral insulin resistance in muscle (Crettaz et al.,1981), WAT (Cushman et al.,1978) and later in the liver (Terretaz et al.,1986a, b). It appears that WAT in the adult obese rat serves mainly as a storage site (Turkenkopf et al.,1980) with the main site of lipogenesis in the liver (Godbole and York, 1978).

A gene dosage effect has been demonstrated in fa/fa and Fa/fa rats with regard to subcutaneous fat pad weight and the susceptibility to gain weight on a high fat diet (Zucker and Zucker, 1963). BAT mitochondrial GDP binding and the metabolic response to a standard meal also show a gene dosage effect (York et al.,1984). Cerebrospinal fluid insulin content (Figlewicz et al.,1985), insulin binding to certain brain areas (Baskin et al.,1985, Figlewicz et al.,1985), insulin binding to liver in adult obese Zucker rats (Figlewicz et al.,1985) and the *in vitro* pancreatic insulin release in response to glucose perfusion (Blonz et al.,1985) all exhibit a proportional relationship to 'fa' gene concentration. These observations suggest that the regulation of DIT through BAT thermogenesis and the binding of insulin to the brain and peripheral organs may be closely linked to the primary gene defect in the Zucker rat.

1.6 Aims of the Project.

The obesity of the Zucker rat arises from a defect in energy expenditure associated with an inability to sympathetically activate BAT in response to dietary signals, although the response to cold is normal in young animals. Adrenalectomy prevents the development of obesity

in the fa/fa rat, abolishes the defects in basal BAT sympathetic tone and thermogenic function, and restores BAT thermogenic responses to dietary stimuli. These improvements in BAT metabolism are thought to be dependent on the removal of corticosterone. The control of food intake in the obese rat is also abnormal. Unlike their lean littermates, obese rats do not become hyperphagic or reduce their metabolic rate after inhibition of central glucose metabolism with 2DG. The defect in DIT and food intake control could involve a lack of dietary signals generated to the central nervous system (CNS), a failure of the CNS to recognise such signals or the inability to couple the signals to effector mechanisms (i.e. BAT) via the CNS (sympathetic) outflow.

This project was designed to investigate the hypothesis that variations in glucose metabolism act as an important signal involved in the activation of BAT thermogenenesis in response to diet, and that this system is disturbed in the obese (fa/fa) Zucker rat, as a result of their apparent sensitivity to adrenal glucocorticoids.

The aim of this project was, therefore:

a) to determine whether BAT thermogenesis in lean rats was sensitive to variations in glucose metabolism;
b) to determine whether obese (fa/fa) rats lack this sensitivity;

and

c) whether this postulated insensitivity of BAT to glucose metabolism in the obese (fa/fa) rat was mediated by adrenal glucocorticoids.

This was investigated by examining the responses of BAT of lean and obese (fa/fa) Zucker rats to thermogenic stimuli (cold, diet, etc.), as determined by the adrenal status of the animal, under manipulation of glucose metabolism. Variations in glucose metabolism or availability were induced experimentally with 2DG or changes in the constitution of the diet.

A further line of study was undertaken to investigate

the role of the brain opiate system in the aberrant feeding behaviour of the obese (fa/fa) Zucker rat. The suggestion has been made that the hyperphagia of the obese (fa/fa) rat is dependent upon the presence of high levels of the endogenous opioid β-endorphin in the brain. The effect of the opiate antogonist naloxone on food intake and BAT thermogenesis in lean and obese (fa/fa) Zucker rats was investigated.

CHAPTER 2. MATERIALS AND METHODS.

2.1 Reagents.

Unless indicated below, chemicals used were of reagent grade and were obtained from BDH Chemicals, Poole, Dorset, UK or Sigma Chemicals, Poole, Dorset, UK.

ABTS - Boehringer Corporation Ltd., Lewes, East Sussex, UK.

Acrylamide - Koch-Light Ltd., Haverhill, Suffolk, England.

ACTH (Synacthen Depot) - CIBA Laboratories, Horsham, West Sussex, UK.

Anti-Rabbit Ig biotinylated whole antibody (from Donkey) - Amersham International PLC, Amersham, Bucks, UK.

Bio-Beads SM2 - BioRad Laboratories, Watford, Herts, UK.

Dextran T_{70} - Pharmacia Fine Chemicals Ltd., Milton Keynes, UK.

Dimethyl Bis-phenyl Oxazoyl Benzene (POPOP) - G. & G. Chemicals Ltd., Berks., UK.

Diphenyloxazole (PPO-Scintillation grade) G. & G. Chemicals Ltd., Berks., UK.

Ensure - Abbot Laboratories Ltd., Queenborough, Kent, UK.

Glucose oxidase (sp.act 200 $U.mg^{-1}$), - Boehringer Corporation Ltd., Lewes, East Sussex, UK.

Glycine - Koch-Light Ltd., Haverhill, Suffolk, UK.

HRP Enzyme substrate (4 -chloro-1-napthol) - BioRad Laboratories, Watford, Herts. UK.

Horseradish peroxidase (sp.act. 200 $U.mg^{-1}$) - Boehringer Corporation Ltd., Lewes, East Sussex, UK.

Hydroxylapetite (Biogel HTP) - BioRad Laboratories, Watford, Herts., UK.

Hypnorm (fentanyl-fluanisone) - Southern Vetinary Supplies Ltd., Lewes, East Sussex, UK.

Insulin Binding Reagent - Wellcome Reagents Ltd., Beckenham, Kent, UK.

Insulin Standard (Rat) - Novo Laboratories, Denmark.

N,N' - Methylenebisacrylamide - Koch-Light Ltd., Haverhill, Suffolk, England.

Naloxone - DuPont Pharmaceuticals, Garden City, New York, USA.

NCS Tissue Solubiliser - Amersham International PLC, Amersham, Bucks, UK.

Non-Esterified Fatty Acids Kit - Boehringer Corporation Ltd., Lewes, East Sussex, UK.

Palmitic Acid (Puriss.) - Koch-Light Ltd., Haverhill, Suffolk, England.

Readysolv NA - Beckman RIIC Ltd., High Wycombe, Bucks, UK.

Streptavidin-Biotinylated Horseradish peroxide complex - Amersham International PLC, Amersham, Bucks, UK.

Radiochemicals.

All radiochemicals were obtained from Amersham International PLC, Amersham, Bucks, UK.

1,2,6,7-[^{3}H]-corticosterone ; 75-105μCi.mmol^{-1} (1mCi.ml^{-1})

8-[^{3}H]-Guanosine 5' diphosphate; 10-15μCi.mmol^{-1} (1mCi.ml^{-1})

[^{125}I]-insulin; 50μCi. g^{-1} (1μCi.ml^{-1})

[^{125}I]-protein A, labelled with Bolton & Hunter reagent; 30mCi.mg^{-1} (100μCi.ml^{-1})

U-[^{14}C] -sucrose; 5-15 mCi.mmol^{-1} (200μCi.ml^{-1})

2.2 Animals.

Lean (Fa/?) and obese (fa/fa) Zucker rats bred in the University animal house from heterozygote parents were used in all experiments. All animals were 4-6 weeks old at the start of each experiment unless otherwise stated in the text. All animals within an experimental group were of the same sex.

2.3.1 Animal Maintenance.

Rats were housed in wire mesh bottomed cages and maintained on a 14-10 hour light dark cycle (lights on 0600-2000 hrs), at an ambient temperature of 22-24^{o}C. Animals were routinely fed laboratory chow (PRD, Labsure, Minea, Cambridgeshire) (table 2.1) and given tap water for drinking.

2.3.2 Temperature Measurement.

Rectal temperature in conscious rats was measured using an electric thermometer (Light Laboratories, Brighton, England) with a flexible probe inserted 2cm into the rectum.

In anaesthetised animals, thermocouples were placed 2cm into the rectum and beneath the interscapular brown adipose tissue depot and held in place with clips. These thermocouples were attached to a Comark microprocessor scanning thermometer (Comark Electronics Ltd., Rustington, West Sussex, England) programmed to read temperatures and print-out at five minute intervals.

2.4 Experimental Diets.

2.4.1 Sucrose Feeding.

Rats were given a drinking solution of 35% (w/v) sucrose in tap water in addition to chow and instead of drinking water.

2.4.2 High Fat and High Carbohydrate Diets.

These diets were prepared in pellet form to the compositions shown in tables 2.2 and 2.3. AIN-76 mineral and vitamin mix compositions are shown in tables 2.4 and 2.5

The high fat diet was designed to have a comparable protein, fibre and mineral content to PRD, but to have an increased fat content, at the expense of carbohydrate. The high carbohydrate diet was designed to be as close as possible in composition to PRD, but the carbohydrate component had the same ratios of simple to complex carbohydrates as the high fat diets.

The values used to calculate the energy density of the diets were 37k J.g^{-1} for fat, 17k J.g^{-1} for protein and 16k J.g^{-1} for carbohydrate.

Table 2.1

Composition of PRD Food Pellets (Manufacturer's data)

	% by weight	% by energy
Protein	19.7	25.8
Fat	2.7	8.1
Carbohydrate	53.5	66.1
Fibre	5.3	-
Minerals) Vitamins)	1.8	-

Metabolisable Energy content 10.79 $MJ.kg^{-1}$

Table 2.2

Composition of Experimental Diets.

	% by weight	
	High Fat	High Carbohydrate
Casein	22	20
Corn Oil	16	3
Bran	9	9
Minerals (AIN-76)	4	4
Vitamins (AIN-76)	2	2
Cornstarch	26	34
Sucrose	20	27
D-L Methionine	0.6	0.6

plus water, to mix. Dried overnight at 65^{o}C after pelleting.

Table 2.3

Macronutrient and Energy compositions of High Fat and High Carbohydrate diets.

	High Fat		High Carbohydrate	
	% weight	% energy	% weight	% energy
Protein	23.7	22.5	21.3	23.7
Fat	16.5	33.9	3.5	8.4
Carbohydrate	48.9	43.6	64.8	67.9
Fibre	4.0	-	4.2	-
Other	6.9	-	5.2	-
Energy Content (Theoretical)	17.94 $MJ.kg^{-1}$		15.29 $MJ.kg^{-1}$	

Table 2.4

Mineral mix recommended by the American Institute of Nutrition 1976 (AIN-76).

	$g.kg^{-1}$ mixture
Dicalcium Phosphate	500
Sodium Chloride	74
Potassium Chloride	42
Potassium Sulphate	52
Magnesium Hydroxide	35
Manganous Carbonate	3.5
Ferric Citrate	6.0
Zinc Carbonate	1.6
Cupric Carbonate	0.3
Potassium Iodide	0.01
Sodium Selenate	0.01
Chromic Potassium Sulphate	0.55
Sodium Molybdate	0.01
Starch	285

Table 2.5

Vitamins mixture recommended by the American Institute of Nutrition 1976 (AIN-76).

		$mg.kg^{-1}$ mixture
Thiamine		600
Riboflavin		600
Vitamin B_6		700
Nicotinic Acid		3,000
Calcium Pantothenate		1,600
Biotin		20
Folic Acid		200
Vitamin B_{12}		1
Vitamin A	(400,000 IU)	800
Vitamin D_3	(10,000 IU)	20
Vitamin E	(5,000 IU)	20,000
Vitamin K	(Menapthone)	5
Inositol		10,000
Choline		10,000
Starch Supplement		952,454

Table 2.6

(a) Composition of CRM(X) Food Pellets and Ensure (Manufacturer's data)

	CRM(X)		Ensure	
	% weight	% energy	% weight	% energy
Protein	18.5	23.1	3.4	14
Fat	3.1	8.9	3.4	33
Carbohydrate	56	65.9	13.3	53
Fibre	3.4	-	-	-
Minerals/vitamins	3.2	-	1.8	-
Water	-	-	77.2	-
Metabolisible energy	13.59 $MJ.kg^{-1}$		4.45 $MJ.dm^{-3}$	

(b) Compositions of mix fed to vagotomised animals.

	% weight	% energy
Protein	7.1	22.5
Fat	1.7	11.9
Carbohydrate	22.0	65.5
Fibre	1.2	-
Minerals/vitamins	1.4	-
Water	60.1	-
Theoretical Energy Content	5.37 $MJ.kg^{-1}$	

Mix 35.5% CRM(X), 48.4% water, 16.1% Ensure by weight.

2.4.3 Feeding After Vagotomy.

All vagotomised and sham-vagotomised animals were fed on CRM(X) laboratory chow (Labsure, Minea, Cambridgeshire) soaked in water and Ensure to the composition shown in table 2.6 (b).

Vagotomised animals had free access to food, sham-vagotomised animals were restricted to the mean daily food intake of the vagotomised group.

2.5 Dose and Site of Injections.

2.5.1 ACTH.

Synacthen Depot was injected intramuscularly (i.m.)at a dose of 50μg.100g^{-1} bodyweight (0.05ml.100g^{-1} bodyweight) 24 hours and 3 hours before sacrifice.

2.5.2 Corticosterone.

Corticosterone in 0.1ml 1:2:7 (v/v/v) dimethylformamide:ethanol:0.9% (w/v) sodium chloride vehicle was injected subcutaneously (s.c.)at a dose of 1mg.100g^{-1} bodyweight per day for 7 days.

2.5.3 2-deoxy-D-glucose (2DG).

(i) 2DG was normally administered in two doses of 360mg.kg^{-1} bodyweight in 0.2ml 0.9% (w/v) sodium chloride intraperitoneally (i.p.) Injections were given 3 hours and 1 hour before sacrifice.

(ii) Where stated, 2DG was administered in a single 360mg.kg^{-1} bodyweight dose in 0.2ml 0.9% (w/v) sodium chloride i.p.,1 hour before sacrifice.

(iii) 2DG was infused into the Hepatic portal vein or carotid artery of anaesthetised animals at a dose of 6mg.100g^{-1} bodyweight in 0.2ml 0.9% (w/v) sodium chloride (sterile) via infusion pump, over a 5 minute period.

2.5.4 Naloxone.

Naloxone hydrochloride was injected s.c. in 0.1ml of an 0.9% (w/v) sodium chloride vehicle at a dose of $1mg.kg^{-1}$ bodyweight at the frequency or times shown in the text.

2.5.5 Noradrenaline.

(-) Arterenol bitartrate in 0.9% (w/v) sodium chloride was injected s.c. at a dose of $50\mu g.100g^{-1}$ bodyweight in an 0.1ml volume 30 minutes before sacrifice.

2.5.6 Propranolol.

Injection of $2mg.100g^{-1}$ bodyweight in 0.1ml 0.9% (w/v) sodium chloride was given s.c. 24, 16, 8 and 1 hour before sacrifice.

All control animals received vehicle only at the same site and volume as experimental groups.

2.6 Surgical Procedures.

2.6.1 Anaesthesia.

All surgery was performed under neuralept-analgesia using $0.05ml.100g^{-1}$ bodyweight Hypnorm, i.m., (fentanyl citrate, $0.315mg.ml^{-1}$ and fluanisone $10mg.ml^{-1}$) and $0.05ml.100g^{-1}$ bodyweight diazepam, i.p. ($4mg.ml^{-1}$ in 13:12 (v/v) ethanol: water) (Green, 1975). Righting reflex was lost within 5 minutes and animals were completely immobilised for 45-75 minutes. Recovery was effected within 2-5 hours.

2.6.2 Adrenalectomy.

Bilateral adrenalectomies were performed from the dorsal approach. After anaesthesia, adrenal glands were removed through two dorsal incisions with minimal damage to the capsule. Wounds were closed with thread, and the animals left to recover in a warm (24-26^{o}C) quiet room.

All adrenalectomised animals were maintained on 0.9% (w/v) sodium chloride instead of drinking water and had free access to chow. Animals were allowed to recover for 5-7 days before experimentation. Success of adrenalectomy was verified by inspection upon sacrifice and by serum corticosterone assay.

2.6.3 Unilateral Sympathectomy of Brown Adipose Tissue.

A 2 cm dorsal incision was made in the skin of anaesthetised animals across the middle of the scapulae. Brown adipose tissue (BAT) was carefully separated from the scapular muscle along the long side of the scapula by blunt dissection and the 5 nerves supplying one side of the pad were isolated. A 2.3 mm section of each nerve was removed. The nerves on the other side were also exposed and isolated, but not severed. Care was taken not to disturb the integrity of the BAT pad more than absolutely necessary. Wounds were stitched with thread and the animals were left to recover in a warm quiet room. Animals were used 48 hours after denervation.

2.6.4 Subdiaphragmatic Vagotomy.

The procedure used was essentially that outlined by Snowden and Epstein (1970). Animals were fasted overnight to reduce the size of the stomach. After anaesthesia a 4 cm midline incision was made, the stomach was gently lifted out of the body cavity and the liver deflected to one side. The caudate lobe of the liver was gently detached from the stomach and deflected aside, and the animal was placed beneath a dissecting microscope. The oesophagus was lifted up from the body cavity and the right branch of the vagus dissected away using watchmakers forceps. Two silk ligatures were tied around the nerve about 5 mm apart and the nerve was cut between them. The lower section was pulled downwards, peeling away the nerve branches down towards the stomach. The top

section was also peeled upwards and the hepatic branches of the vagus nerve were cut. The same procedure was then followed for the left vagus nerve. Any liver-stomach nerves visible were also cut. Vagotomy was taken to be complete if no fibres could be seen between the liver and the oesophagus under 15.75x magnification.

The stomach was returned to the body cavity and all liver lobes were returned to as close as their original position as possible. The wound was closed with thread after ensuring the body cavity was free of fur. Sham-operated animals were treated in the same way, except the caudate lobe of the liver remained attached to the stomach and the nerves were neither separated from the oesophagus nor cut, during gentle oesophagal manipulation.

Animals were allowed to recover in a warm, quiet room at 24-26^{o}C. All animals were used 3 days after surgery, during which time they were fed as described in Section 2.4.3.

2.6.5 Hepatic Portal Vein Infusions.

Rats were anaesthetised as described in Section 2.6.1. A repeat dose of anaesthetic was given to both animals after approximately 1 hour, if necessary.

166U of heparin in 0.1 ml 0.9% (v/v) sodium chloride (sterile) was injected into a femoral vein and the vein tied off. Thermocouples were placed 2 cm into the rectum and under the interscapular BAT pad. A second weight matched animal was prepared in the same way, and both animals were placed ventral surface uppermost on a thermostatically controlled heating pad, the activity of which was regulated by a second rectal probe placed in the control animal to keep its body temperature at 36-37^{o}C.

A 4 cm midline incision was made, the liver was gently deflected upwards and held back with softnet swabs. The pancreas and viscera were spread to the right to expose the hepatic portal vein. The vein

was freed from surrounding tissue by blunt dissection until a length of approximately 1-2 cm was clear. A 21g teflon cannula (H. G. Walker Ltd., Colchester, England) was inserted into the posterior end of the exposed vein, the insertion needle withdrawn and the cannula pushed approximately 1 cm into the vein. Blood was drawn into the cannula line to ensure free flow and to eliminate air bubbles. Blood flow to the liver was not totally occluded. The viscera, pancreas and liver were then returned as close to their original positions as possible and the wound closed with a single stitch through the body wall, which also held the cannula in place. The entire wound was covered with a softnet swab soaked in warm 0.9% (w/v) sodium chloride and a layer of dry cotton wool. The other animal was operated on in the same way. The cannula lines were attached to syringes containing either sterile 0.9% (w/v) sodium chloride (control) or 2DG ($30mg.ml^{-1}$ in sterile 0.9% (w/v) sodium chloride. After an initial 5-10 minute temperature stabilisation period, a 0.2 ml volume (per 100g bodyweight) was administered via infusion pump over a 5 minute period. Rectal and BAT temperatures were monitored for a further 50-60 minutes after initiation of the infusion. All animals were killed by cervical dislocation 1 hour after the infusion began. No animal was permitted to recover consciousness at any time during these experiments.

2.6.6 Carotid Artery Infusions.

Animals anaesthetised as described in Section 2.6.1 were used in these experiments.

166U of heparin in 0.1 ml sterile 0.9%(w/v) sodium chloride was injected into a femoral vein and the vein tied off. A patch of skin 2 cm square was removed from the ventral surface of the neck. The muscles on the right side of the trachea were dissected to expose the right carotid artery. The vessel was carefully separated from the right vagus nerve and internal jugular vein. Two ligatures were placed under the artery at the upper and lower ends

of the exposed vessel. The lower ligature was tied off, occluding further blood flow through the vessel. A small incision was made just above the ligature and a polythene tube (Portex Ltd., Hythe, Kent. ID. 0.4mm, OD. 1.8mm pulled to the same ID as the artery) inserted into the artery. This cannula was tied in place using the second ligature. Care was taken to ensure that this ligature did not slip off the cannula and tie off the artery. The cannula was then taped to the chest, the animal placed ventral surface downwards on a thermostatically controlled heating pad, being careful not to disturb the cannula. A second, weight matched animal was prepared in an identical manner. Thermocouples were placed 2cm into the rectum and under the inter-scapular BAT pad. A second rectal probe was placed in the control animal to regulate the activity of the heating pad to maintain the body temperature of the control rate at 36-37^{o}C.

The cannulae were attached to syringes containing either sterile 0.9% (w/v) sodium chloride (control) or 2DG (30mg.ml^{-1} in sterile 0.9% (w/v) sodium chloride). After an initial 5-10 minutes temperature stabilisation period, a 0.2ml volume (per 100g bodyweight) was administered via infusion pump over a 5 minute period. Rectal and BAT temperatures were monitored for a further 50-60 minute period after initiation of the infusion. Animals were killed by cervical dislocation 60 minutes after the infusion began. No animal was permitted to recover consciousness at any time during these experiments.

2.7 Determination of Body Compositions.

2.7.1 Water Content of Carcasses.

Eviscerated carcasses were weighed in foil boats and placed in a Belling oven at 78-86°C for 3-5 days until constant weight was reached. Body water was measured as the weight difference between wet and dry carcasses.

2.7.2 Carcass Homogenisation.

Dried individual carcasses were chopped and ground with 2-2½x their dry weight of anhydrous sodium sulphate. The first homogenisation was through a Kenwood Chefette electric mincer, and the second through a finer hand-grinder. Ground carcasses were weighed (inclusive of sodium sulphate) and stored in plastic bags at 4°C until required.

2.7.3 Total Fat Extraction.

The total fat content of dried carcasses was estimated by lipid extraction by the method of Folch et al (1977).

Ground tissue samples containing 1g of dried tissue were extracted in chloroform-methanol (2:1, v/v) in a ratio of 20ml per gram homogenate. After thoroughly shaking, the extract was filtered through Whatman No. 1 (qualitative) filter paper which had been presoaked in chloroform. The extract was washed with 0.2 volumes of 0.02% Calcium chloride solution. This was mixed by gentle swirling (more vigorous shaking led to emulsification) and aqueous and non-aqueous layers were allowed to separate out. A 20ml aliquot of the non-aqueous layer was taken into a weighed vessel and the contents dried down to constant weight under a stream of compressed air. Fat content was determined by weighing and multiplying back up to wet weight. Fat content was determined on duplicate samples for each carcass.

2.7.4 Carcass Nitrogen Determination.

Body nitrogen was measured by the method of Kjeldhal (Bradstreet 1965) using the micro-distillation apparatus developed by Markham (1942).

Heating a sample with concentrated sulphuric acid and a catalyst oxidises the carbon and converts any nitrogen present to ammonium sulphate. Ammonium sulphate in alkaline solution yields ammonia gas which can be distilled into acid for subsequent titration.

Samples of ground tissue containing approximately 50mg nitrogen were taken, weighed accurately and placed in a 100ml Kjeldhal flask with 2 copper and 1 selenium Kjeldhal catalyst tablets. 20-30ml concentrated sulphuric acid was added to each flask along with 2 or 3 glass anti-bumping granules. Samples were heated gently in a fume cupboard with the flasks slanted at approximately 30^{o} from the horizontal until white fumes began to appear. Heating was regulated to keep the white fumes refluxing down the neck of the flast without escaping. The samples were heated until the solution turned green, whereupon heating was continued for a further 20 minutes. After cooling, distilled water was added carefully, to make a final volume of 250ml. Duplicate samples of each carcass were digested.

Distillation Reagents.

Boric Acid 2% (w/v)
Hydrochloric acid 0.01 M
Sodium hydroxide 40% (w/v)
Indicator: 1 part 0.1% (w/v) methyl red in ethanol plus 3 parts 0.1% (w/v) bromocresol green in ethanol
Nitrogen Standard:
Ammonium sulphate 0.471g.100ml^{-1} gives a solution containing 1 mg nitrogen.ml^{-1}.

At the beginning of each session, the distillation apparatus was cleaned by passing steam through it for 20 minutes. The first titration of each session was the standardisation of the hydrochloric acid, in triplicate. 2 ml of standard was added to the still, and the still repressurised. 8 ml of the 40% sodium hydroxide (w/v) was added through the funnel, washed through with a little distilled water and the funnel resealed with distilled water. 5 ml boric acid with 3 drops of indicator in a 50 ml conical flask was placed beneath the condenser with the condenser's tip below the surface of the liquid. As distillation began the colour of the flask changed from pink to blue, and distillate was collected for 2 minutes after the colour change. The contents of the flask were titrated against 0.01M hydrochloric acid to an end point of a blue to slate grey colour change. 1 ml of hydrochloric acid normally standardised to 0.13-0.15mgN.ml^{-1}. All standards and samples were assayed in triplicate, 2 to 4 ml aliquots of sample were taken for analysis. After each titration the still was cleaned out with several washes of distilled water, and repressurised.

Total nitrogen content per carcass was calculated and converted to protein content by multiplying by 6.25. This figure is based on the average nitrogen content of a protein being 16%.

2.7.5 Calculations of Energy Balance.

Body composition was converted to energy composition by using the conversion factors 39 $kJ.g^{-1}$ for fat and 17 $kJ.g^{-1}$ for protein. Initial eviscerated carcass composition was extrapolated from data from a control group and scaled to individual bodyweights after an allowance for gut weight had been made.

2.8 Cold Acclimation.

Rats were housed in pairs in wire mesh bottomed cages in a cold room maintained at 4°C. They were allowed free access to laboratory chow and drinking water with lights on from 0830 hours to 1730 hours. Rats were maintained under these conditions for 7 days before use.

2.9 Serum Assays.

2.9.1 Collection of Serum.

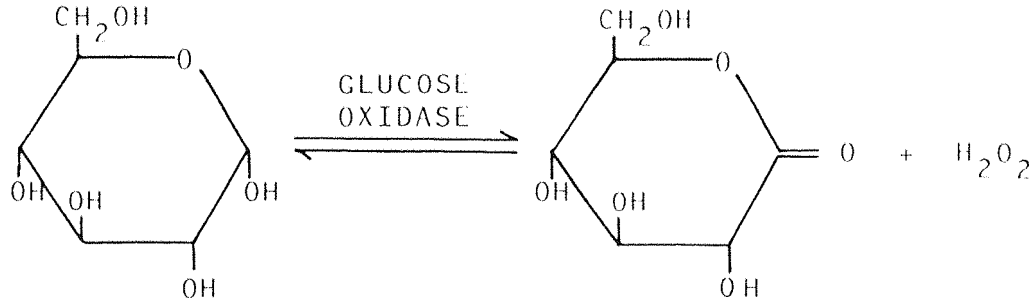
Where blood samples were required, animals were killed by decapitation and trunk blood collected. Blood was allowed to clot for 30 minutes on ice, Centrifuged, the serum was drawn off and stored at -20°C. until required. When blood was not required rats were stunned and killed by cervical dislocation.

2.9.2 Serum Glucose Assay.

Serum glucose was assayed using a glucose oxidase-peroxidase method with ABTS (2.2'-azino-di-3[ethyl-benzthiazolin-sulfonate (6)]. disodium salt) as the chromogen.

Principle.

(i)



(ii) H_2O_2 + $dye_{reduced}$ $\xrightarrow{\text{PEROXIDASE}}$ H_2O + $dye_{oxidised}$.

Reagents.

D-glucose standard	$1mg.ml^{-1}$ in perchloric acid
Perchloric acid	6% (w/v)

Glucose oxidase Reagent

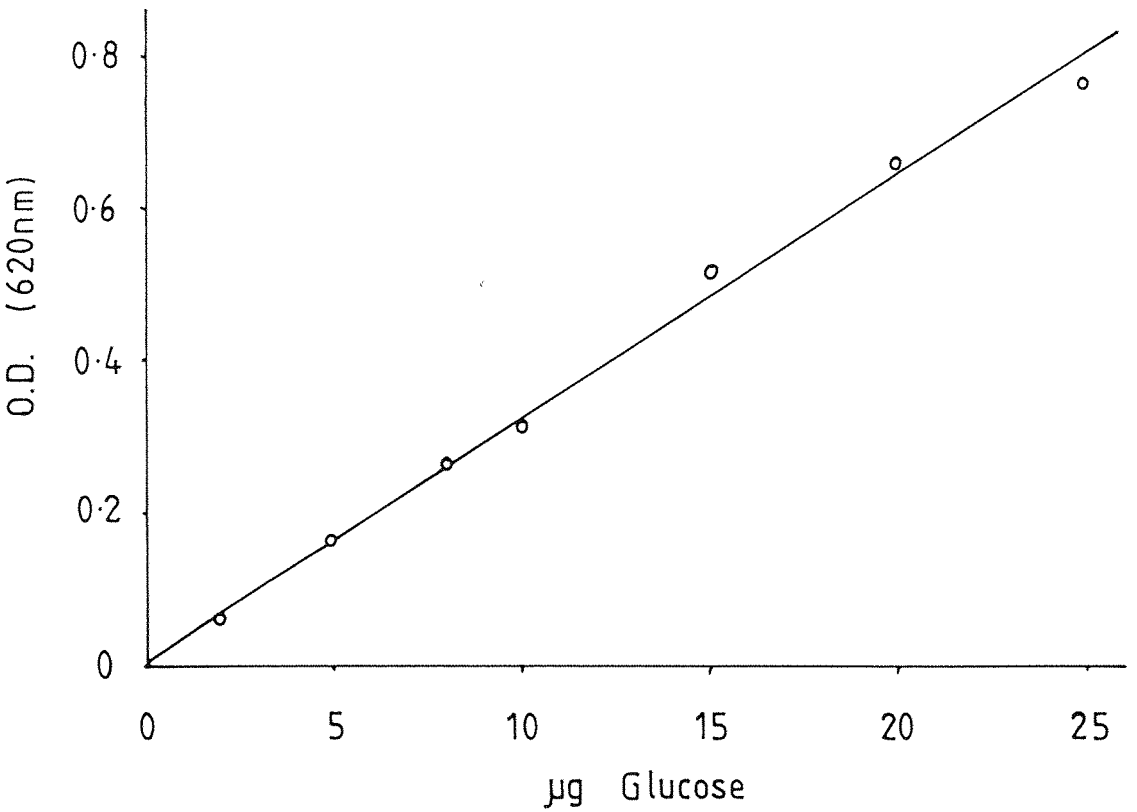
Sodium Phosphate buffer	0.1M pH7.4
Glucose oxidase	$5000U.dm^{-3}$
Horseradish Peroxidase	$8000U.dm^{-3}$
ABTS	$1g.dm^{-3}$

100µl serum samples were added to 900µl 6% perchloric acid, mixed and centrifuged for 10 minutes in a low speed bench centrifuge to precipitate plasma protein. 2.5ml glucose oxidase reagent was added to 100µl aliquots of supernatant, or to 100µl volumes of a 0 - 30µg D-glucose standard curve. After a 15 minute incubation at 37°C assay tubes were allowed to cool and absorbance was determined at 620nm on a Pye-Unicam SP8-400 spectrophotometer against a reagent blank. All samples and standards were prepared and assayed in duplicate. A typical standard curve is shown in figure 2.1

2.9.3 Non-esterified Fatty Acid Determination.

Initially, free fatty acids were assayed using the Boehringer-Mannheim diagnostic kit, but this is no longer available. Subsequent assays were performed using a modification of the methods of Noma et al. (1973) and Chromy et al. (1977), which uses a similar principle to the Boehringer kit.

Figure 2.1 Standard Curve for the Assay of Serum Glucose.



r = 0·996

Fatty acids are extracted from serum samples in a solvent system and are then reacted with a copper reagent resulting in the formation of a stable, solvent soluble fatty acid-copper salt. The concentration of copper is detected using a copper sensitive dye, and is proportional to the fatty acid concentration.

Reagents

Extraction solvent - chloroform:heptane: methanol (28:21:1 (v/v/v))

Stable Copper Reagent

Sodium citrate	0.025M
Triethanolamine	0.45M
Cupric nitrate	0.13M
Sodium Chloride	0.94M in distilled water

Chromagen

2-thiazolylazo-p-cresol(TAC)

0.01% (w/v) in ethanol

Palmitic acid standard

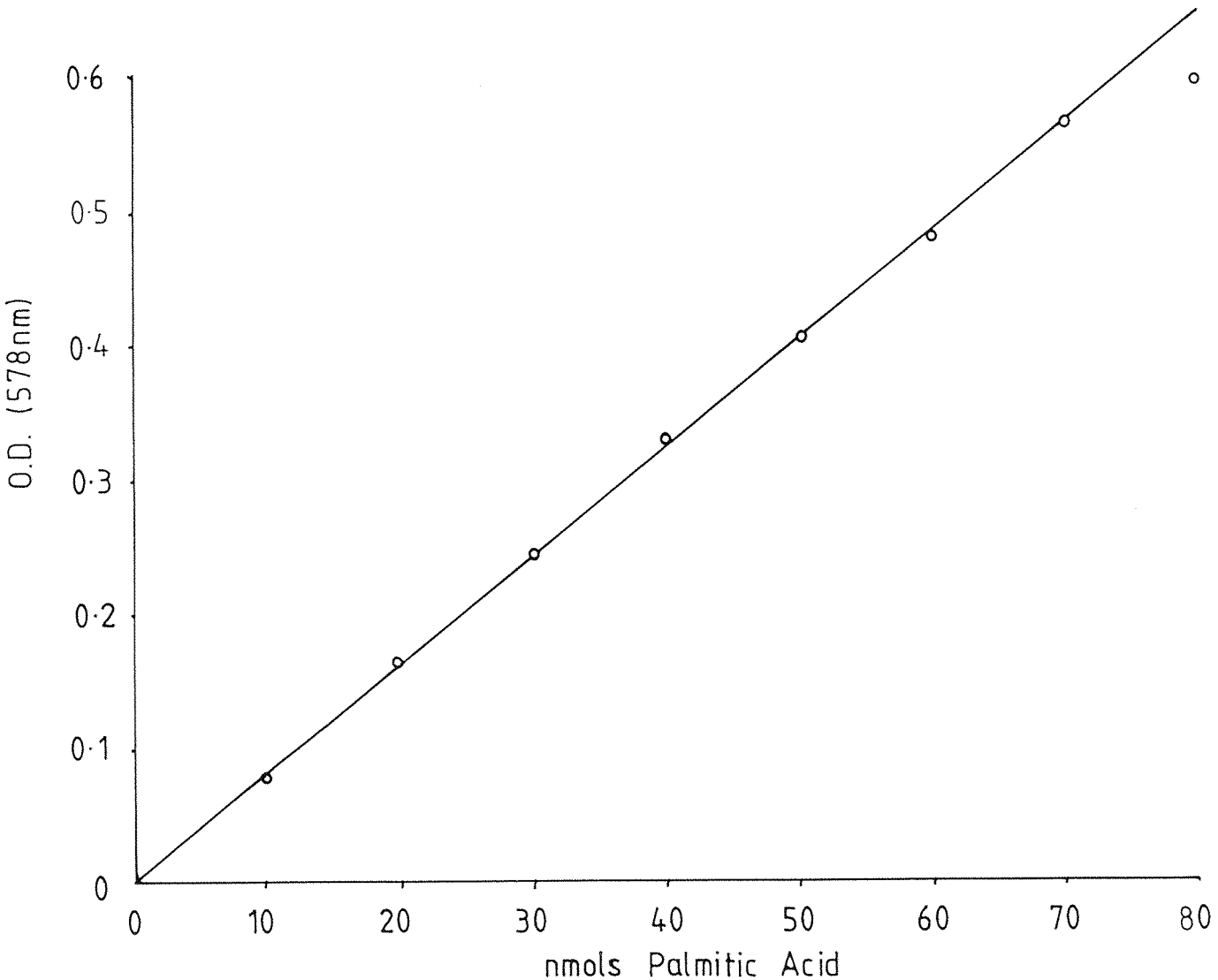
4mM palmitic acid in extraction solvent.

200µl aliquots of serum or standards (0.80nmols per 200µl) were added to 2ml extraction solvent in acid washed ground-glass stoppered tubes and mixed well. 1ml stable copper reagent was added to each tube, and the tubes shaken vigorously for 3 minutes. After centrifugation in a Beckman J6-B centrifuge at 200 rpm and 10°C for 10 minutes, 1ml aliquots of the non-aqueous (upper) layer were added to 2000µl TAC in acid washed glass tubes, mixed immediately and absorption measured at 578nm on a Pye-Unicam SP4-800 spectrophotometer. All samples and standards were prepared and assayed in duplicate. A typical standard curve is shown in figure 2.2.

2.9.4 Insulin Radioimmunoassay.

Serum insulin concentrations were determined by the method of Hales and Randle (1963) using the Wellcome RIA binding reagent. The principle is based on a fixed amount of anti-insulin serum reacting with

Figure 2.2 Standard Curve for the Assay of Non-esterified Fatty Acids.


0·6
0·5
0·4
0·3
0·2
0·1
0
O.D. (578nm)
0
10
20
30
40
50
60
70
80
nmols Palmitic Acid

r = 0·999

an unknown amount of insulin in the sample, in the presence of a known amount of [^{125} I]-insulin. The binding of radiolabelled insulin to the guinea pig anti-insulin antibody is inhibited in proportion to the unknown concentration of insulin in the sample. The soluble insulin-antibody complex is separated from free insulin by precipitation of the complex with a pre-precipitated rabbit-anti-guinea pig antibody.

Insulin Binding Reagent.

This consists of a freeze-dried mixture of guinea pig anti-insulin plus rabbit-anti-guinea pig globulin sera in 40mM sodium phosphate (pH7.4) containing 20mM EDTA, 0.1% (w/v) sodium azide and 0.5% (w/v) Bovine serum albumin, fraction V (BSA). It was reconstituted by the addition of 8ml deionised water.

Buffers.

A.	phosphate buffer	50mM pH7.4
	BSA	0.5% (w/v)
	Thiomersal	0.025% (w/v)
B.	Phosphate buffer	50mM pH7.4
	BSA	0.5% (w/v)
	Thiomersal	0.025% (w/v)
	Sodium Chloride	0.9% (w/v)

Standard Insulin

Rat insulin standard was serially diluted in buffer B to give a range of concentrations between 0.25ng.ml^{-1} to 8ng.ml^{-1} (6.125-196 μU.ml^{-1}).

Radiolabelled Insulin

Stock [^{125} I]-insulin at a concentration of 1μCi.ml^{-1} was diluted in buffer A to a final concentration of 0.125μCi.ml^{-1}.

100μl serum samples or standards were added to 100μl insulin binding reagent, mixed and incubated for 6 hours at 4^{o}C. 100μl [^{125} I]-insulin was added to all tubes which were then vortexed and incubated

at 4°C for 18 hours. Bound [^{125}I]-insulin was separated from free by centrifugation at 2000g and 4°C for 20 minutes in a Beckman J6-B centrifuge. The supernatants were decanted and each tube was counted in a Beckman γ-counter (80% efficiency for ^{125}I). for 1 minute.

Also included in the assay were 3 tubes containing no binding reagent or unlabelled insulin (blanks) for the measurement of radiolabel bound non-specifically to the tubes; 3 tubes containing no unlabelled insulin (zeros) as a zero point on the standard curve; and 3 tubes containing no un labelled insulin and from which the supernatant was not decanted (totals) to ascertain the total amount of radiolabel added to each tube. Volumes were made up with buffer B in those 9 tubes. All standards were assayed in triplicate and samples in duplicate. Bound radioactivity was expressed as a percentage of total [^{125}-I] added (after subtraction of blank counts)

$$\frac{B}{B_o} \% = \frac{\text{sample-blank}}{\text{total -blank}} \times 100$$

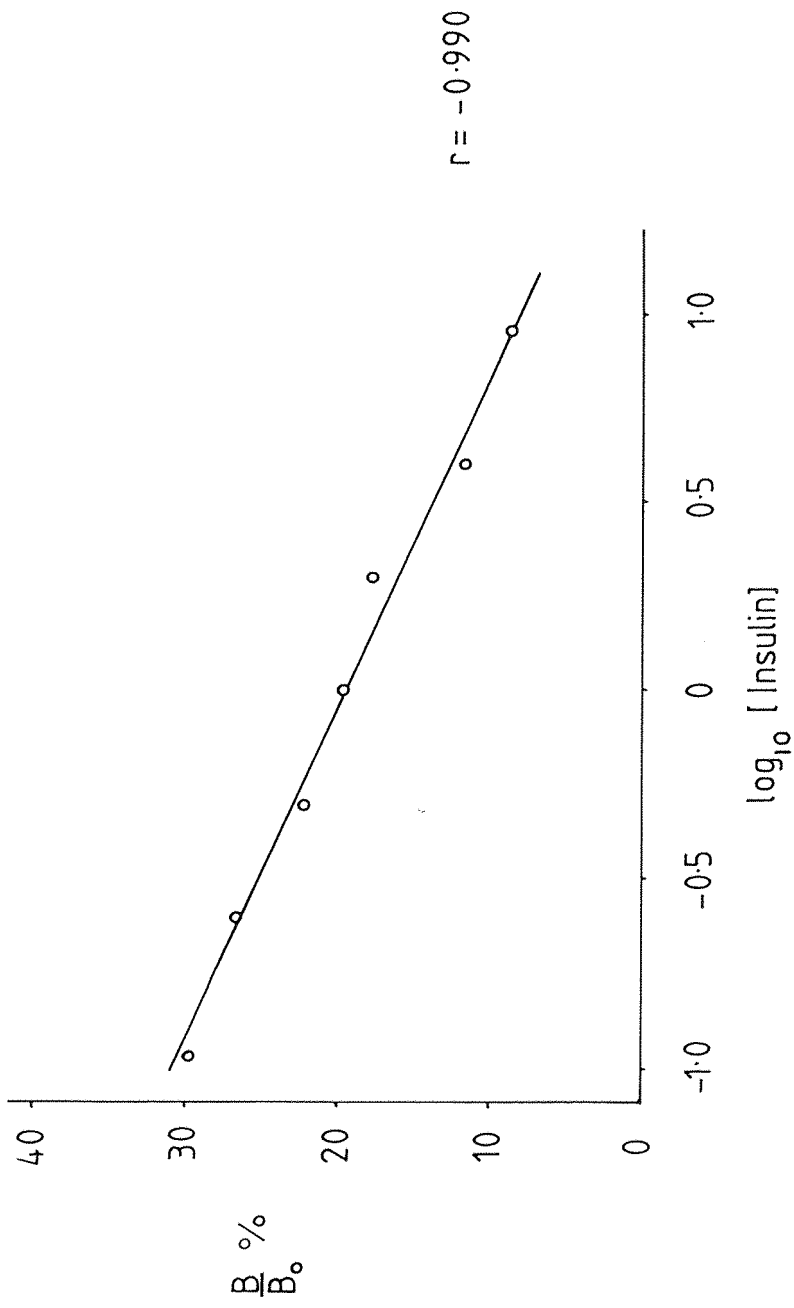
A standard curve was constructed ($\frac{B}{B_o}$ % versus $\log_{10}$ concentration of standard insulin) and used to determine the serum concentrations. A typical standard curve is shown in figure 2.3.

2.9.5 Corticosterone Radioimmunoassay.

The method used for the assay of corticosterone was based on that of Fahmy et al (1975) using a corticosterone antibody produced in rabbits. This antibody was produced within the department. It had no significant cross reactivity with cortisol, testosterone or oestradiol.

A fixed amount of antibody was added to the samples containing an unknown amount of corticosterone. An excess of radiolabelled corticosterone was then added in order to saturate the antibody. Free

Figure 2.3 Standard Curve for the Assay of Serum Insulin.


r = -0·990
40
30
20
10
0
B/B₀ %
-1·0
-0·5
0
0·5
1·0
log₁₀ [Insulin]

corticosterone in solution was precipitated with a mixture of dextran/charcoal and the soluble antibody-corticosterone complex was assayed for radioactivity. The amount of radiolabel present was inversely proportional to the concentration of corticosterone in the sample.

Reagents.

Assay Buffer	Sodium Phosphate Buffer	10mM pH7.4
	Sodium chloride	150mM
	Gelatin	0.1% (w/v)

Dextran/charcoal

Dextran T_{70}	$25\mu g.100ml^{-1}$
Charcoal(Norit GSX)	$250\mu g.100ml^{-1}$ in buffer

Stirred on ice for 1 hour before use.

1,2,6,7-[^{3}H] Corticosterone

A solution of 1,2,6,7-[^{3}H] corticosterone was prepared from stock to a concentration of $10\mu Ci.ml^{-1}$ in ethanol, and stored at 4^oC. For each assay, a portion of this was dried down under a stream of nitrogen and redissolved in assay buffer to a final activity of approximately $2x10^5 dpm.ml^{-1}$.

Corticosterone Standard.

$100ng.ml^{-1}$ corticosterone in ethanol, stored at 4^oC.

Assay Procedure.

100µl serum samples were mixed with 500µl ethanol, vortexed and precipitated protein removed by centrifugation in a Beckman microfuge for 2 minutes. Duplicate 50µl aliquots of supernatant were taken and dried down under nitrogen. A standard curve was prepared by diluting the $100ng.ml^{-1}$ corticosterone standard to give a series of duplicate tubes containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0 and 5.0 ng corticosterone, each in 100µl ethanol. A similar volume of ethanol was added to a further

six tubes; two for a zero standard, measuring the total amount of [^{3}H] -corticosterone bound; two for the measurement of the efficiency of removal of free [^{3}H]-corticosterone from solution by dextran/charcoal in the absence of antiserum (blank); and two for the measurement of total [^{3}H]-corticosterone added (in the absence of antiserum and dextran/charcoal). Standards, zeros, totals and blanks were also evaporated to dryness under nitrogen.

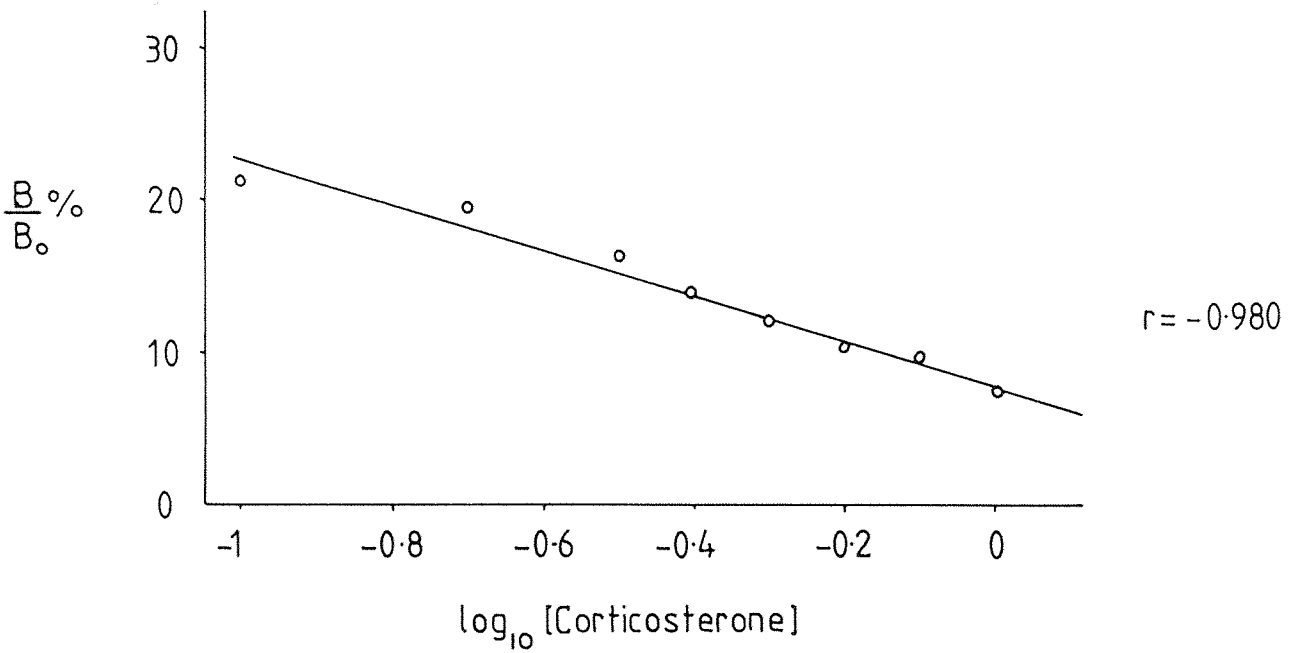
Antiserum of 1:1200 dilution (in assay buffer) was prepared and 100µl was added to all tubes except blanks and totals, which received 100µl of buffer. After brief vortexing, tubes were allowed to stand for 30 minutes at room temperature. 100µl of [^{3}H]-corticosterone was then added to all tubes which were briefly vortexed and incubated at 30^{o}C for 1 hour. At the end of this period, the tubes were placed on ice for 15 minutes. 100µl of dextran/charcoal was added to all tubes except totals, which received 100µl buffer instead. These tubes were then vortexed and allowed to stand on ice for a further 15 minutes. All tubes were then centrifuged for 10 minutes at 1000g and 4^{o}C in a Beckman J6-B centrifuge. 0.5ml aliquots of supernatant were added to 5ml of Tritoscint and then counted for [^{3}H] in a Phillips PW4700 liquid scintillation counter for 1 minute.

Bound radioactivity was expressed as a percentage of the total, after subtraction of the 'blank' counts.

$$\frac{B}{Bo}\ \% = \frac{\text{sample-blank}}{\text{total -blank}} \times 100$$

A standard curve of % bound versus $\log_{10}$ corticosterone added was constructed and used to determine serum concentrations. A typical such curve is shown in figure 2.4

Figure 2.4 Standard Curve for the Assay of Serum Corticosterone.


B/B₀ %
30
20
10
0
-1
-0·8
-0·6
-0·4
-0·2
0
log₁₀ [Corticosterone]
r = -0·980

2.10 Protein Assays.

Protein concentrations were initially determined by the method of Bradford (1976) and latterly by that of Lowry et al (1951). Protein standards were routinely prepared in the medium in which the samples were suspended.

2.10.1 Protein Determination by the Bradford Method.

This assay works on the principle of protein-dye binding. The binding of Coomassie Blue G-250 to protein causes a shift in the absorption maximum of the dye from 465 to 595 nm.

A standard curve of 0-70µg BSA was prepared using a $1mg.ml^{-1}$ BSA standard. Volumes were made up to 70µl with medium. 20µl samples plus 50µl medium were added to duplicate tubes. 2.5ml of dye (Coomassie Blue G-250, 0.01% (w/v) in ethanol: 85% phosphoric acid: water (v/v/v), filtered) were added to each tube. Tubes were mixed by gentle inversion and left for 10 minutes for colour to develop. Absorption at 595nm was measured on a Pye-Unicam SP8-400 spectrophotometer against a reagent blank.

2.10.2 The Lowry method for the Measurement of Protein.

Direct reduction by tyrosine and tryptophan and also by a preformed protein-copper complex of phosphomolybdate present in Folin-Ciocalteau's reagent leads to the formation of a blue colour which is measured by its absorbance at 750nm.

Reagents.

2% sodium carbonate (w/v), in 0.1M NaOH	alkaline reagent
1% copper sulphate (w/v)	
2% sodium potassium tartrate (w/v)	
10% sodium deoxycholate (w/v)	

The above solutions were mixed in a 100:1:1:10 ratio by volume just prior to use.

20µl duplicate samples were taken, and 1µl of the alkaline reagent added. All tubes were vortexed and left to stand at room temperature for 10 minutes.

100µl of Folin-Ciocalteau's reagent (diluted 1:1 (v/v) with water just before use) was added to all tubes. Each tube was vortexed immediately upon addition of this reagent. All tubes were incubated at 37°C for 10 minutes. After cooling, the absorption at 750nm was measured against a reagent blank on a Pye-Unicam SP8-400 spectrophotometer.

A standard curve of 0-70µg was prepared using 4mg.ml^{-1} BSA and included with each assay. All volumes were corrected to 20µl using medium.

A solution of 10% sodium dodecyl sulphate (w/v) was substituted for sodium deoxycholate for the assay of triton-solubilised proteins.

2.11 Preparation of Defatted Bovine Serum Albumin.

Albumin-bound fatty acids were removed from BSA (fraction V) by charcoal treatment as described by Chen (1967).

35g charcoal (Davco G60, activated) was soaked overnight in distilled water (500ml) and the surface residue removed using a vacuum line. 70g of BSA was added slowly to the charcoal suspension, which was stirred continuously on ice, until dissolved. the pH of the protein solution was lowered to 3 by slow addition of 0.4M HCl (approximately 100ml over at least 1 hour), whereupon the suspension was left stirring on ice for 1 hour. The charcoal was then removed by centrifugation in an MSE-21 centrifuge at 30,000g for 1 hour at 4°C. Charcoal-free supernatants were pooled and filtered through a 45µm Millipore filter. The pH of the solution was then raised to 7 by careful addition of 0.4M NaOH (approximately 100ml over at least 1 hour) while stirring continuously on ice. The resulting BSA solution was frozen in 10ml aliquots and stored at -20°C until required. The concentration of protein was determined by the Bradford method using a BSA standard, 1mg.ml^{-1} in distilled water.

2.12 Preparation of Brown Adipose Tissue Mitochondria for the Measurement of Guanosine Diphosphate (GDP) Binding.

Buffer

Sucrose	250mM	pH7.2 @ 4°C.
HEPES	1mM	
EDTA (sodium salt)	0.2mM	

Mitochondria were prepared essentially by the method of Cannon and Lindberg (1979).

The interscapular brown adipose tissue (BAT) depot was removed from animals immediately upon sacrifice and placed in ice cold buffer. The BAT pad was trimmed free of contaminating white adipose tissue and muscle. It was then weighed, minced finely with scissors and homogenised in 5ml ice-cold buffer by 3-4 passes of a teflon pestle in a glass homogeniser. A 300µl sample of homogenate was taken for determination of succinate-cytochrome c oxidoreductase activity, if required.

The homogenate was centrifuged at 700g for 10 minutes at 4°C in an MSE-21 centrifuge. The fat 'cake' which formed at the surface was removed and the supernatant collected. The pellet was re-suspended in 5ml buffer, rehomogenised and centrifuged again as above. The supernatant was pooled with that from the first spin, and then centrifuged at 8500g for 15 minutes at 4°C.

The resultant mitochondrial pellet was then resuspended in 10ml buffer containing 2% (w/v) fatty-acid free BSA (to remove endogenous free fatty acids). Centrifugation at 8500g and 4°C for 20 minutes yielded another mitochondrial pellet. BSA was removed by resuspension of this pellet in 10ml buffer and repeating the above spin for 15 minutes.

The final mitochondrial pellet was then resuspended in 1-2 ml 250mM sucrose solution to an approximate concentration of 2mg.ml^{-1} and kept on ice. Mitochondria were assayed for [^{3}H]-GDP binding within 45 minutes of preparation.

2.13 Measurement of Specific [^{3}H]-Guanosine Diphosphate Binding.

This method is basically the same as that developed by Nicholls (1976).

175µl aliquots of a freshly prepared BAT mitochondrial suspension (prepared as in section 2.12) at a concentration of 1-2 mg.ml^{-1} were added to microfuge tubes containing 300µl of the following incubation medium.

	Final concentration in 500ul assay volume.	
Sucrose	100µM	pH 7.1 @ 25°C
TRIS	20µM	
Choline Chloride	10µM	
EDTA (disodium salt)	1mM	
Rotenone	5µM	
Potassium Atractyloside	100µM	
U - [^{14}C]-Sucrose	0.1µCi	

and either 25µl 4mM GDP or 25µl distilled water.

After a 2 minute incubation period at 25°C, the assay was initiated by the addition of 5nmols GDP containing 0.625µCi 8-[^{3}H]-GDP in a 25µl volume, to give a final assay volume of 500µl. After mixing, the assay tubes were incubated in a shaking water-bath at 25°C for 8 minutes. The reaction was terminated by centrifugation in a Beckman microfuge for 2 minutes.

The supernatant was removed with a Pasteur pipette attached to a vacuum line. NCS tissue solubiliser (0.5ml) was added to each assay tube, which was then placed in a 50°C water bath for 1 hour or until solubilisation was complete. Each solubilised mitochondrial pellet solution was then carefully transferred with a glass Pasteur pipette to a scintillation vial. NCS was neutralised by the addition of 18µl glacial acetic acid to each vial. 7ml of Beckman Readysolv NA scintillant was then added to each vial and [^{3}H] and [^{14}C] labelling was

determined by dual label counting in a Phillips PW4700 liquid scintillation counter. $[^{3}H]$-GDP and $[^{14}C]$-sucrose specific activities were determined for each assay.

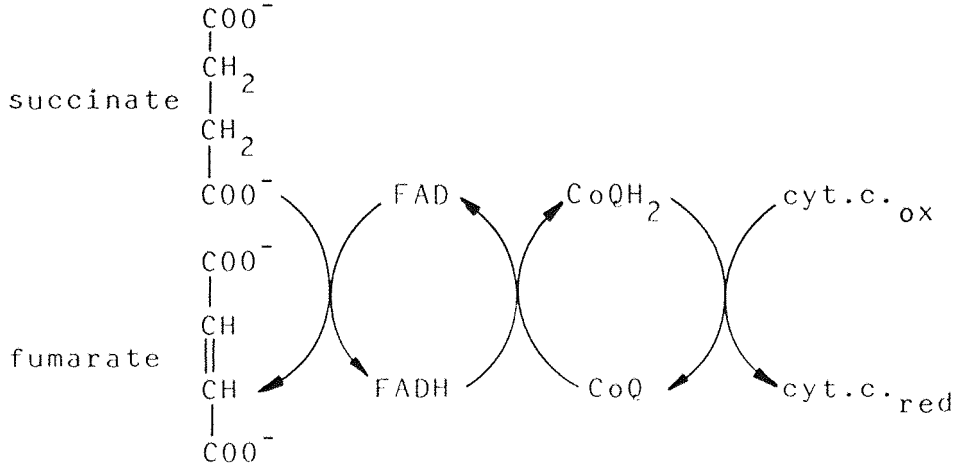
The volume of the extramitochondrial space was determined for each sample from the $[^{14}C]$-sucrose counts. $[^{3}H]$-GDP trapped in the extramitochondrial space was calculated from this and subtracted from the tritium counts for that sample.

Non-specific binding was determined in the tubes containing the 200μM displacer dose of unlabelled GDP. Specific binding was calculated as the difference between the total binding, measured in the absence of unlabelled GDP, and non-specific binding. All assays were performed in duplicate.

Remaining mitochondria were assayed for protein content (using a BSA standard in 250mM sucrose), frozen and stored at -20°C for any further assays.

2.14 Assay of Succinate-cytochrome c Oxidoreductase (Complex I-III) Activity.

The activity of this enzyme was assayed as described by Tisdale (1967).



Enzyme activity was assayed in a final volume of 1ml of phosphate buffer (10mM,pH7.4) containing 1umole sodium azide, 200nmoles EDTA (disodium salt), 5mg BSA, 1mg ferricytochrome c, 10μmoles sodium succinate and 15-40μg of protein, and the assay was performed at 37°C.

The reaction was started by the addition of the sodium succinate. The initial rate of reaction was followed using a Pye-Unicam SP4-800 recording spectrophotometer, recording the rate of increase in optical density at 550nm.

The activity of the enzyme was calculated using an extinction coefficient of $18.5 \times 10^6 . M^{-1} . cm^{-1}$ (at 550nm) and the activities were expressed as μmols cytochrome c reduced per minute per mg protein present, or per depot.

2.15 Preparation of Brown Adipose Tissue Mitochondria for the Isolation of 32K protein.

This method is essentially that of Lin and Klingenberg (1982).

'Standard Buffer'

MOPS	20mM	pH6.7 @ 4°C
Sodium Sulphate	20mM	
EDTA (disodium salt)	1mM	

Rats were killed by cervical dislocation. Interscapular BAT was removed, trimmed clean of contaminating white fat and muscle and placed in ice cold buffer. Depots were pooled from up to 20 rats. The tissue was minced finely with scissors and homogenised in buffer (5ml/depot) by 4-5 passes of a loose fitting teflon pestle in a glass homogeniser.

The homogenised tissue was centrifuged at 700g and 4°C for 10 minutes. After removal of the fat 'cake' the supernatant was collected and centrifuged at 6000g for 10 minutes at 4°C. The resultant pellet was resuspended in a similar volume of buffer and the spin repeated.

This final pellet was resuspended in a small volume of buffer to give an estimated protein concentration of approximately 50mg.ml^{-1}. The mitochondria were either used immediately for protein preparation or frozen at -70^{o}C or in liquid nitrogen for future use.

2.16 Preparation and Purification of 32K Protein.

This procedure was carried out as described by Lin and Klingenberg (1982).

Mitochondria prepared as in section 2.15 were diluted to an approximate concentration of 500mg protein in 18ml standard buffer (see section 2.15 for standard buffer), containing 3.2% (w/v) lubrol WX. This suspension was incubated at 0^{o}C for 30 minutes, then centrifuged at 100,000g and 0^{o}C for 30 minutes in an MSE Pegasus 65 centrifuge. The supernatant containing lubrol-soluble proteins was discarded (except for a 50µl sample for the purification gel). The pellet was resuspended in 0.3M sucrose containing 10mM TRIS and 2mM EDTA (disodium salt), pH7.2 @ 5^{o}C to a similar concentration as before and centrifuged again under the same conditions. This pellet was resuspended in standard buffer containing 5% (w/v) TritonX-100 to a concentration of approximately 25mg.ml^{-1} and kept at 0^{o}C for 30 minutes. The suspension was then centrifuged as before. This supernatant contained a mixture of triton-soluble proteins including the 32K protein. The mixture was applied to a hydroxylapetite column which had been previously equilibrated with standard buffer. The 32K protein was eluted at room temperature with the same buffer. The first two 25ml fractions were collected and concentrated by pressure dialysis in a concentration cell to a final volume of 8-10ml each. The protein content for each concentrated fraction was determined by the Lowry method (with sodium dodecyl sulphate (SDS) replacing sodium deoxycholate). The purified protein was

frozen in 2ml aliquots and stored in liquid nitrogen until required.

The purity of the 32K protein was checked by SDS-polyacrylamide gel electrophoresis on a 12.5%-16% gradient slab gel. A typical such gel is shown in Figure 2.5

Figure 2.5 32K Protein Purification Gel.

25µl samples of solubilised proteins were run in each lane, as follows:-

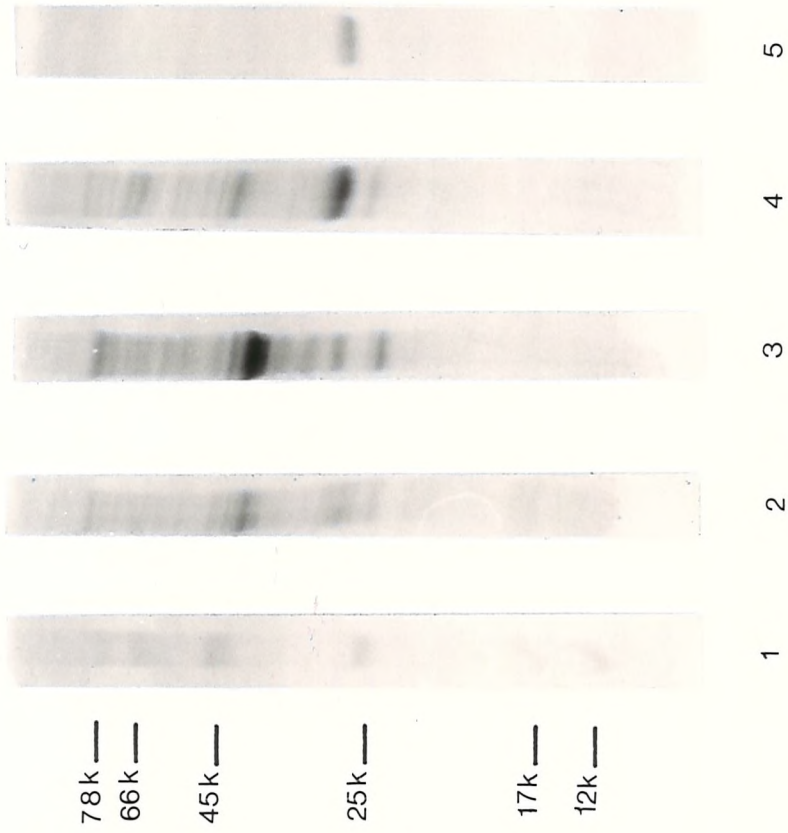
1. Molecular Weight Markers.

2. Solubilised BAT mitochondria.

3. Lubrol soluble proteins.

4. Triton soluble proteins.

5. Purified 32K protein.


78k
66k
45k
25k
17k
12k
1
2
3
4
5

2.17 Characterisation of Antiserum to 32K Protein.

Antiserum to purified 32K protein isolated from rat BAT mitochondria was prepared in rabbits by Dr. R. R. French.

Blood collected from an ear vein of an immunised rabbit was allowed to clot at room temperature for 2 hours. Serum was separated by low speed centrifugation, drawn off and clarified by centrifugation at 90,000g for 30 minutes at 4°C in an MSE Pegasus 65 centrifuge. Antibody specificity was checked by Western Blotting (see section 2.20) against solubilised BAT and liver mitochondria from rats and rabbits, (figure 2.6). Optimum antiserum concentration was determined by a standard dilution curve against purified 32K protein (figure 2.7) by radioimmunoassay as outlined in section 2.18. The antiserum dilution which gave half maximal binding was used in subsequent assays. 1ml aliquots of antiserum were stored at -20°C until required.

2.18 Radioimmunoassay of 32K Protein in Solubilised Mitochondria.

The concentration of 32K protein in triton-solubilised mitochondria samples was determined by solid-phase radioimmunoassay as described by Lean et al (1983).

The principle of the assay is based on the binding of $[^{125}I]$-protein A to antibody-antigen complexes which are bound to a solid matrix. After incubation with samples or standards in solution, any remaining free antibody is allowed to bind to matrix-bound antigen. Free antigen-antibody complex is washed away, and the immobilised complex is incubated with $[^{125}I]$-protein A. The amount of radio-activity present is inversely proportional to the amount of 32K protein in the sample or standard.

Figure 2.6 32K Antibody Characterisation using Western Blotting.

The gel (a) and blot (b) of solubilised rat and rabbit BAT and liver mitochondria are shown.

1. Molecular Weight Markers.

2. Purified 32K protein.

3. Rat BAT mitochondria.

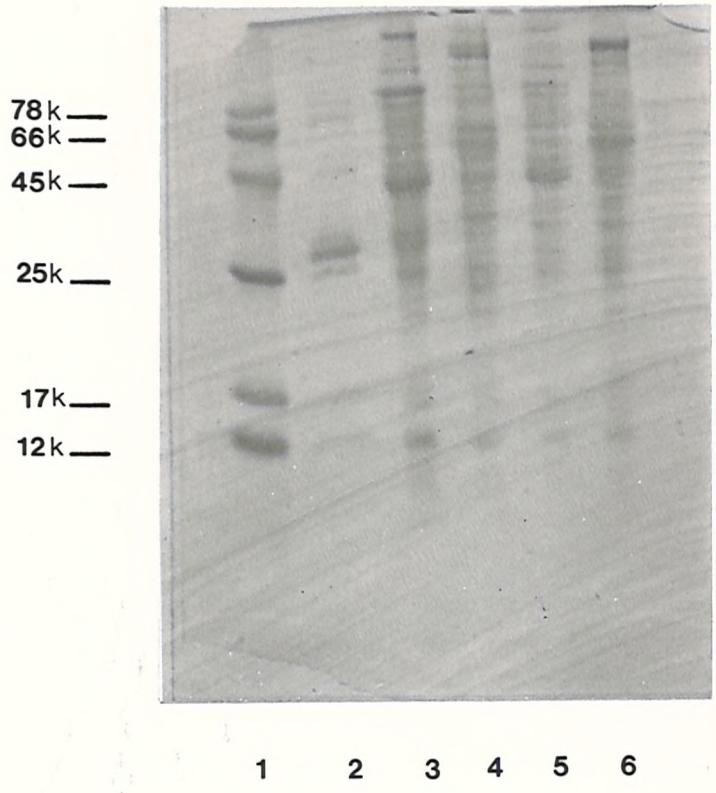
4. Rat liver mitochondria.

5. Rabbit BAT mitochondria.

6. Rabbit liver mitochondria.

Only the rat BAT track of the blot developed a definite protein band. The antiserum cross reacts with no rat liver proteins. The bands on the two rabbit tracks at 38 kDa molecular weight are probably the atractyloside sensitive adenine nucleotide translocator.

(a)



(b)

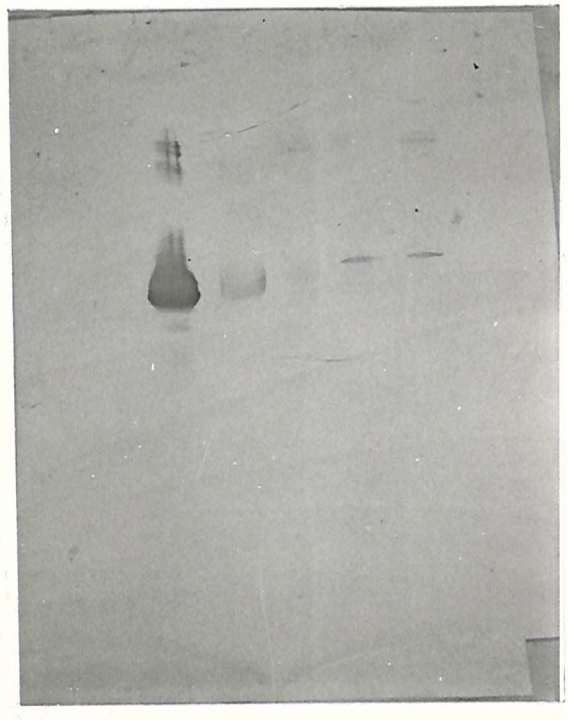
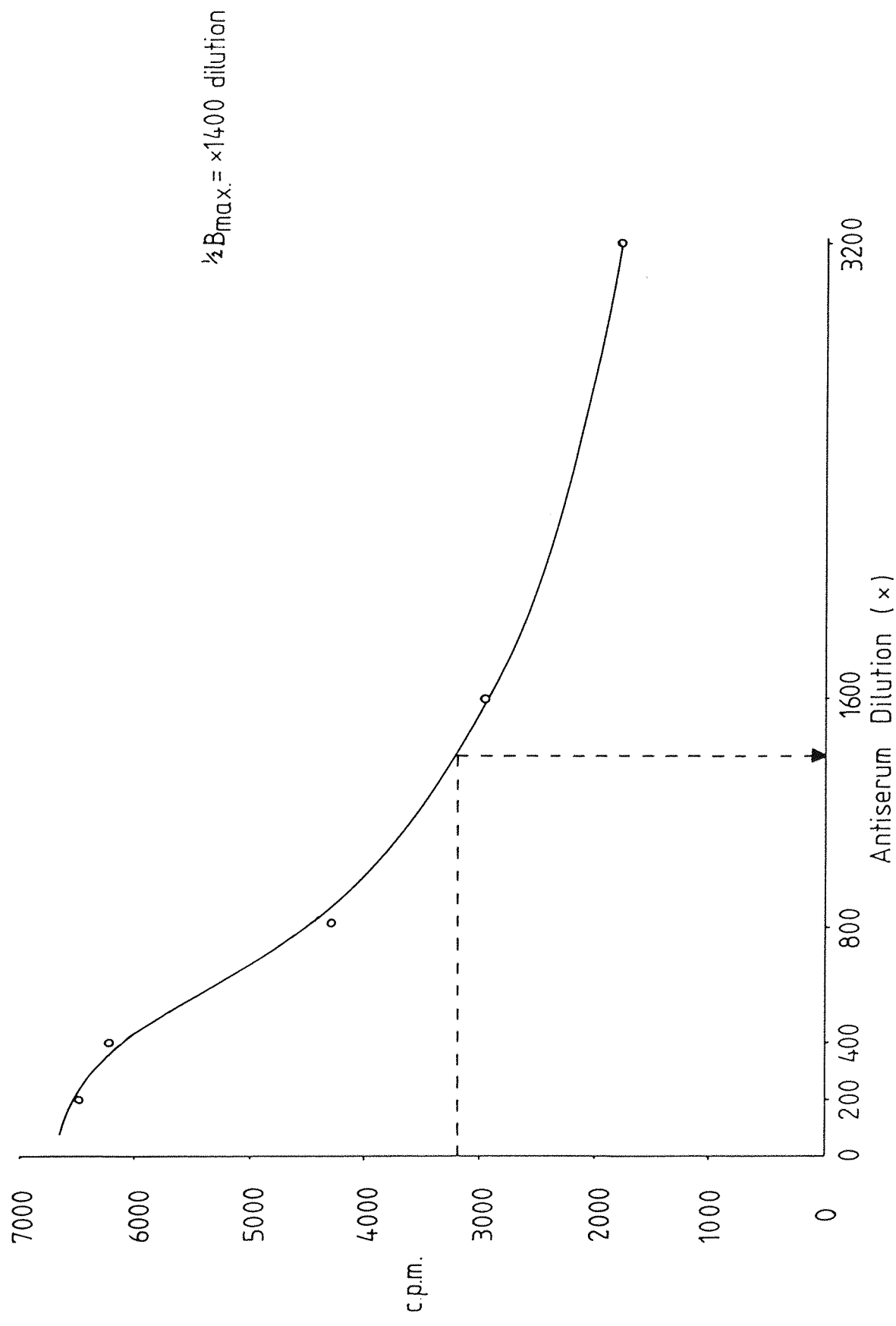


Figure 2.7 32K Antiserum Dilution Curve for the Determination of Optimum Antibody Concentration.


½Bmax. = ×1400 dilution
c.p.m.
7000
6000
5000
4000
3000
2000
1000
0
0 200 400 800 1600 3200
Antiserum Dilution (×)

Phosphate Buffered Saline (PBS)

Sodium Chloride	0.8% (w/v)	pH7.2-7.5 @ RT
Potassium Chloride	0.02% (w/v)	
Disodium hydrogen phosphate	7.5mM	
Potassium dihydrogen phosphate	1.5mM	

Triton X-100 was removed from purified 32K protein by passing through two biobead columns (BioRad). Triton-free protein was coated on to 96-well microtitre plates (Linbro, Flow Laboratories, Rickmansworth, Herts), by adding 50µl of a 20µg.ml^{-1} protein solution to each well (1µg 32K protein per well). Wells were left to coat for 1-2 hours at room temperature, after this time unbound 32K protein was washed from the plates by 3 washes of PBS containing 1% (w/v) BSA (RIA buffer). Wells were drained by shaking and left to dry for 10 minutes.

Standard 32K protein (not Triton-free) was serially diluted with PBS to give a range of standards from 0-30ng. Standards were incubated with antiserum at optimum dilution (1:1200-1:1600) at room temperature for 1 hour, mixing by gentle vortexing at 15 minute intervals. At the end of this period, 50µl aliquots of standard-antiserum mixture were added to coated microtitre wells in triplicate.

Mitochondrial samples were diluted to 1mg.ml^{-1} with PBS and solubilised by the addition of 10% (w/v) of a 5% (w/v) Triton X-100 solution (final concentration of Triton in the samples was 0.5% (w/v)). After incubation at room temperature for 30 minutes, samples were diluted by 5 with PBS and triton insoluble fragments were removed by centrifugation in a Beckman microfuge for 1 minute. Aliquots of supernatant were taken for assay and were diluted with PBS if necessary. Samples were incubated with antiserum in the same manner as standards and were also added in triplicate 50µl

aliquots to coated microtitre wells.

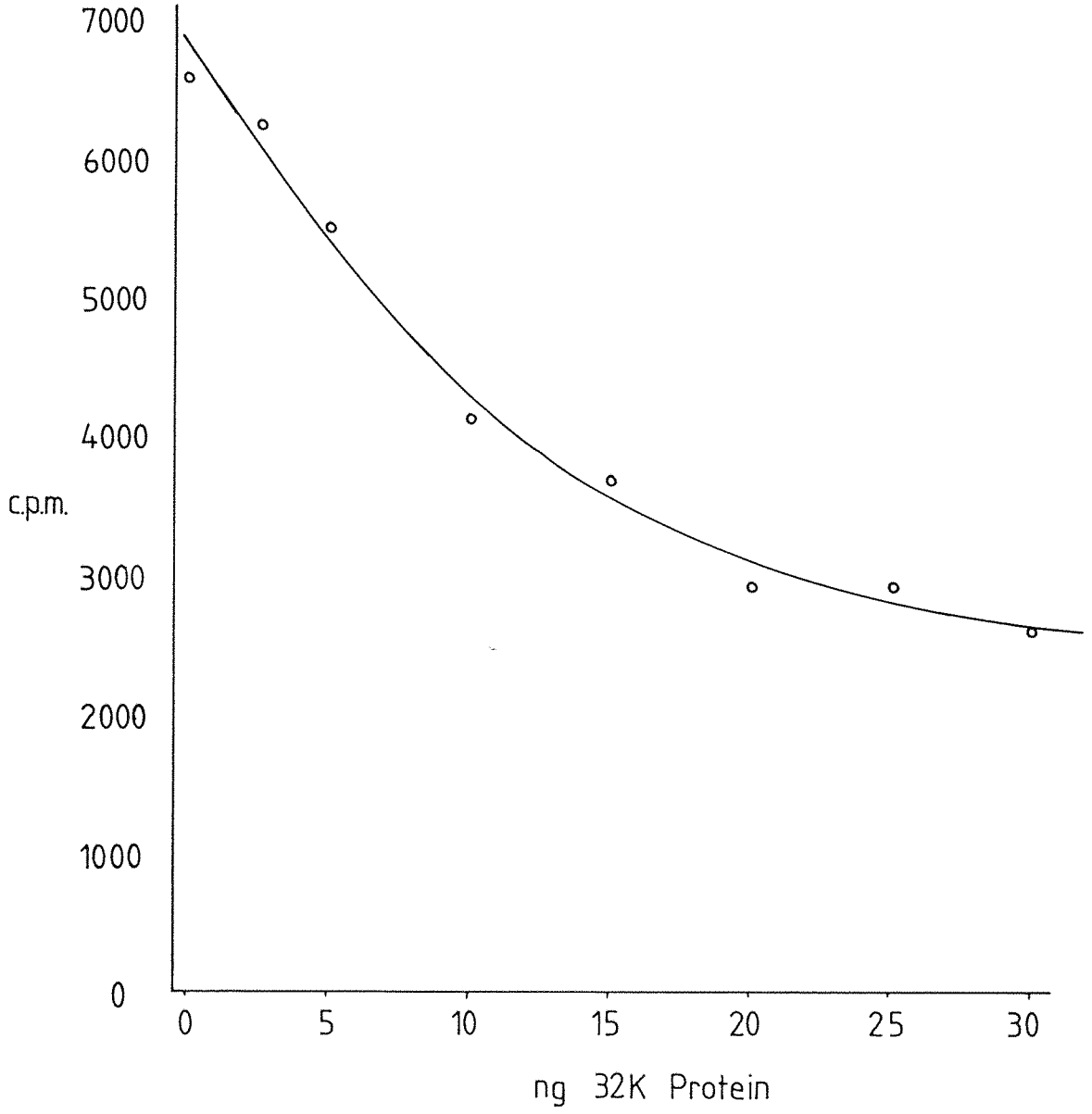
Samples and standards were left overnight at $4^{o}C$ to incubate. Excess liquid was removed from the plates by shaking followed by 3 washes with RIA buffer and allowed to dry as before. [^{125}I]-protein A was diluted to 600,000 cpm.ml^{-1} with PBS and added in 50µl aliquots to each well (30,000 cpm per well). Incubation took place at room temperature over 1 hour. After washing 3 times with RIA buffer and allowing to dry as before, individual wells were cut from the plates into γ-vials and counted for 1 minute on a Beckman Biogamma counter (80% efficiency for ^{125}I). A standard curve of cpm bound versus ng 32K protein added was constructed and used to determine the 32K protein content of the samples. Results were expressed as ng32K protein per mg mitochondrial protein. A standard curve is shown in figure 2.8.

2.19 Polyacrylamide Gel Electrophoresis.

12% polyacrylamide slab gels were usually used, except for the 32K protein purification gels which were 12.5%-16% gradient slab gels. All gels were run using the Laemmli system (Laemmli 1970).

Mitochondrial samples were prepared in 125mM Tris-HCl (pH8.8) containing 1% SDS (w/v) and 1% β-mercaptoethanol (v/v) 60µg of mitochondria in a 30-40µl volume containing 5µl bromophenol blue (1mg.ml^{-1} in ethanol) was loaded per track in all cases. All gels were run in the presence of purified 32K protein and Electran molecular weight markers (BDH Kit No. 44264.2L. 12,800-78,000 M.W.).

Figure 2.8 Standard Curve for the Radioimmunoassay of 32K Protein in Solubilised Mitochondria.



12% Polyacrylamide Gels.

Polyacrylamide	11.68% (w/v)	pH8.8 @ RT
N,N'-methylenebisacrylamide	0.32% (w/v)	
Ammonium Persulphate	0.025 (w/v)	
SDS (10%(w/v) solution)	0.025% (v/v)	
Tris-HCl	0.375M	
TEMED	0.5% (v/v)	

Stacking gel.

Polyacrylamide	4.75% (w/v)	pH6.8 @ RT
N,N'-methylenebisacrylamide	0.375% (w/v)	
Ammonium Persulphate	0.075% (w/v)	
SDS	0.1% (w/v)	
Tris HCl	0.125M	
TEMED	0.5% (w/v)	

12% gel cassettes were placed in an electrophoresis tank containing Laemmli buffer (25mM Tris-HCl,192mM glycine and 0.1% (w/v) SDS) and samples were loaded through a stacking gel. Current was passed at 30mA per gel for approximately 2 hours, until the dye front reached the bottom of the cassette. Gels were always run in pairs, one was stained up for total protein with Coomassie blue R (0.25% w/v in water) and its partner left unstained for Western blotting.

2.20 Preparation and Visualisation of Western Blots.

Unstained gels were washed in Laemmli buffer containing 20% (v/v) methanol and no SDS ('Transfer buffer'). They were then sandwiched against nitrocellulose paper between double layers of protective filter paper and clipped together between electrically wired plastic sheets. This assembly was placed in ice-cold transfer buffer and connected to a power supply, nitrocellulose paper side positive, which delivered a 100mA current. Transfer took place over 3 hours.

After transfer was effected, the nitrocellulose paper was exposed to rabbit anti-rat 32K protein antiserum and the resulting immunoprecipitate was visualised, using the Biotin-Streptavidin system as outlined in Scheme 2.1.

Scheme 2.1

Visualisation of Antibody-Antigen complexes on Nitrocellulose Paper using the Biotin-Streptavidin System.

1. Wash nitrocellulose paper (NCP) either
   (a) overnight in PBS/1% BSA (w/v) at $4^{o}C$

   or

   (b) for 1 hour in PBS/1% BSA (w/v) at $37^{o}C$.

Subsequent steps carried out at $37^{o}C$ with shaking.

2. Incubate NCP for 1 hour with 100ul diluted 32K antiserum in 10ml PBS/1% BSA (w/v).
3. Wash NCP 2 x 15 minutes in PBS/0.05% Tween 20 (w/v).
4. Incubate NCP for 1 hour with 1:500 dilution of anti-rabbit Ig, biotinylated whole Antibody (from donkey) in PBS/1% BSA (w/v).
5. Repeat step 3.
6. Incubate NCP for 30 minutes with 1:400 dilution of Streptavidin-biotinylated Horseradish peroxidase complex in PBS/1% BSA (w/v).
7. Repeat step 3.
8. Develop by adding 6mg HRP Enzyme Substrate dissolved in 2ml cold ethanol:10ml PBS/1% BSA (w/v):6ul 30% H_2O_2. When required colour density is reached, stop reaction by transfer to distilled water.

2.21 Determination of Radioactivity.

Scintillation cocktails used were Beckman Readysolv HA and Tritoscint (0.05% (w/v) POPOP, 0.4% (w/v) PPO, 33.3% Triton X-100 in xylene). Samples were counted in a Phillips 4700 Liquid Scintillation counter with 60% efficiency for ^{14}C and 25% efficiency for ^{3}H in Readysolv and 38% efficiency for ^{3}H in Tritoscint. Variations in quenching were corrected with external standards using preprogrammed quench curves and the counts expressed as disintegrations per minute (dpm).

γ-radiation was measured in a Beckman Biogamma counter with an 80% efficiency for ^{125}I. Results were expressed as counts per minute (cpm).

2.22 Statistics.

All data was analysed by use of Students' Two-tailed t-test. Significant differences were considered to be observed at $P<0.05$.

CHAPTER 3. THE EFFECTS OF 2-DEOXY-D-GLUCOSE ON BAT THERMOGENESIS IN LEAN AND OBESE ZUCKER RATS.

2-deoxy-D-glucose (2DG) is a non-metabolisable glucose analogue which competitively inhibits cellular glucose uptake and metabolism (Wick et al., 1955, 1957). Hexokinase (E.C.2.7.1.1) acts on both glucose and 2DG to form glucose-6-phosphate and 2DG-6-phosphate. Phosphoglucose isomerase (E.C.5.3.1.9.), the second enzyme in the glycolytic pathway is competitively inhibited by 2DG-6-phosphate, levels of glucose-6-phosphate and 2DG-6-phosphate build up within the cell, and the accumulation of glucose-6-phosphate inhibits hexokinase activity (Brown, 1962) leading to a drastic reduction in glucose utilisation and uptake.

2DG administration has many varied physiological effects. A rapid hyperglycaemia develops which is accompanied by enhanced catecholamine release (Brown and Bachrach, 1959; Hokfelt and Bydgeman, 1961), depression of insulin secretion in response to a glucose load (Frohman et al., 1973), and a mobilisation of fatty acids (Coimbra et al., 1979). The enhanced catecholamine release is thought to be due to 2DG-induced stimulation of the adrenal sympathetic nerve (Niijima, 1975). 2DG is not a general sympathetic stimulator, as renal nerve activity is unaffected by 2DG administration (Niijima, 1975), and cardiac noradrenaline turnover is reduced in 2DG treated animals (Rappaport et al., 1982). The elevated levels of catecholamines are thought to be responsible for the inhibition of insulin release in the presence of hyperglycaemia, as adrenalectomy or adrenal demedullation prevents the inhibitory 2DG effect on glucose-stimulated insulin secretion and reduces the hyperglycaemia (Brown and Bachrach, 1959; Frohman et al., 1973). Hyperglycaemia occurs as a result of reduced glucose uptake and utilisation caused by the metabolic effects of 2DG, a direct sympathetic stimulation of hepatic glucose output and a further stimulation of hepatic glucose release by circulating catecholamines (see Himms-Hagen, 1967). The catecholamine stimulated glucose release is mainly due to the effects of adrenaline rather than noradrenaline. Hokfelt and Bydgeman (1961) reported a 40 fold increase in urinary adrenaline output after 2DG administration but only a doubling of noradrenaline levels in the urine.

2DG treated animals exhibit hyperphagia (Smith and Epstein, 1969), increased gastric acid secretion (Hirschowitz and Sachs, 1965), hypothermia (Shiraishi and Mager, 1980a) and reduced metabolic rate (Shiraishi and Mager, 1980a, b; Rothwell et al., 1981, 1982d). 2DG induced gastric acid secretion, hypothermia and reduced metabolic rate can be abolished by bilateral cervical vagotomy or atropine injection (Hirschowitz and Sachs, 1965; Shiraishi and Mager, 1980a, b; Rothwell et al., 1981, 1982d). 2DG also increases the gastric vagal efferent discharge rate when injected into the carotid artery (Hirano and Niijima, 1980) which suggests that a central mechanism exists for the stimulation of gastric acid secretion by 2DG. From these observations it can be seen that the PNS also plays a role in the physiological responses to 2DG. Other central effects of 2DG include an inhibition of glucose effects upon the activities of glucose-sensitive and glucoreceptor neurones in the LH and VMH (Oomura et al., 1978), regions of the hypothalamus which are closely associated with the regulation of food intake and energy balance (see Le Magnen, 1983; Bray, 1984). The VMH has also been demonstrated as being of importance in the sympathetic activation of BAT (Perkins et al., 1981, a, b; Niijima et al., 1984; Holt et al., 1986).

Obese Zucker rats do not show the hyperphagic response normally associated with intracerebroventricular 2DG injection (Ikeda et al., 1980), and the 2DG induced reduction in metabolic rate is attenuated in these animals (Rothwell et al., 1981, 1982d). It is known that the sympathetic drive to BAT is reduced in the obese animals and this is responsible for the lack of DIT which contributes to their obesity (Marchington, 1985; York et al., 1985b). Enhanced PNS activity is also thought to be important for the development of obesity in the Zucker rat with respect to modulating the increase in insulin secretion (Jeanrenaud et al., 1981; Rohner-Jeanrenaud et al., 1983).

As 2DG administration decreases body temperature and metabolic rate, effects that could be associated with depressed BAT activity, the following experiments were undertaken in order to investigate -

a) the possible role for BAT in the physiological responses to 2DG;

b) the role of glucose metabolism in the control of thermogenesis;

and

c) the effect of the hormones of the pituitary-adrenal axis on the thermogenic responses of BAT to 2DG.

Section 3.1 The Effects of 2DG in Lean and Obese Zucker Rats.

3.1.1 The Effects of Varying Doses of 2DG on Core Temperature and BAT Function in the Lean and Obese Zucker Rat.

A single dose of 240, 360 or 480mg 2DG per kg body weight was injected i.p. to lean rats and 360, 450 or 630mg 2DG per kg body weight to obese rats, and the animals sacrificed 1 hour later. All injections were in a 0.2ml volume of 0.9% (w/v) sodium chloride (hereafter referred to as saline). Control animals received a 0.2ml injection of saline only. Food was withdrawn from all animals at the beginning of the experimental period as differential effects of 2DG on food intake in lean and obese rats has previously been demonstrated (Ikeda et al., 1980). Rectal temperatures were measured as described in section 2.3.2 (conscious animals) just prior to 2DG or saline injection, and again just before sacrifice. Animals were sacrificed by cervical dislocation after stunning and BAT mitochondria prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

Results.

Figure 3.1.1.1 shows the change in BAT mitochondrial GDP binding and rectal temperature in response to 2DG in lean rats. The minimum dose ($240mg.kg^{-1}$) was sufficient to produce a maximal depression of both GDP binding and rectal temperature. Figure 3.1.1.2 illustrates the relationship between GDP binding and rectal temperature at each dose of 2DG, and demonstrates a strong correlation ($r=0.988$, $P<0.02$) between these two factors. There were no significant changes in GDP binding or rectal temperature at any dose of 2DG in obese rats (figure 3.1.1.3) and neither was there any dose-dependent correlation between them ($r=0.203$) as can be seen in figure 3.1.1.4.

Figure 3.1.1.1 Changes in BAT Mitochondrial GDP Binding and Rectal Temperature in Response to Increasing Doses of 2DG in Lean Zucker Rats.

A single dose of 2DG, as indicated, in 0.2ml saline was injected i.p. 1 hour before sacrifice. Food was withdrawn from all animals at the beginning of the 1 hour period. Rectal temperature was measured just prior to injection and again at the end of the experimental period. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

The upper diagram shows the GDP binding values 1 hour after injection. The lower diagram illustrates the change (Δ) in rectal temperature over the same period.

Each point represents the $\pm$ S.E.M. for 3 animals in each group.

*	$P < 0.05$	)
**	$P < 0.01$	) compared with saline injected animals.
***	$P < 0.02$	)

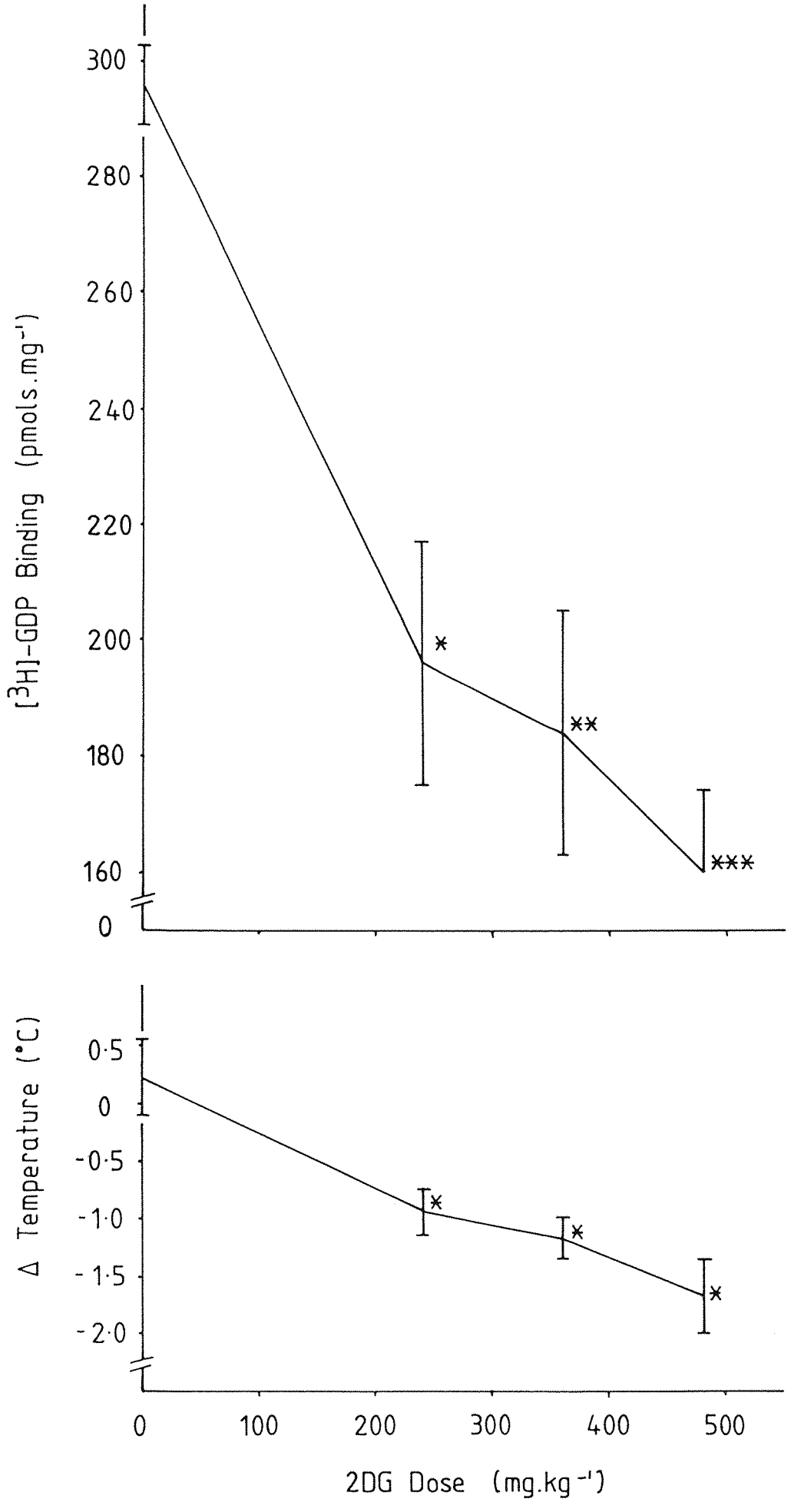

300
280
260
240
220
200
180
160
0
[3H]-GDP Binding (pmols.mg-1)
*
**
***
0·5
0
-0·5
-1·0
-1·5
-2·0
Δ Temperature (°C)
0
100
200
300
400
500
2DG Dose (mg.kg-1)

Figure 3.1.1.2 The Correlation Between Temperature Change and GDP Binding After Increasing Doses of 2DG in Lean Zucker Rats.

A single dose of 2DG (240, 360 or 480 $mg.kg^{-1}$ body weight) in 0.2ml saline was injected i.p. 1 hour before sacrifice. Food was withdrawn from all animals at the beginning of the experimental period. BAT mitochondria were prepared and GDP binding assays were performed as described in sections 2.12 and 2.13 respectively. The change in rectal temperature that occurred over 1 hour at a given dose of 2DG was plotted against the GDP binding level, at the end of the 1 hour period, of animals receiving the same 2DG dose.

● 0 $mg.kg^{-1}$ (saline injected controls)

○ 240 $mg.kg^{-1}$

■ 360 $mg.kg^{-1}$

□ 480 $mg.kg^{-1}$

The correlation coefficient, r, was calculated by linear regression and significance determined by the comparison with the Product-Moment Correlation Coefficient. ΔT_{Re} denotes change in rectal temperature.

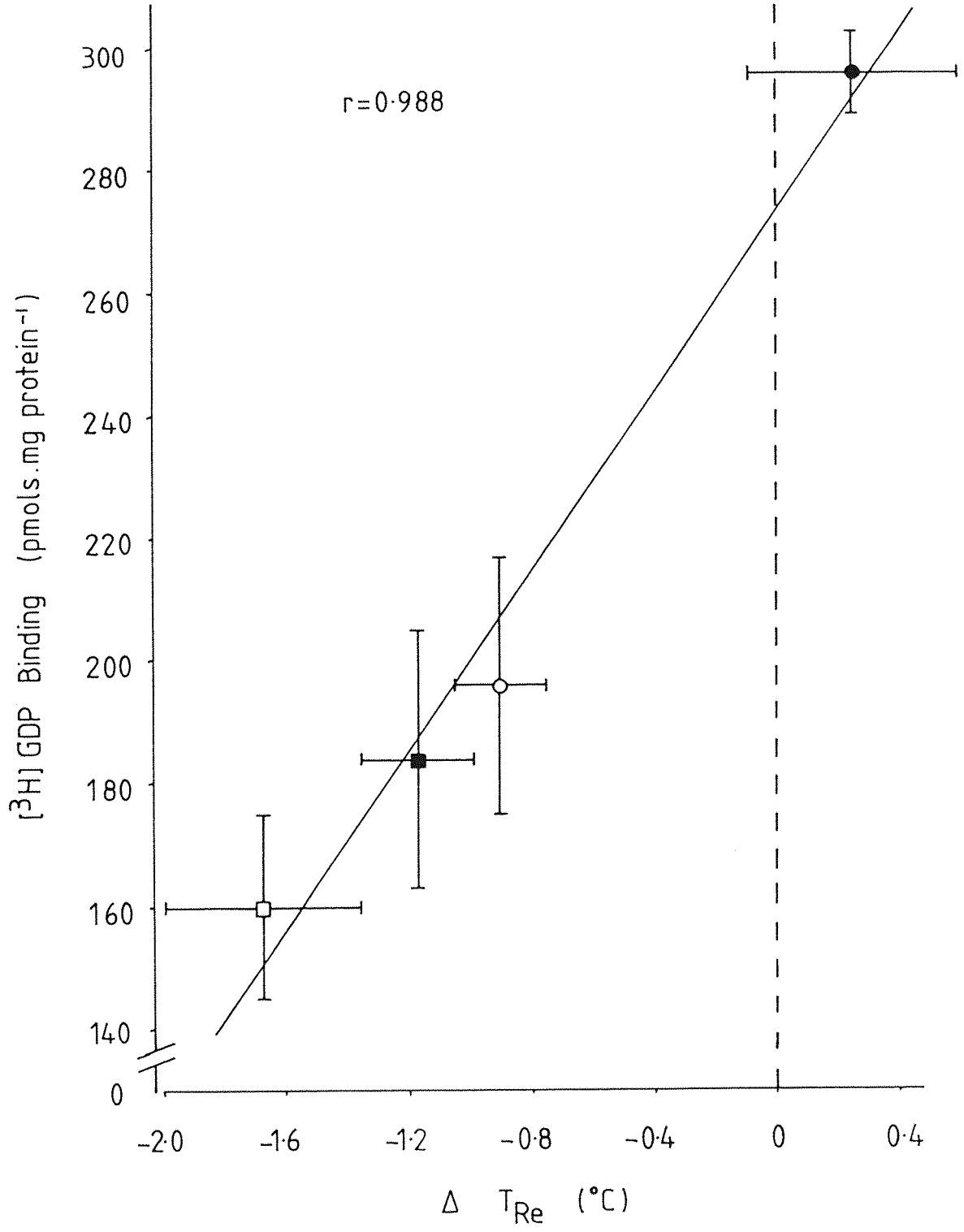

r=0·988
300
280
260
240
220
200
180
160
140
0
[3H] GDP Binding (pmols.mg protein-1)
-2·0
-1·6
-1·2
-0·8
-0·4
0
0·4
Δ TRe (°C)

Figure 3.1.1.3 Changes in BAT Mitochondrial GDP Binding and Rectal Temperature in Response to Increasing Doses of 2DG in Obese Zucker Rats.

A single dose of 2DG, as indicated, in 0.2ml saline was injected i.p. 1 hour before sacrifice. Food was withdrawn from all animals at the beginning of the 1 hour period. Rectal temperature was measured just prior to injection and again at the end of the experimental period. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

The upper diagram shows the GDP binding values 1 hour after injection. The lower diagram illustrates the change (Δ) in rectal temperature over the same period.

Each point represents the $\pm$ S.E.M. for 3 anmials in each group.

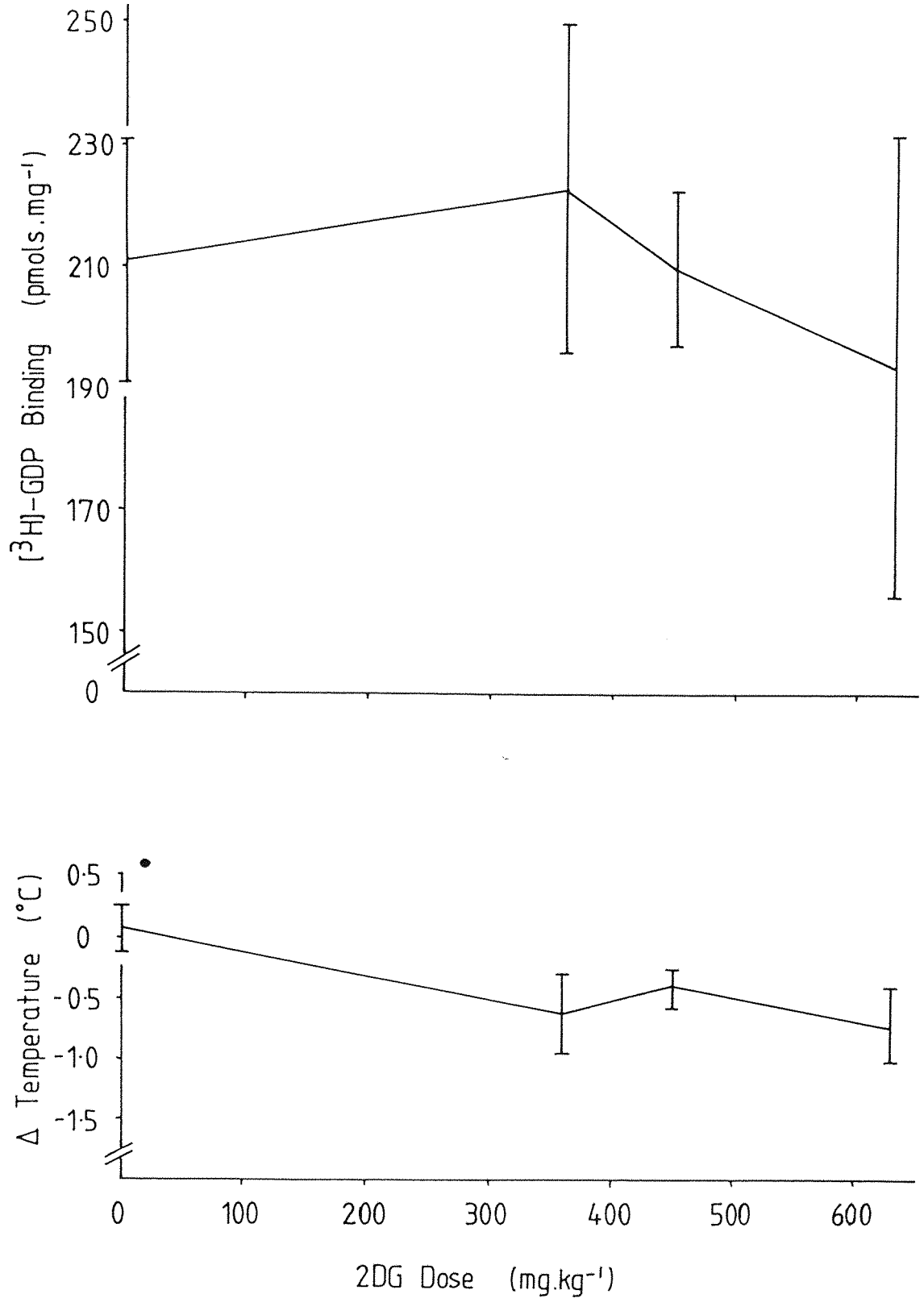

250
230
210
190
170
150
0
[3H]-GDP Binding (pmols.mg-1)
0·5
0
-0·5
-1·0
-1·5
Δ Temperature (°C)
0
100
200
300
400
500
600
2DG Dose (mg.kg-1)

Figure 3.1.1.4 The Correlation Between Temperature Change and GDP Binding After Increasing Doses of 2DG in Obese Zucker Rats.

A single dose of 2DG (360, 450 or 640 $mg.kg^{-1}$ body weight) in 0.2ml saline was injected i.p. 1 hour before sacrifice of the experimental period. BAT mitochondria were prepared and GDP binding assays were performed as described in sections 2.12 and 2.13 respectively. The change in rectal temperature that occurred over 1 hour at a given dose of 2DG was plotted against the GDP binding level, at the end of the 1 hour period, of animals receiving the same 2DG dose.

● 0 $mg.kg^{-1}$ (saline injected controls)

○ 360 $mg.kg^{-1}$

■ 450 $mg.kg^{-1}$

□ 640 $mg.kg^{-1}$

The correlation coefficient, r, was calculated by linear regression and significance determined by the comparison with Product-Moment Correlation Coefficient.

ΔT_{Re} denotes change in rectal temperature.

[3H] GDP Binding (pmols.mg protein-1)
250
230
210
190
170
150
r=0·203
-1·2
-1·0
-0·8
-0·6
-0·4
-0·2
0
0·2
Δ TRe (°C)

3.1.2 The Time Course of the Responses to 2DG in Lean and Obese Zucker Rats.

From the results of the previous section, it was decided to routinely use a dose of 360mg.kg^{-1} body weight 2DG in all following experiments as this dose produced maximal inhibition of BAT mitochondrial GDP binding in lean animals and was low in the range of doses previously used by other workers with this drug (250mg.kg^{-1}, Shiraishi and Mager, 1980b; 750mg.kg^{-1}, Muller et al., 1972). The time course of the effects of a single 360mg.kg^{-1} 2DG i.p. injection on BAT mitochondrial GDP binding, serum hormone and serum metabolite concentrations was investigated over a 3 hour period. All injections were given in a 0.2ml volume, control animals were injected with 0.2ml saline. Food was withdrawn from all animals at the time of injection to avoid differential variations in food intake caused by 2DG in lean and obese animals (Ikeda et al., 1980). Animals were sacrificed 1, 2 or 3 hours after injection, by decapitation. Trunk blood was collected and serum separated as described in section 2.9.1. Serum assays were performed as described in sections 2.9.2 - 2.9.5. BAT mitochondria were prepared and GDP binding assays performed as outlined in sections 2.12 and 2.13 respectively.

Results.

The time course of the changes in BAT mitochondrial GDP binding are shown in figure 3.1.2.1. GDP binding levels in obese rats were unaffected by 2DG at any time, as would be expected from the results in Section 3.1.1. GDP binding in lean rats was depressed after 1 hour to the levels observed in obese rats, and there was no further significant changes throughout the remainder of the experimental period.

Serum glucose and free fatty acid concentrations are shown in figure 3.1.2.2. Serum glucose was significantly increased in both groups, although the increase in obese animals was smaller and only significant at the 1 hour time point. Free fatty acids were increased in lean animals at 2 hours post-injection, but no changes seen in obese rats.

Figure 3.1.2.1 The Time Course of the Change in GDP Binding Due to 2DG in Lean and Obese Zucker Rats.

A single dose of 360mg.kg^{-1} 2DG in 0.2ml saline was injected i.p. and animals sacrified 1, 2 or 3 hours later. Food was withdrawn from all animals 3 hours before sacrifice. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

Each point represents the mean $\pm$ S.E.M. of 3 animals in each group.

o Lean • Obese

** $P < 0.01$ compared with zero time group of the same phenotype.

++ $P < 0.01$ compared with lean group at the same time point.

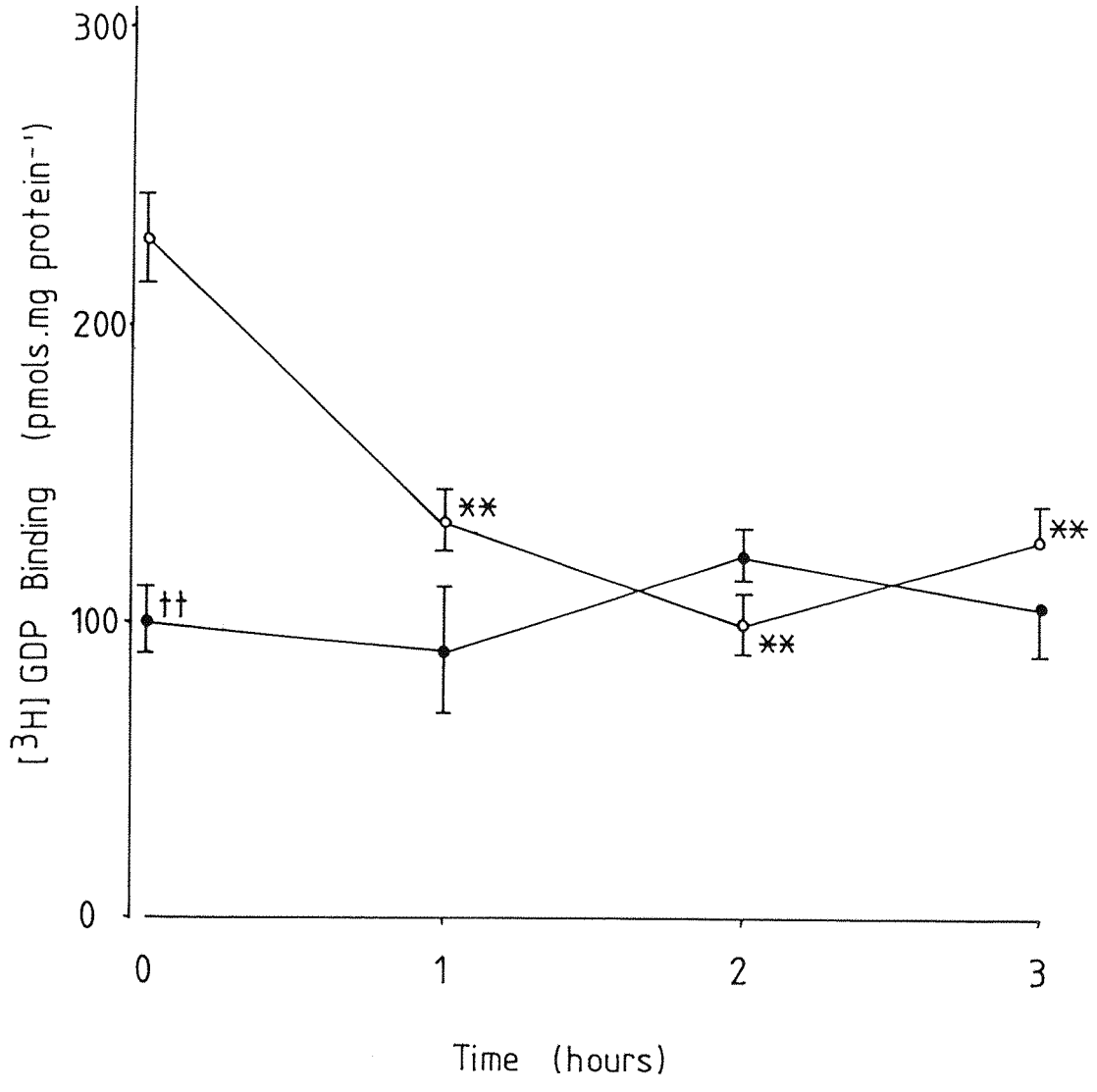

300
200
100
0
[3H] GDP Binding (pmols.mg protein-1)
0
1
2
3
Time (hours)
**
**
**
††

Figure 3.1.2.2 The Time Course of the Changes in Serum Glucose and Free Fatty Acid Concentrations Due to 2DG in Lean and Obese Zucker Rats.

Animals were injected with a single dose of 360mg.kg^{-1} in 0.2ml saline i.p., and sacrificed at the time indicated. Food was withdrawn from all animals 3 hours before sacrifice. Serum was collected as described in section 2.9.1 and assayed for glucose and free fatty acid concentration as described in sections 2.9.2 and 2.9.3 respectively.

Each point represents mean $\pm$ S.E.M. of 3 animals in each group.

o Lean • Obese

* $P < 0.05$
** $P < 0.01$
*** $P < 0.002$
) compared to zero time group of the same phenotype.

+++ $P < 0.002$ compared to lean group at the same time point.

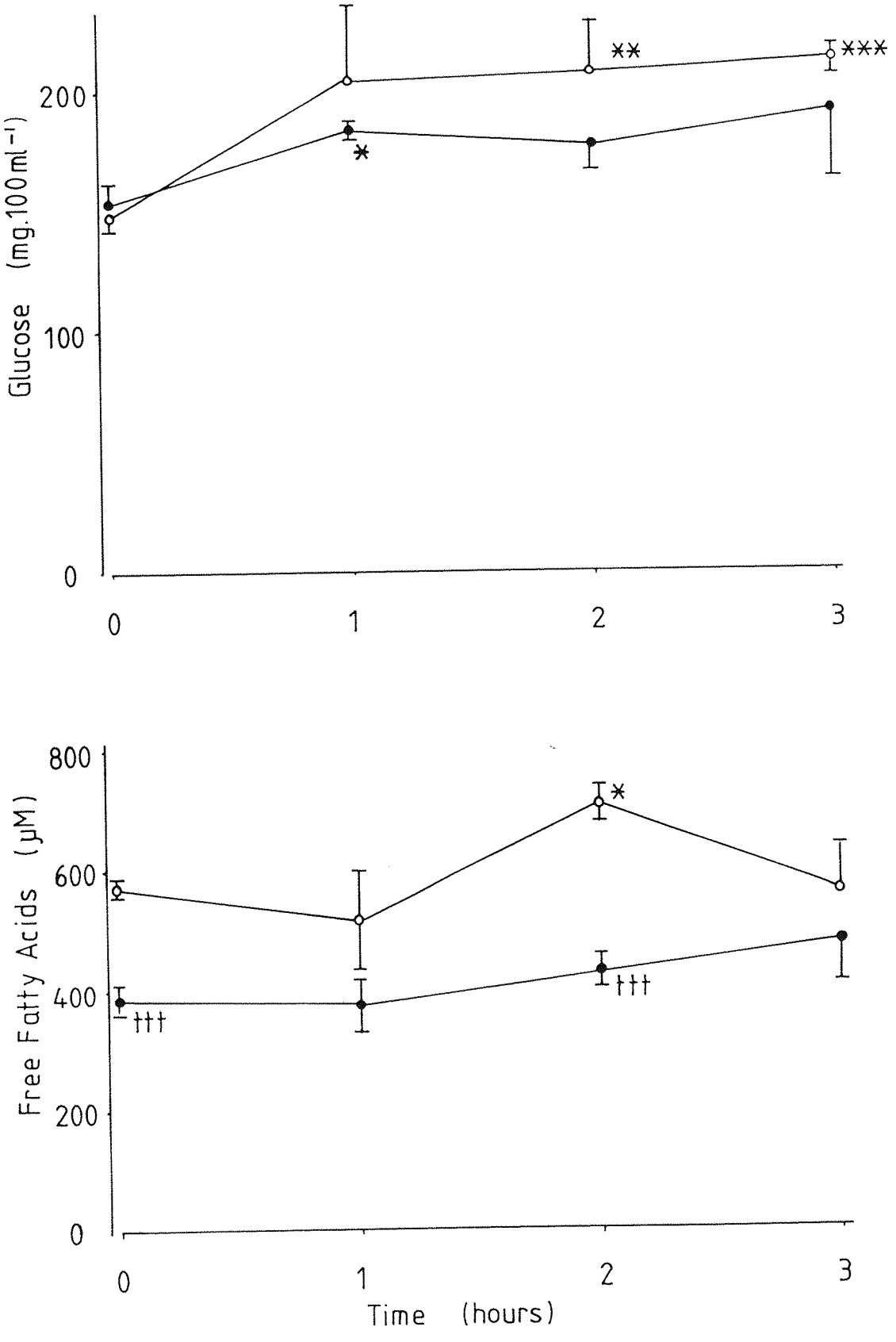

Glucose (mg.100ml⁻¹)
200
100
0
0
1
2
3
**
***
*
800
600
400
200
0
Free Fatty Acids (μM)
*
†††
†††
Time (hours)

Figure 3.1.2.3 shows changes that occurred in insulin and corticosterone levels. No significant changes occurred in insulin levels in either lean or obese animals, however, insulin levels in obese rats dropped dramatically at the 3 hour time point. The large individual variation in insulin concentrations may account for the lack of statistical significance attached to this observation. Corticosterone levels in lean rats doubled within 1 hour of 2DG administration and remained elevated throughout the experimental period. Obese rats' corticosterone levels were transiently increased at 2 hours, but had returned to normal at 3 hours.

In an attempt to synchronise as many of the effects of 2DG as possible, it was decided to give 2DG in two doses of $360mg.kg^{-1}$ i.p., 3 hours and 1 hour before sacrifice in all further experiments.

3.1.3 The Effect of 2DG on Food Intake in Lean and Obese Zucker Rats.

2DG induces hyperphagia in normal rats (Smith and Epstein, 1969; Miselis and Epstein, 1975). The feeding responses to 2DG in lean and obese Zucker rats after intracerebroventricular administration of the drug (Ikeda et al., 1980), but not peripheral administration, have been investigated. Ikeda et al., (1980) found that lean rats became hyperphagic after 2DG treatment, but obese animals' food intake was unaffected and suggested that the effect in lean animals was due to the inhibition of hypothalamic glucoreceptors and that the central hypothalamic gluco-sensitive site for food intake regulation was impaired in the obese rat. The experiment described here was designed to investigate the feeding responses elicited by peripheral 2DG administration in lean and obese animals. Animals were individually caged and allowed ad lib. access to food and water throughout the experimental period. Injections of $360mg.kg^{-1}$ 2DG or saline (0.2ml) were given i.p. 3 hours and 1 hour before sacrifice. Food intake was measured over the 3 hour period by difference in weight of food in the hopper after spilled food had been weighed and discounted from the total.

Figure 3.1.2.3 The Time Course of the Changes in Serum Insulin and Corticosterone Concentrations Due to 2DG in Lean and Obese Zucker Rats.

Animals were injected with a single dose of 360mg.kg^{-1} in 0.2ml saline i.p. and sacrified at the time indicated. Food was withdrawn from all animals 3 hours before sacrifice. Serum was collected as described in section 2.9.1 and assayed for insulin and corticosterone concentrations as described in sections 2.9.4 and 2.9.5 respectively.

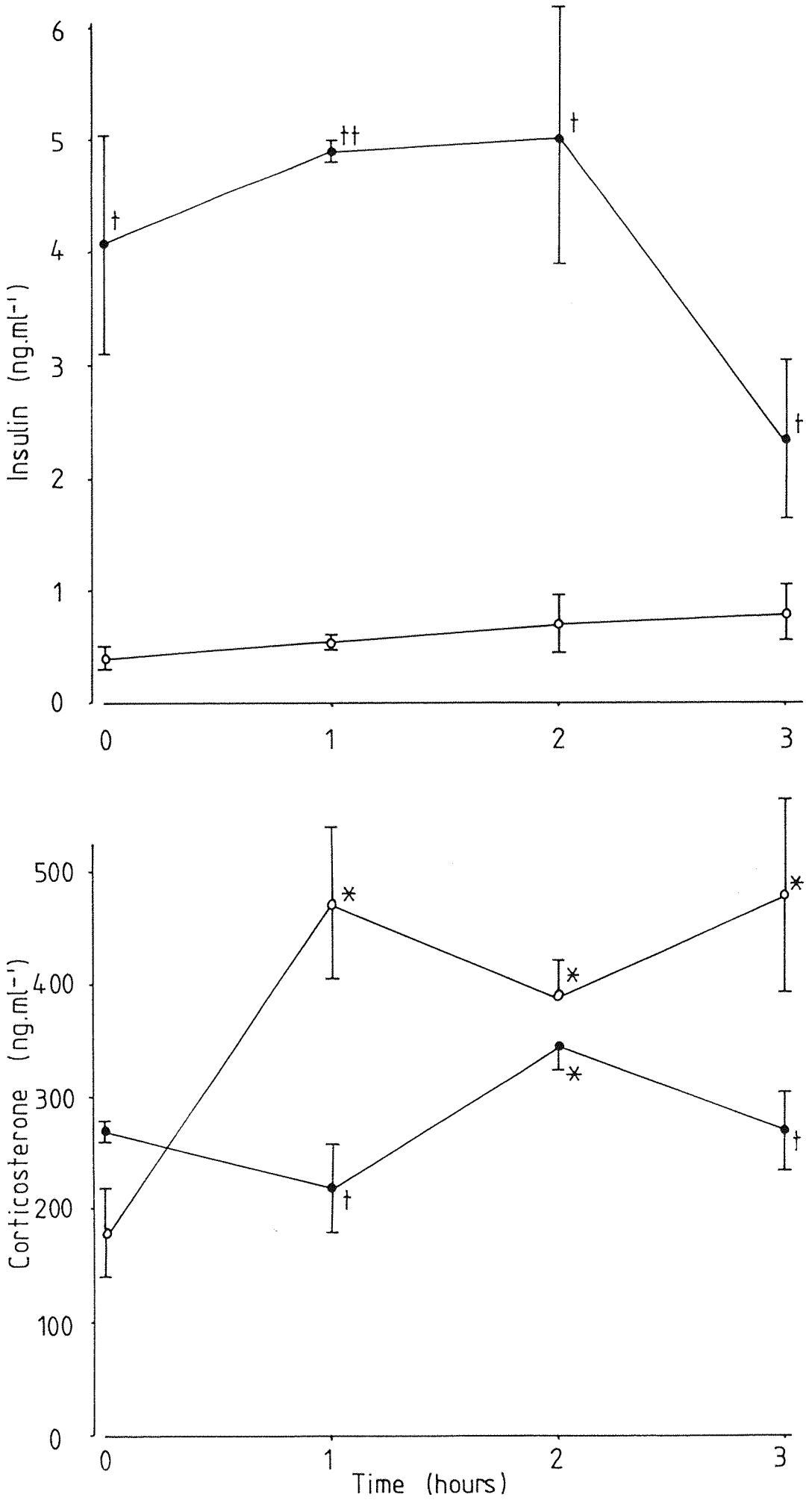
Each point represents mean $\pm$ S.E.M. of 3 animals in each group.

o Lean • Obese

* $P < 0.05$ compared to zero time groups of the same phenotype.

\+ $P < 0.05$) compared to lean groups at the same time point

\+++ $P < 0.002$)


Insulin (ng.ml⁻¹)
6
5
4
3
2
1
0
0
1
2
3
†
††
†
†
Corticosterone (ng.ml⁻¹)
500
400
300
200
100
0
0
1
2
3
Time (hours)
*
*
*
†
†

Results.

Table 3.1.3.1 shows the total food intake of saline injected and 2DG treated animals over the 3 hour experimental period. The food intake of lean animals was doubled compared to their saline-injected controls. Obese animals ate twice as much as their lean counterparts, but their food intake was unaffected by 2DG. These results were similar to those obtained by Ikeda et al. (1980) after central administration of 2DG to young Zucker rats.

3.1.4 The Effect of ad lib. Food Intake on the Responses of BAT and Core Temperature to 2DG in Lean and Obese Zucker Rats.

In view of the differential effects of 2DG on food intake in lean and obese Zucker rats it was decided to investigate BAT thermogenesis in free-feeding animals after 2DG injection. Individually caged animals were allowed ad lib. access to food, or food was withdrawn at the beginning of the experiment. Injections of 360mg.kg^{-1} 2DG or saline (0.2ml) were given 1 hour and 3 hours before sacrifice. Food intake over the entire 3 hour period was measured by difference in weight of food in the hopper, after spilled food had been collected and discounted from the total. Animals were sacrificed by stunning and cervical dislocation. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

Results.

The results are shown in table 3.1.4.1. Food deprivation per se had no significant effect on rectal temperature or BAT mitochondrial GDP binding in either saline injected or 2DG treated animals. The responses to 2DG, however, were affected by the feeding status of the animal. Food intake was increased in lean, but not obese, animals to a similar degree as in the previous section. Rectal temperature was not significantly depressed by 2DG in fed lean animals, but was reduced in lean food-deprived animals. The obligatory component of DIT may partially account for this difference in the response to 2DG. In contrast, the fed obese group

Table 3.1.3.1 The Effect of 2DG on the Food Intake of Lean and Obese Zucker Rats.

	Food Intake (g/3 hours)	
	Saline	2DG
Lean	1.2 ± 0.1	2.5 ± 0.2 ***
Obese	2.9 ± 0.1 +++	2.9 ± 0.4

Animals were individually caged and injected with 360mg.kg^{-1} 2DG 3 hours and 1 hour before sacrifice. Each injection was in a 0.2ml volume of saline. Control animals received 2 injections of 0.2ml saline at the same times as experimental rats. Food intake over the entire 3 hour period was measured by difference in food weight in the cage hopper at the beginning and the end of the experiment. Spilled food was collected on paper beneath the hopper and the food intake measurement adjusted accordingly.

Values represent the mean ± S.E.M. of 4 animals in each group.

*** $P<0.002$ compared to saline injected group of the same phenotype;

+++ $P<0.002$ compared to equivalent lean group.

Table 3.1.4.1 The Effect of ad lib. Food Intake on the Responses of BAT Mitchondrial GDP Binding and Core Temperature to 2DG in Lean and Obese Zucker Rats.

Animals were either allowed ad lib. access to chow (Fed) or food was withdrawn at the beginning of the experiment. 2DG was injected ($360mg.kg^{-1}$) 3 hours and 1 hour before sacrifice in a 0.2ml volume of saline. Control animals received 0.2ml injections of saline at the same times as the experimental rats. Rectal temperature was measured at the beginning and the end of the 3 hour experimental period and the change in rectal temperature (ΔT_{Re}) calculated. Food intake was measured by difference in food weight in the hopper at the beginning and end of the experiment. Food spillage was collected and discounted from the total. BAT mitochondria were prepared and GDP binding assays performed as described in Sections 2.12 and 2.13 respectively. n denotes the number of animals in each group.

Values represent mean $\pm$ S.E.M. for n animals.

* $P<0.05$) compared to saline treated animals
) of the same phenotype.
*** $P<0.002$)

Table 3.1.4.1

		LEAN		OBESE	
		SALINE	2DG	SALINE	2DG
Food Intake (g/3 hours)	Fed	1.9 ± 0.5	3.5 ± 0.5*	3.2 ± 0.4	3.1 ± 0.3
	Deprived	-	-	-	-
ΔT_{Re} (°C)	Fed	0 ± 0.2	-0.8 ± 0.4	+0.2 ± 0.2	-0.6 ± 0.1***
	Deprived	-0.4 ± 0.2	-1.6 ± 0.3*	+0.1 ± 0.2	-0.6 ± 0.3
[^{3}H]-GDP Binding (pmols.mg^{-1})	Fed	345 ± 18	300 ± 22	150 ± 11	145 ± 17
	Deprived	360 ± 32	240 ± 26*	136 ± 13	149 ± 15
n	Fed	5	6	14	14
	Deprived	4	4	4	4

of animals' rectal temperature was significantly depressed by 2DG, but not that of the deprived group. However, the magnitude of depression was similar in both groups but the large individual variability in the deprived 2DG treated group masks any significance in the change. BAT mitochondrial GDP binding was unaffected by either 2DG treatment or food deprivation in these animals,as would be expected, as dietary stimuli do not affect obese rats and no inhibition of GDP binding after 2DG administration to obese rats in previous sections has been observed. Only the food-deprived lean animals showed a statistically significant inhibition of GDP binding in response to 2DG treatment (33%). The fed animals showed a small (13%) but insignificant inhibition of GDP binding, implying that the effects of 2DG as an inhibitor of BAT thermogenesis were attenuated by food.

The inhibition of BAT mitochondrial GDP binding also contributes towards the greater depression in rectal temperature seen in the lean food-deprived rats after 2DG, compared to their fed counterparts. The depression of rectal temperature seen in obese animals after 2DG administration in the absence of any alterations in BAT thermogenesis is probably due to general inhibition of cellular metabolism and therefore, a reduction in obligatory heat production.

In order to avoid the differential effects of 2DG treatment on food intake in lean and obese animals interfering with the effects of the drug on BAT thermogenic function, it was decided to withdraw food from all animals at the start of all subsequent experiments.

3.1.5 The Effect of 2DG on the BAT Thermogenic Response to Noradrenaline Injection in Lean and Obese Zucker Rats.

The depression of BAT mitochondrial GDP binding in response to 2DG administration to lean animals may be due to a direct inhibition of BAT adipocyte metabolism by 2DG. Although free fatty acids are thought to be the major fuel for BAT thermogenesis, it is possible that an inhibition of glycolysis within the cell could cause a significant

reduction in its capacity for thermogenesis (see Gibbins et al., 1985; McCormack et al., 1986). If BAT metabolic function was inhibited directly by 2DG then it would not be possible to fully stimulate BAT thermogenesis with peripheral effectors, such as noradrenaline. To investigate this possibility, the BAT thermogenic response to an acute injection of noradrenaline was measured in 2DG treated lean and obese rats.

Lean and obese animals were treated with two doses of 360mg.kg^{-1} 2DG or 0.2ml saline, i.p., 3 hours and 1 hour before sacrifice, as previously described. A single dose of 50μg.100g^{-1} body weight noradrenaline was given s.c. in a 0.1ml volume 30 minutes before sacrifice (i.e. 30 minutes after the second 2DG injection). Control animals received 0.1ml saline, s.c., at this time. Food was withdrawn from all animals at the beginning of the 3 hour experimental period. Animals were stunned and killed by cervical dislocation. BAT mitochondria were isolated and GDP binding assays were performed as described in sections 2.12 and 2.13 respectively.

Results.

The results are shown in table 3.1.5.1. 2DG treatment of lean and obese rats had no effect on the BAT thermogenic response to noradrenaline injections. These results demonstrated that the inhibitory effects of 2DG on BAT mitochondrial GDP binding were not due to a direct effect on the tissue, as maximal activity could be induced by peripheral stimulation of BAT during 2DG treatment.

3.1.6 Discussion.

2DG treatment significantly inhibited BAT mitochondrial GDP binding and rectal temperature after 1 hour in lean animals at all doses of 2DG used, and the degree of significance increased in direct proportion to the dose (240mg.kg^{-1} $P<0.05$; 360mg.kg^{-1}, $P<0.01$; 630mg.kg^{-1}, $P<0.002$). A linear dependence was observed in lean animals between GDP binding and rectal temperature in relation to the dose of 2DG given,

Table 3.1.5.1 The Effects of Noradrenaline on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Treated with 2DG.

BAT [^{3}H] GDP Binding. (pmols.mg protein^{-1})			
Noradrenaline		Saline	2DG
LEAN	-	228 ± 19 (4)	139 ± 11** (5)
	+	305 + 14+ (4)	332 + 22+++ (5)
OBESE	-	169 ± 6 (4)	183 ± 19 (5)
	+	426 ± 49++ (4)	427 ± 57++ (5)

Values represent means ± S.E.M. for the number of animals shown in parenthesis.

** $P < 0.01$ compared to saline injected animals (for 2DG treatment.

+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$, compared to saline injected animals of the same phenotype (for noradrenaline treatment).

Animals were injected with two doses of 2DG (360mg.kg^{-1} i.p.) 3 hours and 1 hour before sacrifice. Noradrenaline (50µg.100g^{-1} s.c.) was injected 30 minutes before sacrifice. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

with the greatest depression of mitochondrial GDP binding and rectal temperature at the greatest 2DG dose. A dose response effect of 2DG has been demonstrated on rectal temperature over a range of doses (250-562.5mg.kg^{-1} 2DG i.p.), in Sprague-Dawley rats (Shiraishi and Mager, 1980b). The failure of 2DG to suppress BAT mitochondrial GDP binding in obese rats is consistent with the lack of depression of metabolic rate after peripheral 2DG administration in obese Zucker rats (Rothwell et al., 1981, 1982d). The dose of 2DG used by these workers was 360mg.kg^{-1}, equivalent to the lowest dose used here in obese rats. The depression in rectal temperature observed in 2DG treated obese rats was not correlated to GDP binding levels and did not show linear dose dependence (figure 3.1.1.4). This core temperature depression (which was observed as statistically significant in section 3.1.4 in fed animals) probably reflected the effects of the general inhibition of glucose metabolism and the reduction of obligatory heat production associated with this.

The time course of the BAT thermogenic response to 2DG is similar to that described by Shiraishi and Mager (1980b), who observed maximal depression in rectal temperature by 1 hour post-injection, which then recovered throughout a 6 hour period, although temperature recovery was not complete at the end of this time (6 hours). Previous investigations have shown that serum glucose concentration is increased 2-3 hours after peripheral 2DG injection, and free fatty acids, after 3 hours (Haito et al., 1984; Weidenfeld et al., 1984). Serum levels of corticosterone have also been previously shown to peak 1 hour after 2DG injection, and remain elevated for some time (Sun et al., 1979; Weidenfeld et al., 1984). These timings are similar to the time courses of the changes in serum glucose, free fatty acid and corticosterone levels observed here in lean rats. Central administration of 2DG to Wistar rats has no effect on insulin levels (Coimbra et al., 1979) but attenuates the insulin response to an i.p. glucose load (Frohman et al., 1973). No changes in insulin levels of lean rats were observed here, which is consistent with the

observations of Coimbra et al., (1979). The changes in serum metabolites and hormones which occurred in lean animals were attenuated, absent or delayed in obese rats. The 2DG induced hyperglycaemia, increase in free fatty acids and changes in hormone levels, are all thought to be due to the actions of 2DG at different brain regions. Free fatty acid mobilisation in response to 2DG injection is inhibited by VMH lesion (Nishizawa and Bray, 1978) and completely blocked by globus pallidus lesions (Grunion et al., 1984), possibly through the inactivation of a pathway running through this area and into the lateral hypothalamus another area which, when lesioned, prevents fatty acid mobilisation in response to 2DG (Teixiera et al.,1973). VMH lesion has no effect on 2DG-induced hyperglycaemia or suppression of insulin levels (Nishzawa and Bray, 1978). The 2DG stimulated hyperglycaemia and inhibition of insulin release are thought to be due to separate effects of 2DG acting at different brain regions, which elevate sympathetic activity to the liver, adrenal medulla and pancreas (Nishizawa and Bray, 1978; Le Magnen, 1983). The absence of responses in the obese rat is perhaps indicative of a general lack of sensitivity to glucose metabolism in these animals. Glucose metabolism was apparently inhibited by 2DG in obese rats, to a sufficient extent to cause a decrease in body temperature, which suggests that the lack of physiological effects of 2DG in the obese rat was not due to an inability of the drug to alter cellular glucose metabolism in these animals. The apparent depression of insulin levels in the obese rat could be due to an imbalance in the autonomic responses to 2DG. Both Rothwell et al., (1981) and Shiraishi and Mager, (1980b), found their 2DG induced decreases in metabolic rate and rectal temperature respectively were blocked by atropine injection or cervical vagotomy, suggesting a vagal involvement in these responses. If the parasympathetic nervous system was activated by 2DG, this might be expected to cause a mild hyperinsulinaemia. However, other effects of 2DG acting through the SNS, specifically adrenaline release from the adrenal medulla, would result in an α-adrenergic inhibition of insulin release (Storlein et al., 1985). The reduced hyperglycaemia and

absence of free fatty acid release in obese animals could be accounted for by an impaired sympathetic drive to the liver and white adipose tissue. However, an increase in adrenaline release from the adrenal medulla stimulating α-adrenergic mechanisms in WAT (in the presence of a reduced SNS activation of WAT lipolysis) might also account for the absence of free fatty acid release in these animals.

Corticosterone release after 2DG is thought to be due to a 2DG-stimulated ACTH secretion from the pituitary gland (Weidenfeld et al., 1984). Lean Zucker rats maintain elevated corticosterone levels throughout the experimental period, but obese animals show a delayed and attenuated increase in corticosterone levels in response to 2DG. ACTH elicits corticosterone secretory responses in obese Zucker rats (Yukimura et al., 1978), so it is possible that ACTH release in response to central 2DG actions is impaired in obese rats.

The failure of 2DG to affect food intake in obese animals suggests that these animals are unresponsive to glucosensitive inputs involved in the control of food intake (Ikeda et al., 1980), however, whether the site of action of 2DG was peripherally or centrally located was impossible to ascertain from this study. Infusions of 2DG into the hepatic portal system can elicit feeding responses in conscious animals almost immediately (Novin and VanderWeele, 1977), as can intraperitoneally injected 2DG (Smith et al., 1972). 2DG administered centrally at very low doses (3-6mg per 200g rat) which have no effects when peripherally injected, also increase food intake (Miselis and Epstein, 1975; Ikeda et al., 1980). It is possible, therefore, that the feeding responses elicited in lean rats in this study were caused by 2DG acting at both peripheral and central sites.

The attenuation of the BAT thermogenic response to 2DG in lean animals given free access to food could simply be due to increased availability of dietary glucose competing more effectively with 2DG to inhibit the analogue's uptake at

both the intestine-blood and blood-tissue levels. The smaller depression of rectal temperature which occurred in fed animals treated with 2DG could be explained as a direct result of an attenuated depression of GDP binding in BAT, and from the contribution of obligatory heat production due to the maintenance (and elevation) of food intake and absorption.

The observation that noradrenaline could fully stimulate BAT mitochondrial GDP binding in 2DG treated lean and obese rats seems to indicate that 2DG is not acting to inhibit BAT metabolism at the tissue level, by inhibiting the utilisation of glucose as a potential thermogenic substrate. It appears that the maximal capacity for noradrenaline stimulated BAT mitochondrial GDP binding still exists in 2DG treated animals without the capacity to utilise glucose. This suggests that the inhibitory effects of 2DG on BAT mitochondrial GDP binding may be mediated at a central site. The recent observation that 2DG reduces blood flow through BAT in animals housed at normal temperatures supports the suggestion that a centrally mediated inhibition of BAT thermogenesis occurs in response to 2DG (Girardier and Benzi, 1986).

In summary, the results presented in this section demonstrate that 2DG inhibits BAT mitochondrial GDP binding in lean, but not obese, Zucker rats and that the inhibition of BAT function does not appear to be a direct effect of 2DG at the tissue level. The varied physiological effects of 2DG that were measured were attenuated or absent in the obese rat, suggesting that the obese Zucker rat lacks sensitivity to many of the 2DG induced effects of glucoprivation.

Section 3.2 The Effects of 2DG on the BAT Thermogenic Responses to Cold and Diet in Lean and Obese Zucker Rats.

The results presented in Section 3.1 showed that BAT of lean and obese Zucker rats responds differently to 2DG administration, and that the effect is not mediated at the tissue level. It is known that young obese rats can respond normally to cold acclimation (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983) with an increase in BAT thermogenic function, but not to dietary stimuli (Holt et al., 1983; Marchington et al., 1983). The experiments presented in this section were designed to investigate the effects of 2DG as a glucoprivic agent, on the responses of BAT to physiological thermogenic stimuli - cold and diet - to determine any differences in responsiveness in lean and obese rats.

3.2.1 The Effect of 2DG on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Acclimated to 4°C.

Lean and obese Zucker rats were caged in pairs and maintained at 4°C, as described in Section 2.8, for 7 days. 2DG ($360mg.kg^{-1}$ i.p.) was administered in two doses 3 hours and 1 hour before sacrifice, control animals were injected with 0.2ml saline i.p. at the same times. Food was withdrawn from all animals at the beginning of the experiment. Animals were stunned and killed by cervical dislocation in the cold room. BAT mitochondria were isolated and GDP binding assays performed, as described in Sections 2.12 and 2.13 respectively.

Results.

The effects of 2DG on BAT mitochondrial GDP binding in cold acclimated rats are shown in table 3.2.1.1. 2DG treatment had no effect on GDP binding in either lean or obese rats. Cold acclimation significantly increased GDP binding in both phenotypes (approximately 4 fold and 9 fold

Table 3.2.1.1 The Effects of 2DG on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Acclimated to 4°C.

Mitochondrial [^{3}H] GDP Binding (pmols.mg protein^{-1})			
	Saline	2DG	(Warm Controls)
Lean	831 ± 59	752 ± 21	(224 ± 14)
Obese	1130 ± 44 $^{++}$	1219 ± 40^{+++}	(129 ± 10)

Values represent mean ± S.E.M. for 5 animals in each group.

++ $P < 0.01$, +++ $P < 0.002$ compared to equivalent lean group.

'Warm control' values are shown for reference only.

Animals were maintained at 4°C for 7 days and treated with 2DG as described in the text. BAT mitochondria were prepared and GDP binding assays performed as described in Sections 2.12 and 2.13 respectively.

in lean and obese rats respectively), and the cold stimulated obese mitochondrial GDP binding value was significantly greater in both saline treated and 2DG treated obese rats compared to the equivalent lean group.

3.2.2 The Effect of 2DG on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Fed with Supplementary Sucrose.

The previous section investigated the effects of 2DG on BAT thermogenic function in animals with thermogenically active BAT due to NST. This section was designed to investigate 2DG effects on BAT thermogenesis in animals with BAT activated by dietary means. The effects of 2DG on BAT mitochondrial GDP binding and rectal temperature were attenuated in free-feeding animals (section 3.1.4). 2DG glucoprivation mimics the effects of a physiological insufficiently of glucose, that is, the hungry state. Over-feeding lean rats tends to increase the level of BAT thermo-genesis in order to dissipate the excess caloric load (Holt et al., 1983), so it was of interest to determine the effects of glucoprivation in animals exhibiting DIT.

Lean and obese animals were caged individually and given ad lib. access to laboratory chow and 35% (w/v) sucrose drinking solution in place of water. This feeding regime was maintained for 24 hours or for 7 days, at the end of which animals were injected with two doses of 2DG ($360mg.kg^{-1}$) or saline (0.2ml) 3 hours and 1 hour before sacrifice. Chow was withdrawn throughout the experimental period, and sucrose solutions were replaced with water for drinking. Animals were killed by stunning and cervical dislocation, BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Food intake in lean rats was monitored over the final 24 hour period of sucrose feeding. The amount of chow eaten was measured as previously described (section 3.1.3), and sucrose intake was measured by the weight difference of the feeding bottle. Food intake was not measured in detail in obese rats, as previous work in this laboratory has shown that the increased sucrose intake in obese rats has no

effects on BAT mitochondrial GDP binding (Holt et al., 1983). The sucrose bottle in a cage housing 3 or 4 animals was weighed to ensure that some sucrose had been taken.

Results.

The energy intake of lean rats is shown in table 3.2.2.1 for the entire 24 hour feeding period, or for the final 24 hours of the 7 day period. Total energy intake was elevated in both 24 hour and 7 day fed groups due to a slight decrease in chow intake and a consistent sucrose intake. Table 3.2.2.2 shows the effects of acute (24 hour) or chronic (7 day) sucrose overfeeding on BAT of lean and obese rats. BAT mitochondrial GDP binding was elevated by both 24 hour and 7 day sucrose feeding in lean rats, although the increase appears to be somewhat larger after 7 days feeding. Obese rats' GDP binding levels were unaffected by acute sucrose feeding, but appeared to be significantly depressed after 7 days access to sucrose. Other parameters of BAT measured were largely unaffected by sucrose feeding; depot size was slightly increased in obese rats, but protein content was unaffected. Lean rats interscapular BAT depot size was not significantly affected by either acute or chronic sucrose feeding, but depot protein was slightly increased after 7 days of elevated energy intake. 2DG had no effect on either of these factors in either phenotype. Mitochondrial GDP binding was not affected by 2DG treatment in obese rats, but was significantly reduced in the 7 day sucrose fed lean group. The magnitude of the reduction in binding was similar to that seen in control animals, (37% and 38% for control and 7 day sucrose fed animals respectively), although the basal level remained elevated. In contrast, no effect of 2DG on BAT binding was seen in acutely sucrose fed lean animals. It would appear from these results that 2DG is capable of inhibiting the elevated levels of BAT mitochondrial GDP binding associated with diet, but only on prolonged dietary stimulation. To confirm the effects seen on 7 day sucrose feeding, the effects of 2DG on BAT of animals fed a high fat diet for 10 days, were investigated.

Table 3.2.2.1 The Effect of Sucrose Feeding on Energy Intake in Lean Zucker Rats.

ENERGY INTAKE (kJ/24 hours)	CHOW	SUCROSE	TOTAL
Chow-fed	118 ± 9	-	118 ± 9
24 Hour Sucrose Fed	98 ± 11	76 ± 12	174 ± 6**
7 day Sucrose Fed	86 ± 11	82 ± 13	170 ± 6**

35% (w/v) sucrose was supplied instead of drinking water and in addition to chow.
Food intake was measured over the last 24 hour period in the case of 7 day fed animals.

Values represent mean ± S.E.M. of 4 animals per group.
** < P 0.01 compared to chow fed group.

Table 3.2.2.2 The Effects of 2DG on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Fed With Supplementary Sucrose.

		CONTROL		24 HOUR SUCROSE		7 DAY SUCROSE	
		SALINE	2DG	SALINE	2DG	SALINE	2DG
[^{3}H] GDP Binding (pmols.mg protein^{-1})	L	247 ± 9	156 ± 10***	316 ± 20^{++}	307 ± 24^{+++}	476 ± 33^{+++}	297 ± 21^{***+++}
	O	167 ± 11	155 ± 19	198 ± 10	209 ± 18	102 ± 14^{++}	150 ± 14
Interscapular BAT depot wet weight (g)	L	0.20 ± 0.02	0.20 ± 0.02	0.16 ± 0.01	0.17 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
	O	0.47 ± 0.02	0.41 ± 0.02	0.54 ± 0.02^{+}	0.53 ± 0.07	0.49 ± 0.05	0.54 ± 0.04^{+}
Interscapular BAT depot protein content (mg)	L	23.4 ± 1.2	24.5 ± 1.4	21.7 ± 0.6	23.0 ± 1.5	32.8 ± 3.3^{+}	31.4 ± 3.6
	O	20.6 ± 0.8	19.4 ± 1.2	23.2 ± 1.0	23.3 ± 2.1	16.1 ± 3.6	16.5 ± 3.3
n	L	8	6	7	6	4	4
	O	8	6	7	7	4	4

Values represent means ± S.E.M. of n animals per group.

L - Lean animals O - Obese animals.

*** P<0.002 compared to saline injected animals on the same feeding regime and of the same phenotype.

+ P<0.05, ++ P<0.01, +++ P<0.002 compared to chow fed animals of the same phenotype and treatment group.

BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Depot protein content was measured as described in section 2.10.2.

3.2.3 The Effect of 2DG on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Maintained on a High Fat Diet.

Lean and obese Zucker rats were housed in pairs and given ad lib. access to the high fat semisynthetic diet described in section 2.4 for 10 days. Food intake was measured over the final 24 hour period for individual animals. After 10 days on the high fat diet, animals were injected with two doses of 2DG ($360mg.kg^{-1}$) 3 hours and 1 hour before sacrifice. Control animals received saline injections (0.2ml) at the same time as 2DG-injected animals. Food was withdrawn from all animals at the beginning of the experimental period. Animals were stunned and killed by cervical dislocation. Interscapular BAT was removed and mitochondria prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

Results.

The control data shown in table 3.2.3.1 is the same as that in table 3.2.2.2 and is repeated for convenience. Table 3.2.3.1 shows the energy intake and the responses of BAT to 2DG in high fat fed animals. Energy intake was significantly increased in both lean and obese rats, but depot protein content was not significantly affected by high fat feeding, so the increase in BAT mass was probably due to excess lipid deposition. BAT total protein content was increased in lean animals by high fat feeding, but depot weight was unaffected. BAT mitochondrial GDP binding was neither increased by diet nor decreased by 2DG in obese animals, but lean GDP binding levels were elevated by high fat feeding and reduced by 2DG (25% reduction). These results are similar to those obtained in the previous section for chronically sucrose-fed animals.

Table 3.2.3.1 The Effects of 2DG on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats Maintained on a High Fat Diet.

Animals were maintained on a high fat diet (see section 2.4) for 10 days. Food intake was measured over the last 24 hour period. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Depot protein content was measured as described in section 2.10.2.

Values represent means $\pm$ S.E.M. of n animals per group.

* $P < 0.05$, *** $P < 0.002$ compared to saline treated group on the same diet and of the same phenotype.

++ $P < 0.01$, +++ $P < 0.002$ compared to chow fed control animals in the equivalent treatment group of the same phenotype.

Table 3.2.3.1

		CONTROL		HIGH FAT	
		SALINE	2DG	SALINE	2DG
[^{3}H] GDP Binding (pmols.mg protein^{-1})	LEAN	247 ± 9	156 ± 10***	321 ± 23^{++}	239 ± 16^{*+++}
	OBESE	167 ± 11	155 ± 19	135 ± 20	135 ± 6
Interscapular BAT depot wet weight (g)	LEAN	0.20 ± 0.02	0.20 ± 0.02	0.26 ± 0.04	0.28 ± 0.05
	OBESE	0.47 ± 0.02	0.41 ± 0.02	0.69 ± 0.05^{+++}	0.68 ± 0.05^{+++}
Interscapular BAT depot protein content (mg)	LEAN	23.4 ± 1.2	24.5 ± 1.4	42.9 ± 0.6^{+++}	48.2 ± 6.2^{++}
	OBESE	20.6 ± 0.8	19.4 ± 1.2	28.5 ± 0.6	23.3 ± 2.1
n	LEAN	8	6	10	10
	OBESE	8	6	6	6
Energy intake (kJ/24 hours)	LEAN	118 ± 9		204 ± 9^{+++}	
	OBESE	173 ± 11		279 ± 12^{+++}	

3.2.4 The Effect of Age on the Response of BAT Mitochondrial GDP Binding to 2DG Treatment in Intact and Adrenalectomised Lean Zucker Rats.

It has been demonstrated that the capacity of normal animals to resist dietary-induced obesity declines with age (Rothwell et al., 1984b), and can be restored by adrenalectomy. It was, therefore, of interest to investigate the effects of 2DG, as an inhibitor of dietary signals, in adult animals. Two groups of lean animals were taken at 3 and 6 months of age, half of each group was adrenalectomised as described in section 2.6.2 and the responses of BAT to 2DG administration investigated. Animals received two doses of 2DG ($360mg.kg.^{-1}$) in a 0.3ml volume of saline 3 hours and 1 hour before sacrifice. Control animals received two 0.3ml saline injections at the same times. The larger volume was required due to the size of the animals. Animals were stunned and killed by cervical dislocation, BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

Results.

Table 3.2.4.1 summarises the effects of 2DG in 3 and 6 month old rats. BAT mitochondrial GDP binding was inhibited in 3 month old rats by 45% in intact animals and by 42% in adrenalectomised rats, with no significant effects of adrenalectomy on either control or 2DG inhibited binding levels. Similar effects were found in 6 month old animals with reductions of 40% in intact and 41% in adrenalectomised groups' GDP binding levels after 2DG. Again, no effects of adrenalectomy on basal or inhibited levels were apparent.

These results are very similar to those obtained with 5-6 week old animals. BAT mitochondrial GDP binding in intact lean rats falls from 224 $\pm$ 14 pmols per mg protein to 148 $\pm$ 13 pmols per mg after 2DG treatment (34% reduction) and GDP binding drops from 261 $\pm$ 35 pmols per mg protein to 161 $\pm$ 19 pmols per mg protein (38% reduction) in young adrenalectomised lean rats.

Table 3.2.4.1 The Effects of Age on the Response of BAT Mitochondrial GDP Binding to 2DG Treatment in Intact and Adrenalectomised Lean Zucker Rats.

	AGE	INTACT		ADRENALECTOMISED.	
		SALINE	2DG	SALINE	2DG
[^{3}H] GDP Binding (pmols.mg protein^{-1})	3 months	264 ± 13	143 ± 21	254 ± 36***	147 ± 7*
	6 months	220 ± 30	131 ± 7	219 ± 14	130 ± 26*
BAT depot wet weight (g)	3 months	0.53 ± 0.04	0.50 ± 0.05	0.48 ± 0.03	0.44 ± 0.05
	6 months	0.62 ± 0.05	0.60 ± 0.06	0.48 ± 0.03	0.50 ± 0.07
Total BAT depot protein content (mg)	3 months	60.5 ± 5.9	64.1 ± 9.5	51.3 ± 4.3	56.7 ± 5.4
	6 months	48.2 ± 3.4	58.8 ± 3.7	55.1 ± 2.3	57.2 ± 1.5
BAT depot mitochondrial protein (mg)	3 months	14.8 ± 1.5	14.1 ± 2.8	14.8 ± 1.4	16.2 ± 0.9
	6 months	20.1 ± 1.8	19.2 ± 3.4	26.0 ± 1.7	23.8 ± 1.8

Values represent mean ± S.E.M. of 4 animals per group.

* $P < 0.05$, *** $P < 0.002$ compared to saline injected animals of the same group.

BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Total depot protein content was measured as described in section 2.10.2. Mitochondrial protein content was calculated from succinate cytochrome c oxidoreductase activities, as described in section 2.14.

These results seem to indicate that no change in response to this dose of 2DG occurred with age, or was improved by adrenalectomy in older animals. However it must be remembered from section 3.1.1 that the dose of $360mg.kg^{-1}$ body weight was not the minimal dose required for a significant inhibition of BAT thermogenesis in lean rats and it may be that lower doses which elicit a response in younger animals have no effect on older rats.

3.2.5 Discussion.

2DG treatment had no inhibitory effects on BAT mitochondrial GDP binding in cold acclimated animals of either phenotype. In animals with BAT GDP binding elevated by chronic dietary means (7 day sucrose or 10 day high fat feeding), 2DG treatment significantly reduced GDP binding levels in lean rats. GDP binding levels were not elevated by diet or inhibited by 2DG in obese animals. These results suggest that 2DG preferentially inhibits DIT in lean animals and has no effect on NST in either phenotype. This would be consistent with the role for 2DG as an inhibitor of dietary signals. The increase in BAT mitochondrial binding in young obese rats in response to cold is normal (Holt et al., 1983), a finding that has been confirmed in the present study. The failure of 2DG to affect cold-stimulated GDP binding suggests that the effects of 2DG are not due to a general inhibition of SNS activity and implies a central site of action for its effects on BAT. The failure of 2DG to inhibit BAT mitochondrial GDP binding in acutely sucrose fed animals may possibly be due to a powerful initial sympathetic stimulation of BAT which cannot be overridden by acute dietary signals, such as that provided by 2DG. As over-feeding persists, the sensitivity of the controlling system could adapt to the level of dietary stimulation and acute dietary signals, such as glucoprivation, might again be able to influence its outputs. Support for this suggestion is, as yet, minimal. Chronic cold acclimation provides another powerful stimulus to BAT which cannot be overriden by 2DG. There may be humoral factors involved in the acute response which exert direct effects on BAT. Feeding

elicits increases in corticosterone secretion and also presumably, ACTH (Brindley et al., 1979). ACTH is known to have a direct stimulatory effect on BAT mitochondrial GDP binding (York and Al-Baker, 1984) which does not involve activation of the SNS (Marchington, 1985). Investigations of the involvement of the SNS in the response of BAT to acute and chronic sucrose overfeeding are reported in section 3.4, at which point the difference in the ability of 2DG to inhibit BAT mitochondrial GDP binding with the duration of sucrose feeding will be discussed further.

The 2DG-induced inhibition of BAT mitochondrial GDP binding which occurs in young lean animals does not appear to diminish with age, unlike the responses to overfeeding (York et al., 1984), and is not increased by adrenalectomy, as is the response to cafeteria feeding in older rats (Rothwell et al., 1984b). The dose of 2DG used ($360mg.kg^{-1}$) was not the minimum dose required to elicit a significant inhibition of BAT GDP binding in young lean animals. It could be that the sensitivity to dietary stimuli which appears to reduce with age would result in a reduced sensitivity of older rats to 2DG at minimal doses which produce a response in younger rats, however, such an effect remains to be demonstrated.

Section 3.3 The Effect of Adrenal Status on the Responses to 2DG in Lean and Obese Zucker Rats.

Adrenalectomy is known to correct the thermogenic defects of the obese Zucker rat, including the ability to respond to dietary stimuli by increasing BAT mitochondrial GDP binding (Holt et al., 1983). The effects of adrenalectomy on BAT thermogenesis are thought to be due to the removal of corticosterone, rather than an increase in circulating ACTH concentration . Corticosterone replacement to adrenalectomised obese Zucker rats restores the defective BAT thermogenesis and noradrenaline turnover rate, body weight gain and deranged feeding patterns (Freedman et al., 1985, 1986a; York et al., 1985a, b), suggesting that corticosterone is required for the expression of the genetic defect in fa/fa rats. Hypophysectomy, which removes ACTH and thus decreases corticosterone secretion, improves body weight gain in obese Zucker rats (Powley and Morton, 1976). It has been demonstrated in Sprague-Dawley rats that BAT mitochondrial GDP binding is increased after hypophysectomy but that ACTH treatment reduced BAT GDP binding levels due to the increase in circulating corticosterone concentrations (Rothwell and Stock, 1985a). ACTH treatment did not depress BAT mitochondrial GDP binding completely down to control levels, there was still a slight stimulatory component which may have been due to the effects of centrally released CRF. It has recently been demonstrated that CRF administered centrally can stimulate BAT mitochondrial GDP binding (Arase, York and Bray; unpublished observations). It has been suggested, as corticosterone is required in permissive amounts for the survival of cold exposure (Deavers and Mussachia, 1979; Fellenz et al., 1982), that corticosterone inhibits only the diet-related sympathetic activation of BAT in the fa/fa rat. The results of the previous section suggest that 2DG also preferentially inhibits diet-related BAT function.

This series of experiments was devised to investigate the role of the pituitary-adrenal axis in the 2DG-mediated inhibition of BAT function.

3.3.1 The Effects of Adrenal Status on the Response of BAT Mitochondrial GDP Binding and Serum Metabolites to 2DG in Lean and Obese Zucker Rats.

On the experimental day, animals received two doses of 360mg.kg^{-1} body weight 2DG in 0.2ml saline, i.p., 3 hours and 1 hour before sacrifice. Control animals received injections of 0.2ml saline, i.p., at the same times as the experimental animals. Food was withdrawn from all animals at the beginning of the experimental period. Animals were killed by decapitation, trunk blood was collected and serum prepared as described in section 2.9.1. Serum concentrations of glucose, free fatty acids, insulin and corticosterone were determined as described in sections 2.9.2 to 2.9.5. Upon sacrifice, BAT mitochondria were prepared and GDP binding assays performed, as described in sections 2.12 and 2.13 respectively. Protein concentrations were determined as outlined in section 2.10.1 or 2.10.2, and succinate cytochrome c oxidoreductase activities were measured as described in section 2.14.

5 groups of animals were taken through the above protocol:

(1) Control (intact) lean and obese rats.

(2) Lean and obese rats adrenalectomised and maintained as described in section 2.6.2. Animals were used 6-7 days after surgery and success of adrenalectomy was verified visually on sacrifice, and by the presence of undetectable serum corticosterone.

(3) Lean and obese rats injected with 1mg.100g^{-1} body weight corticosterone per day, as described in section 2.5.2, for 7 days.

(4) Lean and obese rats injected with 50µg.100g^{-1} body weight ACTH as described in section 2.5.1 for 24 hours.

(5) Lean and obese rats adrenalectomised and maintained as described in section 2.6.2, injected with 1mg.100g^{-1} body weight corticosterone (section 2.5.2) daily, for 7 days.

Results.

a. Brown Adipose Tissue.

Tables 3.3.1.1 to 3.3.1.5 show the detailed data of the effects of 2DG on BAT from these experiments. Of the parameters measured, 2DG only affected BAT mitochondrial GDP binding, however, the manipulation of adrenal status had independent effects which will be summarised here. Tissue wet weight was decreased by adrenalectomy in lean and obese animals, but tissue protein content was only depressed in the lean group. Succinate cytochrome c oxidoreductase activity per depot was increased in obese, and decreased in lean, adrenalectomised rats. Corticosterone treatment reduced BAT depot protein content in obese rats, succinate cytochrome c oxidoreductase activity in lean rats and had no other effects on BAT composition. The composition of BAT was largely unaffected by ACTH treatment, although mitochondrial population (succinate cytochrome c oxidoreductase activity) was increased in obese animals. Adrenalectomy with corticosterone replacement had little effect on BAT in lean or obese animals compared to intact rats. In lean animals total depot protein content and mitochondrial population were both depressed by corticosterone replacement. Obese adrenalectomised corticosterone-replaced animals showed all the BAT characteristics (of the parameters measured) of intact obese animals. The effects of adrenalectomy (decreased BAT weight, increased mitochondrial and depot GDP binding and increased succinate cytochrome c oxidoreductase activities) were all abolished in corticosterone replaced obese rats.

Figure 3.3.1.1 summarises the changes that occurred in BAT mitochondrial GDP binding in response to 2DG in varied conditions of adrenal function. The changes that occurred in total BAT depot GDP binding were similar, although they did not always attain statistical significance. The open bars represent the 'control' BAT GDP binding value for each experimental condition. Basal GDP binding levels were decreased in corticosterone treated lean rats, and increased after ACTH treatment. Basal BAT mitochondrial GDP binding was also depressed by corticosterone treatment in

Table 3.3.1.1 The Effects of 2DG on BAT of Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
BAT Depot Weight (g)	0.27 ± 0.01	0.25 ± 0.02	$0.53 \pm 0.03^{+++}$	$0.45 \pm 0.04^{+++}$
BAT Depot Protein Content (mg)	28.6 ± 1.3	28.2 ± 1.3	29.6 ± 2.1	26.7 ± 1.5
BAT Depot Succ.cyt.c O-R activity (μmols.min^{-1}. depot^{-1})	4.38 ± 0.53	5.18 ± 0.73	$1.98 \pm 0.2^{+++}$	$2.55 \pm 0.22^{++}$
BAT [^{3}H]GDP binding (pmols.mg protein^{-1})	224 ± 14	$148 \pm 13^{***}$	$129 \pm 10^{+++}$	128 ± 7
BAT Depot [^{3}H]GDP binding (pmols.depot^{-1})	2608 ± 537	$1083 \pm 409^{*}$	$1130 \pm 313^{+}$	1303 ± 237
n	8	8	6	8

Values represent means $\pm$ S.E.M. of n animals in each group.

* $P < 0.05$, *** $P < 0.002$ compared to saline injected group of the same phenotype.

+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to the equivalent lean group.

Animals were injected with 2 doses of 2DG (360 mg.kg^{-1}), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. After sacrifice BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were measured as described in section 2.10.1 or 2.10.2. Depot GDP binding was calculated on the basis of 100% mitochondrial recovery as assessed by succinate cytochrome c oxidoreductase (succ.cyt c O-R) activities, assayed as described in section 2.14.

Table 3.3.1.2 The Effects of 2DG on BAT of Adrenalectomised Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
BAT Depot Weight (g)	0.13 ± 0.02	0.14 ± 0.01	$0.29 \pm 0.02^{+++}$	$0.28 \pm 0.03^{+++}$
BAT Depot Protein Content (mg)	18.5 ± 1.3	20.2 ± 1.4	$30.0 \pm 1.6^{+++}$	$28.5 \pm 1.7^{++}$
BAT Depot succ.cyt.c O-R activity (μmols.min^{-1} depot^{-1})	3.31 ± 0.32	3.71 ± 0.36	3.51 ± 0.48	3.80 ± 0.50
BAT [^{3}H]GDP binding (pmols.mg protein^{-1})	261 ± 35	$161 \pm 19^{*}$	247 ± 18	$177 \pm 13^{**}$
BAT Depot [^{3}H]GDP binding (pmols.depot^{-1})	1569 ± 218	1173 ± 118	$2561 \pm 367^{+}$	$1783 \pm 245^{+}$
n	5	4	12	12

Values represent means $\pm$ S.E.M. of n animals in each group.

* $P < 0.05$, ** $P < 0.01$ compared to control group of same phenotype.

\+ $P < 0.05$, +++ $P < 0.002$ compared to equivalent lean group.

Animals were injected with 2 doses of 2DG (360mg.kg^{-1}), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. After sacrifice BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were measured as described in section 2.10.1 or 2.10.2. Depot GDP binding was calculated on the basis of 100% mitochondrial recovery as assessed by succinate cytochrome c oxidoreductase (succ.cyt c O-R) activities, assayed as described in section 2.14.

Table 3.3.1.3 The Effects of 2DG on BAT of Corticosterone Treated Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
BAT Depot Weight (g)	0.28 ± 0.02	0.23 ± 0.02	0.47 ± 0.02^{+++}	0.38 ± 0.05^{+}
BAT Depot Protein Content (mg)	28.0 ± 2.3	26.5 ± 2.0	19.2 ± 1.9^{++}	18.5 ± 1.6^{++}
BAT Depot Suc.cyt.c O-R activity (μmols.min^{-1}.depot^{-1})	2.35 ± 0.46	2.30 ± 0.42	1.51 ± 0.29	2.11 ± 0.49
BAT [^{3}H]GDP binding (pmols.mg protein^{-1})	159 ± 17	136 ± 7	95 ± 8^{++}	104 ± 7^{++}
BAT Depot [^{3}H]GDP binding (pmols.depot^{-1})	1456 ± 360	1030 ± 196	745 ± 11	1083 ± 301
n	12	12	7	8

Values represent means ± S.E.M. of n animals in each group.

No 2DG dependent differences.

+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to equivalent lean group.

Animals were injected with 2 doses of 2DG (360mg.kg^{-1}), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. After sacrifice BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were measured as described in section 2.10.1 or 2.10.2. Depot GDP binding was calculated on the basis of 100% mitochondrial recovery as assessed by succinate cytochrome c oxidoreductase (succ.cyt.c O-R) activities, assayed as described in section 2.14.

Table 3.3.1.4 The Effects of 2DG on BAT of ACTH Treated Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
BAT Depot Weight (g)	0.30 ± 0.01	0.29 ± 0.03	0.51 ± 0.05^{+++}	0.43 ± 0.03^{++}
BAT Depot Protein Content (mg)	31.1 ± 1.4	32.7 ± 2.5	33.4 ± 2.6	27.4 ± 2.7
BAT Depot Succ.cyt.c O-R activity (μmols.min^{-1}.depot^{-1})	3.58 ± 0.35	4.08 ± 0.35	4.07 ± 0.32	4.41 ± 0.50
BAT [^{3}H]GDP binding (pmols.mg protein^{-1})	290 ± 20	211 ± 11**	221 ± 10^{++}	284 ± 17^{++**}
BAT Depot [^{3}H]GDP binding (pmols.depot^{-1})	3399 ± 412	2230 ± 266*	2464 ± 177	2622 ± 347
n	8	8	8	8

Values represent means ± S.E.M. of n animals in each group.

* $P < 0.05$, ** $P < 0.01$ compared to control group of same phenotype.

++ $P < 0.01$, +++ $P < 0.002$ compared to equivalent lean group.

Animals were injected with 2 doses of 2DG (360mg.kg^{-1}), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. After sacrifice BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were measured as described in section 2.10.1 or 2.10.1. Depot GDP binding was calculated on the basis of 100% mitochondrial recovery as assessed by succinate cytochrome c oxidoreductase (succ.cyt c O-R) activities, assayed as described in section 2.14.

Table 3.3.1.5 The Effects of 2DG on BAT of Adrenalectomised Corticosterone-Replaced Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
BAT Depot Weight (g)	0.20 ± 0.01	0.19 ± 0.01	0.46 ± 0.03^{+++}	0.41 ± 0.02^{+++}
BAT Depot Protein Content (mg)	22.4 ± 1.4	23.1 ± 11.9	26.2 ± 1.3	26.9 ± 1.6
BAT Depot succ.cyt.c O-R activity (μmols.min^{-1}.depot^{-1})	1.58 ± 0.13	1.67 ± 0.05	2.20 ± 0.55	2.25 ± 0.43
BAT [^{3}H]GDP binding (pmols.mg.protein^{-1})	199 ± 17	164 ± 11	144 ± 11^{+}	117 ± 11^{+}
BAT Depot [^{3}H]GDP binding (pmols.depot^{-1})	1999 ± 180	1605 ± 185	914 ± 159	1251 ± 159
n	4	4	8	8

Values represent means ± S.E.M. of n animals in each group.

No 2DG dependent differences.

+ $P < 0.05$, +++ $P < 0.002$ compared to equivalent group.

Animals were injected with 2 doses of 2DG (360mg.kg^{-1}), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. After sacrifice BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were measured as described in section 2.10.1 or 2.10.2. Depot GDP binding was calculated on the basis of 100% mitochondrial recovery as assessed by succinate cytochrome c oxidoreductase (succ.cyt.c O-R) activities, assayed as described in section 2.14.

Figure 3.3.1.1 The Effect of Adrenal Status on the Response of BAT Mitochondrial GDP Binding to 2DG in Lean and Obese Zucker Rats.

Animals were injected with 2 doses of 2DG ($360mg.kg^{-1}$), i.p., in 0.2ml saline, or 0.2ml saline, 3 hours and 1 hour before sacrifice. At sacrifice, BAT mitochondria were prepared and GDP binding assays performed, as described in sections 2.12 and 2.13 respectively.

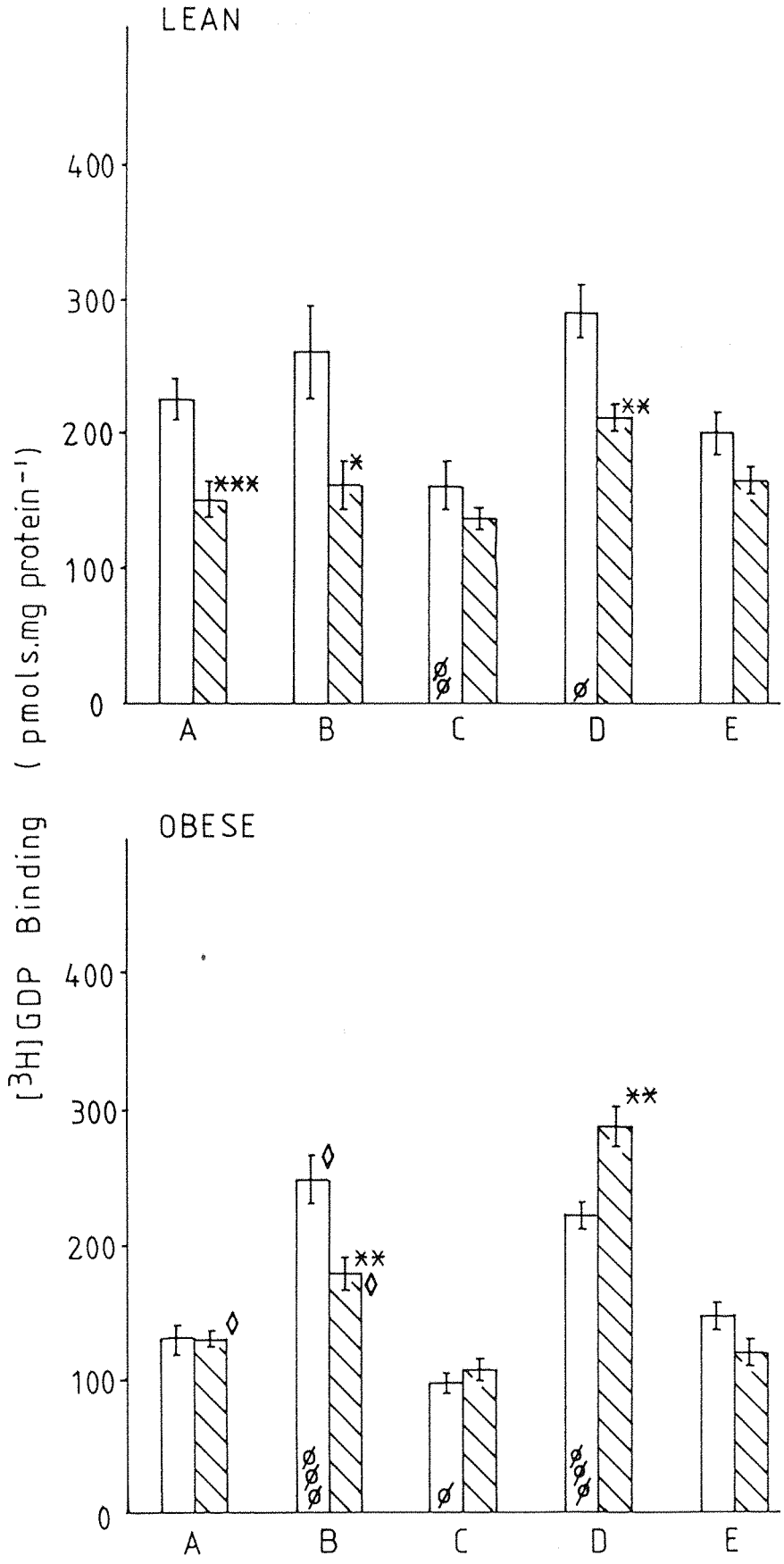
The experimental groups are as follows:-

□ saline treated ▨ 2DG treated

A. Control (intact) animals.
B. Adrenalectomised animals (surgery was performed and animals maintained as described in section 2.6.2).
C. Corticosterone treated animals (injected with $1mg.100g^{-1}$ body weight daily, as described in section 2.5.2).
D. ACTH treated animals (injected with $50\mu g.100g^{-1}$ body weight 24 hours and 1 hour before sacrifice, as described in section 2.5.1).
E. Adrenalectomised animals treated with $1mg.100g^{-1}$ body weight corticosterone daily (see section 2.6.2 for surgical procedure and maintenance, and 2.5.2 for details of corticosterone injection).

Upper diagram, lean animals; lower diagram, obese animals. Bars represent means $\pm$ S.E.M. of 4-12 animals per group. ϕ $P < 0.05$, ϕϕ $P < 0.01$, ϕϕϕ $P < 0.002$ compared to control (intact) animals of the same phenotype.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$ compared to saline injected animals of the same phenotype and the same adrenal status.

◊ denotes not significantly different from equivalent lean group. All other cases were significantly lower than the equivalent lean group except ACTH treated 2DG injected obese rats (D ▨; higher).


LEAN
OBESE
[3H]GDP Binding (pmols.mg protein-1)
400
300
200
100
0
A
B
C
D
E
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obese rats, but both adrenalectomy and ACTH treatment stimulated basal BAT GDP binding. The replacement of corticosterone to obese adrenalectomised rats depressed GDP binding levels to control values. The effects of 2DG are illustrated by the cross-hatched bars. BAT mitochondrial GDP binding was significantly depressed by 2DG in lean control rats, lean and obese adrenalectomised rats and in lean ACTH treated rats. 2DG stimulated BAT GDP binding in obese ACTH treated rats. Corticosterone replacement to adrenalectomised rats not only abolished the effects of adrenalectomy on basal BAT GDP binding in obese rats, but abolished the effects of 2DG in both phenotypes. The significant inhibition of basal BAT mitochondrial GDP binding in lean rats by corticosterone apparently abolished the effects of 2DG in lean animals. The effects of corticosterone and 2DG in lean rats did not appear to be additive, but it could be that either corticosterone or 2DG alone, at the doses used, ($10mg.kg^{-1}$ and $360mg.kg^{-1}$ respectively) was sufficient to maximally suppress GDP binding in lean rats.

b. Serum Measurements.

Tables 3.3.1.6 to 3.3.1.10 show details of changes in serum hormones and metabolites which occurred on adrenal changes and after 2DG. The changes due to altered adrenal function were as follows: Adrenalectomy reduced serum glucose levels in both phenotypes. Free fatty levels were increased in obese rats and the normally elevated serum insulin levels of obese animals were reduced towards lean levels. Corticosterone treatment raised serum glucose levels in both phenotypes but had no other effects on the parameters measured. ACTH treatment increased serum levels of every measured factor, except glucose in lean animals. Corticosterone replacement to adrenalectomised animals led to an increase in serum insulin in lean rats and a lower level of serum corticosterone in obese rats.

2DG had no effects on serum free fatty acid or insulin levels under any of the experimental conditions. Serum glucose

Table 3.3.1.6 The Effects of 2DG on Serum Metabolite and Hormone Concentrations in Lean and Obese Zucker Rats.

SERUM	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
Glucose ($mg.100ml^{-1}$)	118 ± 2	199 ± 7***	122 ± 1	213 ± 22***
Free Fatty Acids (μM)	552 ± 65	600 ± 52	446 ± 31	425 ± 33+
Insulin ($ng.ml^{-1}$)	0.82 ± 0.13	1.05 ± 0.31	4.12 ± 0.96+	4.05 ± 1.16+
Corticosterone ($ng.ml^{-1}$)	177 ± 38	387 ± 36*	273 ± 6	351 ± 21*
n	4	4	4	4

Values represent means ± S.E.M. of n animals in each group.

* $P < 0.05$, *** $P < 0.002$ compared to saline injected animals of the same phenotype.

\+ $P < 0.05$ compared to the equivalent lean group.

Animals were injected with 2 doses of 2DG ($360mg.kg^{-1}$), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. Animals were killed by decapitation, trunk blood was collected and serum prepared as described in section 2.9.1. Serum concentrations of glucose, free fatty acids, insulin and corticosterone were determined as described in sections 2.9.2, 2.9.3, 2.9.4 and 2.9.5, respectively.

Table 3.3.1.7 The Effects of 2DG on Serum Metabolite and Hormone Concentrations in Adrenalectomised Lean and Obese Zucker Rats.

SERUM	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
Glucose ($mg.100ml^{-1}$)	89 ± 5	$129 \pm 1^{***}$	98 ± 2	$148 \pm 4^{+++***}$
Free Fatty Acids (μM)	431 ± 76	327 ± 23	$846 \pm 107^{++}$	$740 \pm 94^{+++}$
Insulin ($ng.ml^{-1}$)	1.00 ± 0.16	0.92 ± 0.12	1.18 ± 0.16	1.15 ± 0.12
Corticosterone ($ng.ml^{-1}$)	n/d	n/d	n/d	n/d
n	5	4	12	8

Values represent means $\pm$ S.E.M. of n animals in each group.

*** $P < 0.002$ compared to saline injected animals of the same phenotype.

++ $P < 0.01$, +++ $P < 0.002$ compared to the equivalent lean group.

Animals were injected with 2 doses of 2DG ($360mg.kg^{-1}$), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. Animals were killed by decapitation, trunk blood was collected and serum prepared as described in section 2.9.1. Serum concentrations of glucose, free fatty acids, insulin and corticosterone were determined as described in sections 2.9.2, 2.9.3, 2.9.4 and 2.9.5, respectively.

Table 3.3.1.8 The Effects of 2DG on Serum Metabolite and Hormone concentrations in Corticosterone Treated Lean and Obese Zucker Rats.

SERUM	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
Glucose ($mg.100ml^{-1}$)	146 ± 5	$261 \pm 11^{***}$	143 ± 4	$220 \pm 15^{+***}$
Free Fatty Acids (μM)	508 ± 70	475 ± 57	542 ± 86	$730 \pm 97^{+}$
Insulin ($ng.ml^{-1}$)	0.92 ± 0.19	0.96 ± 0.16	$3.84 \pm 0.61^{+++}$	$5.28 \pm 0.84^{+++}$
Corticosterone ($ng.ml^{-1}$)	240 ± 28	264 ± 17	189 ± 34	239 ± 19
n	12	12	8	8

Values represent means $\pm$ S.E.M. of n animals in each group.

*** $P < 0.002$ compared to saline injected animals of the same phenotype.

+ $P < 0.05$, +++ $P < 0.002$ compared to the equivalent lean group.

Animals were injected with 2 doses of 2DG ($360mg.kg^{-1}$), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. Animals were killed by decapitation, trunk blood was collected and serum prepared as described in section 2.9.1. Serum concentrations of glucose, free fatty acids, insulin and corticosterone were determined as described in sections 2.9.2, 2.9.3, 2.9.4 and 2.9.5 respectively.

Table 3.3.1.9 The Effects of 2DG on Serum Metabolite and Hormone Concentrations in ACTH Treated Lean and Obese Zucker Rats.

SERUM	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
Glucose ($mg.100^{-1}$)	108 ± 4	$181 \pm 17^{***}$	$155 \pm 6^{+++}$	165 ± 5
Free Fatty Acids (μM)	1176 ± 180	864 ± 91	1060 ± 85	$1239 \pm 116^{+}$
Insulin ($ng.ml^{-1}$)	4.53 ± 0.93	4.70 ± 0.97		
Corticosterone ($ng.ml^{-1}$)	335 ± 33	288 ± 29		
n	8	8	4	4

Values represent means $\pm$ S.E.M. of n animals in each group.

*** $P < 0.002$ compared to saline injected animals of the same phenotype.

+ $P < 0.05$, +++ $P < 0.002$ compared to the equivalent lean group.

Animals were injected with 2 doses of 2DG ($360mg.kg^{-1}$), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. Animals were killed by decapitation, trunk blood was collected and serum prepared as described in section 2.9.1. Serum concentrations of glucose, free fatty acids, insulin and corticosterone were determined as described in sections 2.9.2, 2.9.3, 2.9.4 and 2.9.5, respectively.

Table 3.3.1.10 The Effects of 2DG on Serum Metabolite and Hormone Concentrations in Adrenalectomised Corticosterone-Replaced Lean and Obese Zucker Rats.

SERUM	LEAN		OBESE	
	SALINE	2DG	SALINE	2DG
Glucose ($mg.100ml^{-1}$)	134 ± 11	139 ± 15	112 ± 5	136 ± 4**
Free Fatty Acids (μM)	400 ± 34	473 ± 47	474 ± 60	492 ± 33
Insulin ($ng.ml^{-1}$)	2.34 ± 0.55	2.93 ± 0.61	3.60 ± 0.52	3.89 ± 0.77
Corticosterone ($ng.ml^{-1}$)	133 ± 6	111 ± 19	109 ± 8+	135 ± 26
n	4	4	8	8

Values represent means ± S.E.M. of n animals in each group.

** $P < 0.01$ compared to saline injected animals of the same phenotype.

+ $P < 0.05$ compared to the equivalent lean group.

Animals were injected with 2 doses of 2DG ($360mg.kg^{-1}$), i.p., in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. Animals were killed by decapitation, trunk blood was collected and serum prepared as described in section 2.9.1. Serum concentrations of glucose, free fatty acids, insulin and corticosterone were determined as described in sections 2.9.2, 2.9.3, 3.9.4 and 2.9.5, respectively.

levels were elevated in most cases by 2DG administration, the exceptions being in obese, ACTH treated rats and lean corticosterone replaced-adrenalectomised rats. 2DG elevated serum corticosterone levels only in control animals of both phenotypes.

3.3.2 The Effects of Adrenalectomy on the 2DG Dependent Stimulation of Food Intake in Lean and Obese Zucker Rats.

The results from the previous section suggest that adrenalectomised obese Zucker rats respond normally to 2DG in that BAT mitochondrial GDP binding is inhibited to a similar extent as seen in lean adrenalectomised animals (38% and 28% inhibition in lean and obese adrenalectomised rats respectively). Section 3.1.3 demonstrated that, in contrast to lean animals, obese rats did not increase their food intake in response to 2DG administration. It was decided to investigate further the restorative effects of adrenalectomy on the responses to 2DG in obese rats, by examining the food intake of adrenalectomised lean and obese Zucker rats over the 3 hour period of 2DG treatment.

Lean and obese Zucker rats were adrenalectomised and maintained as described in section 2.6.2. After a 7 day recovery period animals were individually housed, and injected with 2 doses of 2DG ($360mg.kg^{-1}$) in 0.2ml saline, i.p., at zero time and after two hours. Control animals received 2 injections of 0.2ml saline at the same times as control animals. Fresh, weighed, food was placed in the cage hoppers at time zero, and food intake measured 3 hours later. Success of adrenalectomy was confirmed by the demonstration of undetectable corticosterone levels.

Results.

Table 3.3.2.1 shows the food intake of lean and obese adrenalectomised animals in response to 2DG. Food intake was increased in intact lean animals by 2DG, but obese rats' food intake was not stimulated. Adrenalectomy of lean rats did not affect 3 hour food intake in lean rats, but significantly reduced the food intake of obese rats to

Table 3.3.2.1 The Effects of Adrenalectomy on the 2DG Dependent Stimulation of Food Intake in Lean and Obese Zucker Rats.

FOOD INTAKE (g/3 HOURS)

	INTACT		ADRENALECTOMISED	
	SALINE	2DG	SALINE	2DG
LEAN	1.2 ± 0.1	$2.5 \pm 0.2^{**}$	1.4 ± 0.2	$2.5 \pm 0.3^{**}$
OBESE	$2.9 \pm 0.1^{\phi\phi\phi}$	2.9 ± 0.4	$1.5 \pm 0.3^{++}$	$2.6 \pm 0.2^{*}$

Values represent means $\pm$ S.E.M. of 4 animals in each group.

* $P < 0.05$, ** $P < 0.01$ compared to saline injected animals of the same adrenal status and phenotype.

++ $P < 0.01$ compared to intact animals in the same treatment group and of the same phenotype.

$\phi\phi\phi$ $P < 0.002$ compared to equivalent lean group.

Animals received injections at zero time and after 2 hours, of $360 m.kg^{-1}$ 2DG, i.p., in 0.2ml saline, or 0.2ml saline. Food intake was measured over the 3 hour period. 'Intact' data is repeated from table 3.1.3.1 for ease of comparison.

approximately half that of their intact counterparts, an intake equivalent to that of the lean adrenalectomised group. 2DG increased food intake in both adrenalectomised groups to a similar level, and the magnitude of the increase was similar to that seen in intact lean animals (82%, 75% and 106% increase in lean adrenalectomised, obese adrenalectomised and lean intact animals, respectively).

3.3.3 Discussion.

The alteration of adrenal status produced similar changes in BAT function and serum metabolites in this study as have been previously reported. Adrenalectomy improved BAT mitochondrial GDP binding, mitochondrial population and tissue GDP binding capacity in obese rats which is consistent with previous observations (Holt and York, 1982; Marchington et al., 1983). Serum glucose was reduced in both lean and obese adrenalectomised rats, accompanied by a decrease from the high levels of insulin normally seen in the obese Zucker rat, effects confirming previous reports (York and Godbole, 1979; Freedman et al., 1986b). The increase in free fatty acids that occurred after adrenalectomy in obese rats was probably due to the increase in serum ACTH after adrenalectomy. ACTH is a lipolytic agent which acts on both brown and white adipose tissue (Bertin and Portet,1976). This effect of ACTH was probably only seen in obese rats due to their greater WAT stores. Corticosterone depressed BAT function in both lean and obese rats, an effect only previously reported in lean animals (York et al., 1985a). Serum glucose levels were increased in corticosterone treated animals, probably as a result of glucocorticoid mediated inhibition of glucose uptake in WAT and muscle (Fain 1979). ACTH treatment stimulated BAT function and increased serum glucose, free fatty acids, insulin and corticosterone levels in both phenotypes. The beneficial effects of ACTH on BAT have only previously been demonstrated in obese rats (York and al-Baker, 1984) and that report did not demonstrate the increase in mitochondrial population in obese rats seen here. BAT mitochondrial GDP binding in lean animals has only been previously demonstrated to increase in response to ACTH in the absence of endogenous

corticosterone (York and Al-Baker, 1984). However, ACTH is known to increase metabolic rate and BAT blood flow (Heim and Hull, 1966; Laury and Portet, 1977; Kuroshima et al., 1968), as well as to provoke an increase in BAT lipolysis through activation of adenylate cyclase (Bertin and Portet, 1976), so an increase in BAT mitochondrial GDP binding in lean rats caused by ACTH treatment is not unlikely. Corticosterone replacement to adrenalectomised animals had little effect in lean rats, and the replacement dose produced slightly reduced serum corticosterone levels. In obese rats, however, BAT function was depressed back to control levels, even though serum corticosterone levels in these animals were lower than the intact group (273 ± 6 and 109 ± 8 $ng.ml^{-1}$, for intact and corticosterone replaced animals respectively). The inhibitory effect of corticosterone replacement on BAT function in adrenalectomised obese Zucker rats is well documented (Holt et al., 1983; York et al., 1985a; Freedman et al., 1986a) and is again confirmed here.

The sympathetic stimulation of the adrenal medulla and direct sympathetic stimulation of the liver are thought to be the two major effectors of the hyperglycaemia that occurs after 2DG administration (Rappaport et al., 1982; Storlein et al., 1985). The hyperglycaemia after 2DG was reduced in the absence of the adrenal medulla, i.e. in adrenalectomised and adrenalectomised-corticosterone replaced animals of both phenotypes (lean animals 69%, 45% and 4% increase; obese animals 75%, 51% and 21% increase for control, adrenalectomised, and adrenalectomised corticosterone-replaced animals respectively). Only in lean corticosterone-replaced adrenalectomised animals and obese ACTH treated animals was the 2DG dependent increase in serum glucose absent. The increase in serum corticosterone observed in control animals was probably due to 2DG-induced ACTH release, and thus ACTH-mediated stimulation of corticosterone secretion (Weidenfeld et al., 1984). Corticosterone levels were not increased in any other group in either phenotype. Adrenalectomised animals had no target organ for ACTH to stimulate, ACTH treated rats

had high levels of exogenous ACTH, so any endogenous ACTH released would probably be ineffective, and similarly, injected corticosterone would probably suppress both endogenous ACTH and corticosterone release.

The only situations investigated in which 2DG failed to depress BAT mitochondrial GDP binding in lean rats, was in intact or adrenalectomised animals treated with exogenous corticosterone. In contrast, obese animals only responded to 2DG by an inhibition of BAT GDP binding in the complete absence of corticosterone, whereas in ACTH treated obese rats there was a stimulation of BAT mitochondrial GDP binding after 2DG. It appears, therefore, that corticosterone inhibits the effects of 2DG induced glucoprivation on BAT GDP binding in obese rats. 2DG acts centrally to cause the release of ACTH, which then acts to increase corticosterone secretion (Weidenfeld et al., 1984), however, hypophysectomised Sprague-Dawley rats still show a marked reduction in rectal temperature in response to centrally injected 2DG (Muller et al., 1973). This suggests that the effects of 2DG on thermogenesis are not mediated through corticosterone release per se and this suggestion is supported by the present observations that adrenalect-omised rats of both phenotypes responded to 2DG with a reduction in BAT mitochondrial GDP binding, and the depression of BAT GDP binding after 2DG in ACTH-treated lean rats was not associated with an increase in serum corticosterone levels. Although it appears that the 2DG-inhibition of BAT GDP binding is not mediated directly by corticosterone release, there seems to be an interaction of corticosterone and 2DG effects on BAT. Serum corticosterone levels in corticosterone treated and corticosterone-replaced animals were either normal or depressed, suggesting that circulating corticosterone levels were not responsible for the restoration of insensitivity to 2DG in obese adrenalect-omised-corticosterone treated rats, or the depression of 2DG sensitivity in lean corticosterone injected or corticosterone-replaced animals. It is possible that corticosterone treatment maintained, or elevated central corticosterone levels and that an interaction between corticosterone and glucose metabolism occured centrally. The

effectsof 2DG and corticosterone do not appear to be additive but that could be because both 2DG and corticosterone were maximally depressing GDP binding at the doses used. The failure of 2DG to significantly depress BAT mitochondrial GDP binding in lean adrenalectomised-corticosterone-replaced rats could be due to the replacement dose of corticosterone to these rats slightly inhibiting basal GDP binding, so that the effect of 2DG was no longer significant.

Adrenalectomy of obese rats, as well as restoring BAT mitochondrial GDP binding to lean levels, normalises noradrenaline turnover in BAT (York et al., 1985a, b). Corticosterone treatment of lean animals depresses BAT sympathetic activity (noradrenaline turnover) (York et al., 1985a) so it appears that in instances in lean animals, where BAT sympathetic activity is depressed, 2DG does not affect BAT GDP binding and in the instances where BAT sympathetic activity is normalised in obese rats, that is after adrenalectomy, 2DG inhibits GDP binding. A possible mechanism by which 2DG suppresses BAT mitochondrial GDP binding is by inhibition of the VMH glucoreceptors leading to reduction in the efferent activity of the sympathetic nerves to BAT. The recent demonstration by Niijima (1986) that intravenous administration of glucose causes an increase in the firing rate of the BAT nerves, lends support to this suggestion. It is possible that corticosterone inhibits the central effects of 2DG, as obese rats respond to 2DG only in the complete absence of corticosterone and the 2DG inhibition of BAT mitochondrial GDP binding in lean rats is inhibited in the presence of exogenous corticosterone which may lead to an elevation in central corticosterone levels. Corticosterone is known to reduce the uptake of glucose into WAT and muscle by the inhibition of the cellular glucose transport system (Fain 1979). It could be that the postulated hypersensitivity of the obese rat to the effects of corticosterone (Yukimura et al., 1978; Freedman et al., 1986a) is responsible for the reduced sensitivity of central glucoreceptor mechanism in relation to BAT activation.

The mechanism by which ACTH stimulates BAT mitochondrial GDP binding in obese rats is unclear. ACTH treatment increases BAT mitochondrial GDP binding in lean and obese rats, but no concomitant increase in tissue noradrenaline turnover is seen (Marchington, 1985) suggesting that ACTH has a direct effect on the tissue. If, as suggested above, 2DG acts by inhibiting the sympathetic drive to BAT, the absence of such a tonic drive in obese or obese ACTH treated rats would explain the failure of 2DG to affect BAT mitochondrial GDP binding in these animals. In addition, if corticosterone does act to inhibit the central effects of 2DG, increased ACTH levels would induce an increase in circulating corticosterone which might then inhibit any central effects of 2DG on BAT function. ACTH secretion is mediated by CRF release from the hypothalamus. The stimulation of ACTH secretion caused by centrally acting 2DG (Weidenfeld et al., 1984) may result from CRF release. Recent observations suggest that CRF, when injected intracerebroventricularly, stimulates BAT mitochondrial GDP binding (Arase, York and Bray: unpublished observations). It is possible, therefore, that 2DG-induced CRF release causes a centrally mediated stimulation of BAT mitochondrial GDP binding.

Section 3.4 The Involvement of the SNS and PNS in the Responses to 2DG, and the Site of Action of 2DG on the Reduction of Body Temperature and BAT Mitochondrial GDP Binding.

Although the results in section 3.1.5 suggest that the effects of 2DG are not mediated by direct inhibition of BAT metabolism at the tissue level, the experiments described so far have not elucidated the means by which 2DG mediates its effects on BAT mitochondrial GDP binding or body temperature. It has been previously demonstrated that 2DG has effects on the activity of both the SNS and PNS. The activity of the sympathetic adrenal nerve is stimulated by 2DG administration but the renal nerve is unaffected and cardiac noradrenaline turnover is decreased in 2DG treated animals (Niijima, 1975; Rappaport et al., 1982). The 2DG induced depression in metabolic rate and rectal temperature was abolished by atropine treatment or cervical vagotomy (Rothwell et al., 1981; Shiraishi and Mager, 1980b, respectively), suggesting that the PNS is involved in these effects of 2DG. 2DG infused into the hepatic portal vein stimulates abdominal afferent vagal activity (Niijima 1981) increases feeding (VanderWeele, 1985) and stimulates gastric acid secretion (Hirschowitz and Sachs , 1965), effects which are reduced or abolished by subdiaphragmatic vagotomy. The varied physiological effects of 2DG are, therefore, attributable to actions of both the PNS and SNS.

BAT is innervated primarily by fibres of the sympathetic nervous system, and a functional sympathetic link has been demonstrated between the VMH and BAT (Perkins et al., 1981,a,b; Holt et al., 1985, 1986). The VMH contains glucoreceptors which are sensitive to 2DG application (Oomura et al., 1978) and it has been recently demonstrated that administration of glucose via the jugular vein increases BAT sympathetic nerve activity (Niijima, 1986). These experiments were designed, therefore, to investigate the involvement of the PNS and SNS in the effects of 2DG on BAT mitochondrial GDP binding, and to attept to distinguish between peripheral and central effects of 2DG

on rectal temperature and BAT thermogenesis. The experiments in this section were carried out only in lean animals, as no effects of 2DG on BAT have been demonstrated in previous sections in intact obese animals.

3.4.1 The Effects of Propranolol on BAT Mitochondrial GDP Binding in Lean Zucker Rats Fed with Supplementary Sucrose.

The acute response of BAT to dietary stimuli is thought to be sympathetically mediated (Landsberg et al., 1984). BAT of chow fed lean, but not of obese, Zucker rats is sensitive to the β_1-antagonist propranolol and undergoes a 40% reduction in BAT mitochondrial GDP binding after injection of this drug (Holt 1984). In view of the results in section 3.2.2, where lean animals were sensitive to the effects of 2DG on BAT after 7 days of sucrose feeding, but not after 24 hours, it was of interest to examine the effects of propranolol under similar conditions, to elucidate the role of the SNS in the 2DG-induced decrease in BAT mitochondrial GDP binding.

Lean animals were individually caged, and given ad lib. access to a 35% (w/v) sucrose solution instead of water and in addition to chow for 24 hours or for 7 days. Food and sucrose intake was measured over the last 24 hour period. Propranolol ($2mg.100g^{-1}$) or saline (0.1ml) was injected, s.c., over the entire 24 hour sucrose-feeding period, or over the final 24 hours of the 7 day sucrose feeding period at 8 hourly intervals, as described in section 2.5.6. The final injection of propranolol or saline was given 1 hour before sacrifice, which took place at the normal time at which 2DG treated animals were killed (12.00 - 12.30 hours). Animals were sacrificed by decapitation and trunk blood collected. Serum was prepared as described in section 2.9.1, and assayed for glucose and insulin concentrations as outlined in sections 2.9.2 and 2.9.4 respectively. BAT mitochondria were prepared and GDP binding assays performed, as described in sections 2.12 and 2.13 respectively. Protein concentrations were determined by the method of Lowry (section 2.10.2).

Results.

Table 3.4.1.1 shows the effects of propranolol treatment on energy intakes of sucrose or chow fed lean animals. Sucrose feeding increased energy intakes in all groups, however, propranolol treatment of 24 hour sucrose fed rats led to a significant decrease in energy intake but this decreased level was still higher than in chow fed propranolol treated animals. Table 3.4.1.2 shows the effects of propranolol on BAT and serum metabolites in sucrose-fed animals. 7 day sucrose feeding increased BAT wet weight and protein content. BAT mitochondrial GDP binding and serum glucose levels were increased in both sucrose fed groups, but insulin levels were unaffected. Propranolol treatment significantly reduced BAT GDP binding in 24 hour sucrose fed animals (26%), but had no significant inhibitory effect in 7 day sucrose fed animals (14% reduction), although sucrose feeding increased BAT mitochondrial GDP binding to similar levels in both 24 hour and 7 day fed groups. These findings were in contrast to effects of 2DG on 24 hour and 7 day sucrose fed animals, in which BAT mitochondrial GDP binding was inhibited by 2DG in animals that had been sucrose fed for 7 days, but not for 24 hours.

The mode of action of 2DG in its effects on BAT mitochondrial GDP binding is still unclear. Two possible mechanisms of action are a direct effect on the hypothalamus affecting the sympathetic neural output controlling BAT activity (Niijima, 1986), or peripheral effects of 2DG acting through the hepatic glucoreceptor system and the vagus nerve (Niijima 1981, 1983) causing a parasympathetically mediated inhibition of thermogenesis. To investigate these possibilities it was decided to examine the effects of BAT sympathectomy and subdiaphragmatic vagotomy on the response of BAT to 2DG treatment.

Table 3.4.1.1 The Effect of Propranolol on Energy Intakes of 24-hour or 7 day Sucrose-Supplemented Lean Zucker Rats.

ENERGY INTAKE (kJ/24 hours)	PROPRANOLOL	CHOW	SUCROSE	TOTAL	n
CHOW-FED	-	118 ± 11	-	118 ± 11	4
	+	106 ± 6	-	106 ± 6	4
24 HOUR SUCROSE FED	-	97 ± 11	76 ± 12	174 ± 6^{++}	8
	+	96 ± 11	47 ± 12	144 ± 8^{*++}	8
7 DAY SUCROSE FED	-	86 ± 11	82 ± 13	170 ± 6^{++}	12
	+	89 ± 9	59 ± 16	150 ± 10^{++}	12

Values represent the means ± S.E.M. of n animals in each group.

* $P < 0.05$ compared to saline injected animals on the same feeding regime.

++ $P < 0.01$ compared to chow fed controls in the same treatment group.

A 35% (w/v) sucrose drinking solution was supplied instead of water and in addition to chow. Food intake was measured over the last 24 hour period in the case of 7 day sucrose fed animals. Propranolol was injected over the final 24 hour period of sucrose feeding as described in the text.

Table 3.4.1.2 The Effects of Propranolol on BAT Mitochondrial GDP Binding and Serum Metabolites in Lean Zucker Rats Fed Supplementary Sucrose.

	CHOW	24 HOUR SUCROSE		7 DAY SUCROSE	
		SALINE	PROPRANOLOL	SALINE	PROPRANOLOL
BAT [^{3}H] -GDP binding (pmols.mg protein^{-1})	247 ± 9	456 ± 35^{+++}	338 ± 20*	432 ± 30^{+++}	373 ± 19
BAT Depot wet weight (mg)	0.14 ± 0.02	0.15 ± 0.01	0.18 ± 0.01	0.22 ± 0.01^{++}	0.24 ± 0.01
BAT Depot protein content (mg.depot^{-1})	23.4 ± 1.2	21.8 ± 1.2	23.1 ± 1.6	32.4 ± 1.6^{+++}	29.8 ± 1.3
Serum Glucose (mg.100ml^{-1})	118 ± 2	174 ± 4^{+++}	172 ± 4	183 ± 8^{+++}	177 ± 5
Serum Insulin (ng.ml^{-1})	0.41 ± 0.18	0.28 ± 0.09	0.46 ± 0.14	0.42 ± 0.09	0.43 ± 0.13
n	6	8	8	12	12

Values represent means ± S.E.M. of n animals.

* $P < 0.05$ compared to saline treated animals on the same feeding regime.

++ $P < 0.01$, +++ $P < 0.002$ saline treated sucrose fed animals compared to chow fed controls.

BAT mitochondria were prepared, GDP binding and protein assays performed, as described in sections 2.12, 2.13 and 2.10.2 respectively. Serum assays were performed as outlined in sections 2.9.2 and 2.9.4.

3.4.2 The Effects of BAT Sympathectomy on the Response of BAT Mitochondrial GDP Binding to 2DG Treatment in Lean Zucker Rats.

6 week old Lean Zucker rats were individually caged and their interscapular BAT depots were unilaterally denervated as described in section 2.6.3. Animals were allowed to recover for 48 hours, during which time their food intake was measured to ensure that any observed effects were not affected by a post-operative depression in food intake. Animals were injected with two doses of 2DG ($360mg.kg^{-1}$) in 0.2ml saline, i.p., 3 hours and 1 hour before sacrifice. Control animals received 0.2ml saline at the same times as experimental animals. Food was withdrawn from all animals at the beginning of the experimental period. Animals were killed by stunning and cervical dislocation. BAT was dissected out and the depot carefully divided in half. BAT mitochondria were prepared and GDP binding assays performed separately on the denervated and intact half of each depot, as described in sections 2.12 and 2.13 respectively.

Results.

The effects of unilateral BAT denervation on the 2DG-induced inhibition of BAT mitochondrial GDP binding are shown in table 3.4.2.1. Unilateral denervation of BAT caused a slight increase in tissue weight, possibly due to the deposition of lipid in the absence of SNS activation. Denervated BAT specific mitochondrial GDP binding was depressed significantly, as was depot GDP binding. 2DG significantly inhibited both specific and depot mitochondrial GDP binding in functionally innervated BAT, but had no effect on GDP binding in BAT without an intact nerve supply. Food intake in these rats was not significantly depressed for the 24 hours preceding the experiment; (food intakes $10.5 \pm 2.1g.day^{-1}$ and $9.6 \pm 1.8g.day^{-1}$, for control animals and unilaterally denervated animals respectively.)

Table 3.4.2.1 The Effects of Unilateral BAT Denervation on the Response of BAT Mitchondrial GDP Binding to 2DG in Lean Zucker Rats.

	INTACT		DENERVATED	
	SALINE	2DG	SALINE	2DG
BAT $[^3H]$GDP Binding (pmols.mg protein^{-1})	325 ± 35	240 ± 15*	225 ± 12+	218 ± 12
½ depot wet weight (g)	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.17 ± 0.01++
½ depot protein content (mg)	13.8 ± 1.3	12.9 ± 1.1	12.7 ± 0.9	15.5 ± 0.7
½ depot succ.cyt.c O-R activity (pmols.red.min^{-1}.mg^{-1})	5.58 ± 0.42	5.51 ± 0.35	4.76 ± 0.29	5.40 ± 0.24
½ depot $[^3H]$GDP Binding (pmols.½ depot^{-1})	2206 ± 527	1476 ± 243	861 ± 106+	986 ± 63

Values represent means ± S.E.M. of 6-8 animals.

* $P < 0.05$ compared to saline treated animals in the same state of innervation.

\+ $P < 0.05$, ++ $P < 0.01$ compared to intact animals in the same treatment group.

Animals were unilaterally BAT denervated as described in section 2.6.3 and maintained for 48 hours. 2DG was administered in 2 doses of 360mg.kg^{-1} in 0.2ml saline 3 hours and 1 hour before sacrifice. Control animals received 0.2ml saline injections at these times. BAT mitochondria were prepared and GDP binding assays performed on separate ½ depots, as described in sections 2.12 and 2.13 respectively. Protein assays were performed as in section 2.10.2, and succinate cytochrome c oxidoreductase activities (succ.cyt.c.O-R)measured as described in section 2.14. ½ depot GDP binding was calculated on the basis of 100% mitochondrial recovery as assessed from succ.cyt.c O-R activities.

3.4.3 The Effects of Subdiaphragmatic Vagotomy on the Response of BAT Mitochondrial GDP Binding to 2DG in Lean Zucker Rats.

It appears from the last section that the depression in BAT mitochondrial GDP binding that occurs after 2DG treatment is dependent upon an intact sympathetic nerve supply to BAT. However, denervation itself significantly decreased GDP binding and it was possible that para-sympathetic mechanisms contributed to the hypothermic effects of 2DG, so subdiaphragmatically vagotomised animals were investigated with regard to the effects of 2DG on BAT.

7-8 week old lean Zucker rats were starved overnight and subdiaphragmatically vagotomised or sham-vagotomised. Slightly older animals were used due to the intricacy of the surgical procedure, as described in section 2.6.4. Animals were housed individually in a warm room and given 3 days to recover from surgery. During the recovery period, vagotomised animals were fed the wet, palatable mash described in section 2.4.3. Sham operated animals were pair-fed to the ad lib. intake of the vagotomised group to eliminate the effects of reduced food intake. At the end of the recovery period, animals were injected with two doses of 2DG ($360mg.kg^{-1}$) in 0.2ml saline, or with 0.2ml saline, 3 hours and 1 hour before sacrifice. Animals were killed by decapitation. BAT was removed, BAT mitochondria were prepared and GDP binding assays performed, as described in sections 2.12 and 2.13 respectively. Upon sacrifice vagotomised animals were visually inspected for success of vagotomy (any nerve fibres running between the liver and the oesophagus counted against a successful vagotomy). All apparently successfully vagotomised animals had distended stomachs and lost weight ($-4 \pm 2g$) over the 3 day recovery period (Sham operated rats gained $15 \pm 1g$).

Results.

The results of this experiment are presented in table 3.4.3.1. Subdiaphragmatic vagotomy reduced BAT mitochondrial GDP binding by 20%, which is comparable to the vagotomy dependent decrease in GDP binding, observed by Andrews et

Table 3.4.3.1 The Effects of Subdiaphragmatic Vagotomy on the Response of BAT Mitochondria GDP Binding to 2DG in Lean Zucker Rats.

Values represent means $\pm$ S.E.M. of n animals in each group.

* $P < 0.05$ compared to saline treated animals of the same vagal status.

\+ $P < 0.05$, ++ $P < 0.01$ compared to sham-operated animals in the same treatment group.

ϕ $P < 0.05$ compared to restricted food intake.

ad lib. food intake (shown in parenthesis) was measured in sham-operated animals over the first 24 hour post operative period, after which time these animals were pair-fed to the food intake ov the vagotimsed group. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were assayed as outlined in section 2.10.2. Depot GDP binding values were calculated on the basis of 100% mitochondrial recovery as assessed from succinate cytochrome c oxidoreductase activities (succ.cyt.c O-R) measured as in section 2.14.

Table 3.4.3.1 The Effects of Subdiaphragmatic Vagotomy on the Response of BAT Mitochondrial GDP Binding to 2DG in Lean Zucker Rats.

	SHAM		VAGOTOMISED	
	SALINE	2DG	SALINE	2DG
BAT [^{3}H]GDP Binding (pmols.mg protein^{-1})	340 ± 24	279 ± 7*	270 ± 16^{+}	220 ± 16^{*++}
BAT Depot wet weight (g)	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
BAT Depot protein content (mg)	22.6 ± 1.7	22.6 ± 0.7	21.7 ± 1.8	17.6 ± 1.3
BAT Depot total succ.cyt. c OR activity (μmols.min^{-1}.depot^{-1})	8.47 ± 1.21	7.66 ± 0.35	8.11 ± 0.66	7.48 ± 0.69
BAT Depot total [^{3}H]GDP binding (pmols.depot^{-1})	3277 ± 251	2460 ± 180*	2216 ± 227^{++}	1525 ± 227^{*++}
Food intake (g/day)	32.6 ± 1.8 (39.3 ± 1.8$^{\phi}$)		29.4 ± 3.1	
Energy intake (kJ/day)	175 ± 10 (211 ± 10$^{\phi}$)		158 ± 17	

al (1985; 27%). However, 2DG inhibited BAT GDP binding in both sham-operated and vagotomised animals to a significant degree (18% in both cases). Depressed food intake was not responsible for the depression in basal BAT GDP binding levels in vagotomised rats as the pair-fed energy intakes were similar (as expected), although lower than the *ad lib.* intake of sham-operated animals.

These results suggest that an intact vagal input from the liver/stomach is not necessary to the inhibition of BAT function caused by 2DG administration.

3.4.4 The Effects of 2DG Infused into the Carotid Artery or Hepatic Portal Vein on BAT Thermogenesis and Body Temperature in Anaesthetised Lean Zucker Rats.

In order to further elucidate the site of action of 2DG as peripheral or central, infusions of 2DG were made into the hepatic portal vein or the carotid artery to deliver the drug directly to potential peripheral and central sites of action respectively. Changes in BAT and rectal temperatures after infusion of 2DG via these routes were monitored and BAT mitochondrial GDP binding was measured. Pairs of animals were anaesthetised as described in section 2.6.1 and surgery performed as described in section 2.6.5 for hepatic portal infusions, and 2.6.6. for carotid artery infusions. During experiments animals were kept on a thermostatically controlled heating pad, regulated to keep the body temperature of the control rat constant. Thermistors were placed under the interscapular BAT pad and 2cm into the rectum of each animal to continuously monitor temperatures. After a 5-10 minute temperature stabilisation period 6mg.100g^{-1} body weight in 0.2ml.100g^{-1} body weight saline was delivered via infusion pump over a 5 minute period. Control animals received 0.2ml.100g^{-1} body weight saline only. BAT and rectal temperatures of both animals were monitored for a further 55-60 minutes. At this point, animals were killed by cervical dislocation after checking that the cannulae had not slipped out. BAT was removed, mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13

respectively. If, at any point during the experiment, either of the animals began to regain consciousness, a further half dose of anaesthetic was given to each animal. Animals were not permitted to recover consciousness at any time during these experiments.

Results.

Figure 3.4.4.1 shows the changes in BAT and rectal temperatures that occurred after infusion of 2DG or saline into the carotid artery. Both BAT and rectal temperatures began to fall within 10-15 minutes of initiation of the infusion, but temperatures were only significantly depressed after 50 and 55 minutes. Figure 3.4.4.2 shows more clearly the relative changes that occurred in BAT and rectal temperatures in 2DG treated animals. Figure 3.4.4.3 shows the changes in BAT and rectal temperatures after infusion of 2DG or saline into the hepatic portal vein In contrast to the effects seen after intracarotid infusion, no significant changes occurred in either rectal or BAT temperatures. Figure 3.4.4.4 shows the changes that occurred in 2DG infused animals relative to saline treated rats and it can be seen more clearly that no 2DG dependent hypothermia occurred after hepatic portal infusion.

Figure 3.4.4.5 shows that BAT mitochondrial GDP binding was not significantly changed by either intracarotid or intraportal infusion of 2DG, although the general trend in the intracarotid group was to decrease GDP binding (-21%) and the intrahepatic group tended to increase GDP binding (+9%). The wide range of individual values for BAT mitochondrial GDP binding might account for the lack of statistical significance attached to these observations, an effect that might have been due to the effects of anaesthesia.

These observations tend to support the suggestion that 2DG is mediating its inhibitory effect on BAT mitochondrial GDP binding, BAT temperature and rectal temperature through central mechanisms.

Figure 3.4.4.1 The Effects of Intracarotid Infusion of 2DG on BAT and Rectal Temperatures in Anaesthetised Lean Zucker Rats.

Weight-matched pairs of animals were prepared for carotid artery infusions as described in section 2.6.6. 6mg.100g^{-1} body weight 2DG was administered in a 0.2ml volume of saline over the 5 minute period, as marked (▬). Rectal and BAT temperatures were measured for 55 minutes at 5 minute intervals.

The changes in temperature relative to individual starting temperatures are shown.

——— Saline treated animals (0.2ml saline.100g^{-1} body weight) ($n=4$)

------- 2DG treated animals (6mg.100g^{-1} body weight) ($n=4$)

* $P<0.05$, ** $P<0.01$ compared to saline treated animals at the same time point.

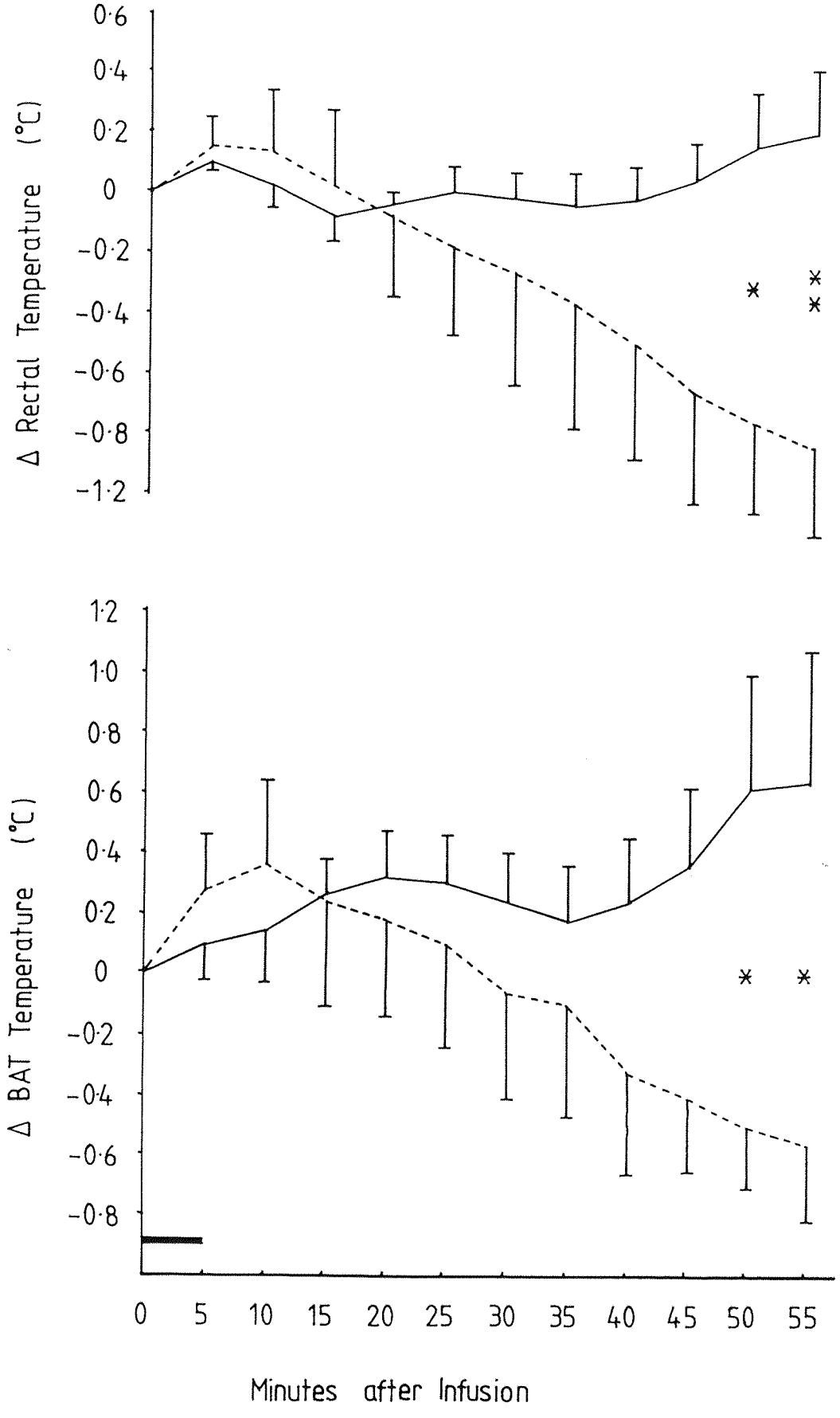

Δ Rectal Temperature (°C)
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·2
Δ BAT Temperature (°C)
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
0 5 10 15 20 25 30 35 40 45 50 55
Minutes after Infusion

Figure 3.4.4.2 The Relative Change in BAT and Rectal Temperature After Intracarotid Infusion of 2DG to Anaesthetised Lean Zucker Rats.

Weight matched pairs of animals were prepared for carotid artery infusions as described in section 2.6.6. $6mg.100^{-1}$ body weight 2DG in 0.2ml saline, or 0.2ml saline was infused over a 5 minute period, as marked (▬). Rectal and BAT temperatures were monitored over a further 55 minute period. The relative temperature differences between 2DG infused and saline infused animals are shown.

——— Rectal temperatures (n=4)

- - - - - - - BAT temperatures (n=4)

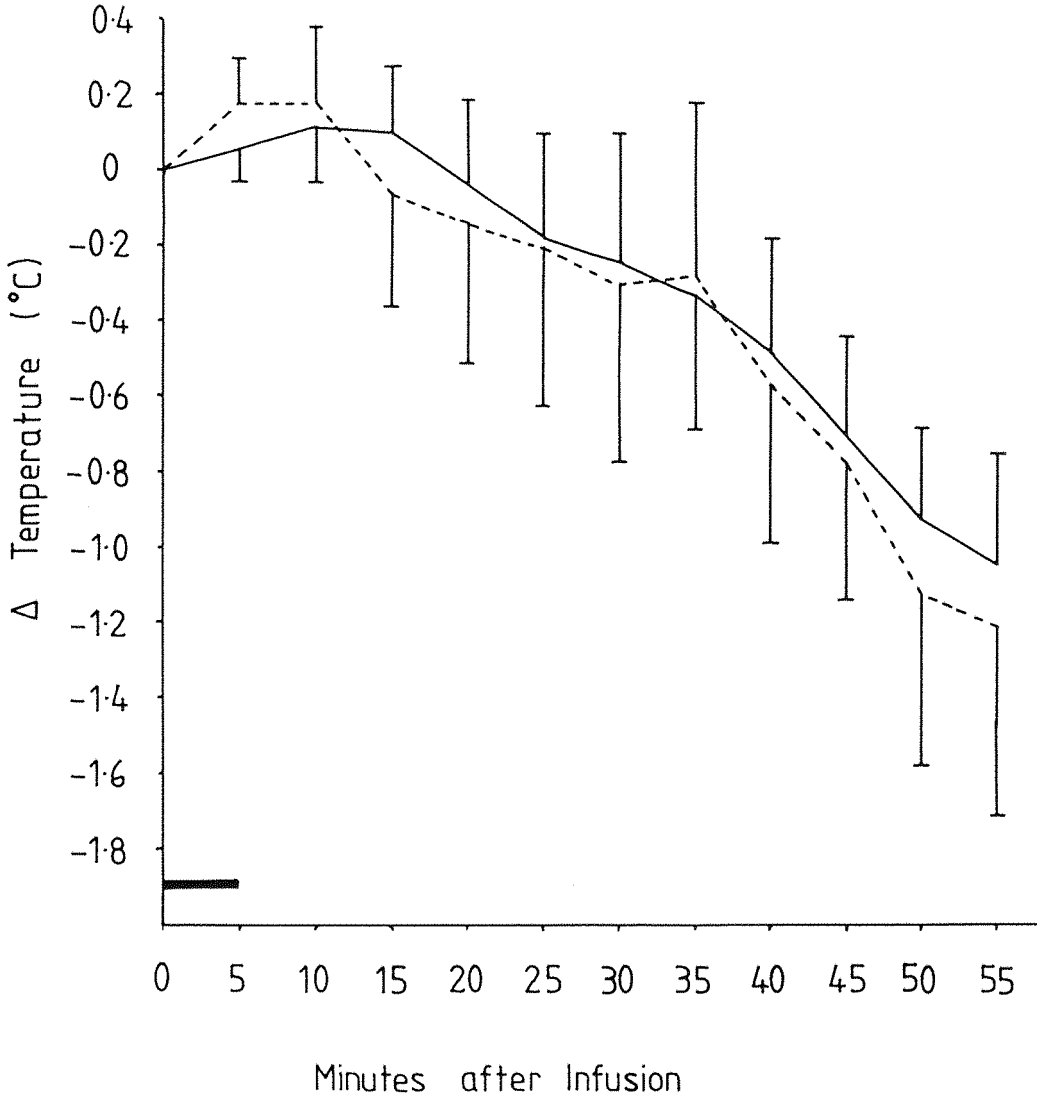

0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
-1·2
-1·4
-1·6
-1·8
Δ Temperature (°C)
0
5
10
15
20
25
30
35
40
45
50
55
Minutes after Infusion

Figure 3.4.4.3 The Effects of Intrahepatic Portal Infusion of 2DG on BAT and Rectal Temperatures in Anaesthetised Lean Zucker Rats.

Weight-matched pairs of animals were prepared for hepatic portal vein infusions as described in section 2.6.6. 6mg.100g^{-1} body weight 2DG was administered in a 0.2ml volume of saline over the 5 minute period, as marked (▬). Rectal and BAT temperatures were measured for 50 minutes at 5 minute intervals.

The changes in temperature relative to individual starting temperatures are shown.

——— Saline treated animals (0.2ml saline.100g^{-1} body weight) ($n=3$)

------ 2DG treated animals (6mg.100g^{-1} body weight) ($n=3$)

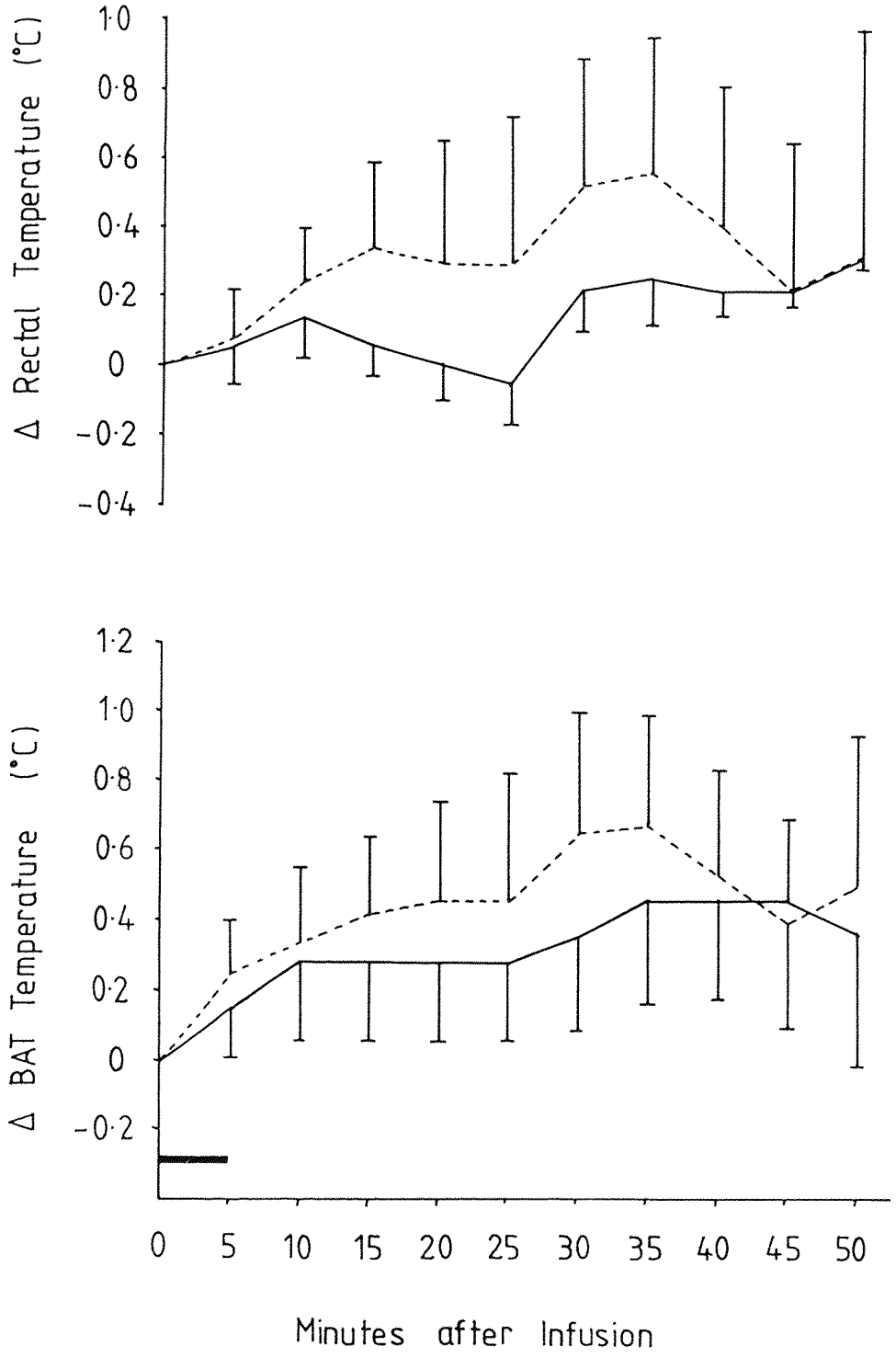

Δ Rectal Temperature (°C)
1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
Δ BAT Temperature (°C)
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·2
0
5
10
15
20
25
30
35
40
45
50
Minutes after Infusion

Figure 3.4.4.4 The Relative Change in BAT and Rectal Temperature After Intrahepatic-Portal Infusion of 2DG to Anaesthetised Lean Zucker Rats.

Weight matched pairs of animals were prepared for hepatic portal vein infusions as described in section 2.6.6. $6mg.100^{-1}$ body weight 2DG in 0.2ml saline, or 0.2ml saline was infused over a 5 minute period, as marked (▬). Rectal and BAT temperatures were monitored over a further 50 minute period. The relative temperature differences between 2DG infused and saline infused animals are shown.

——— Rectal temperatures (n=3)

- - - - - - BAT temperatures (n=3)

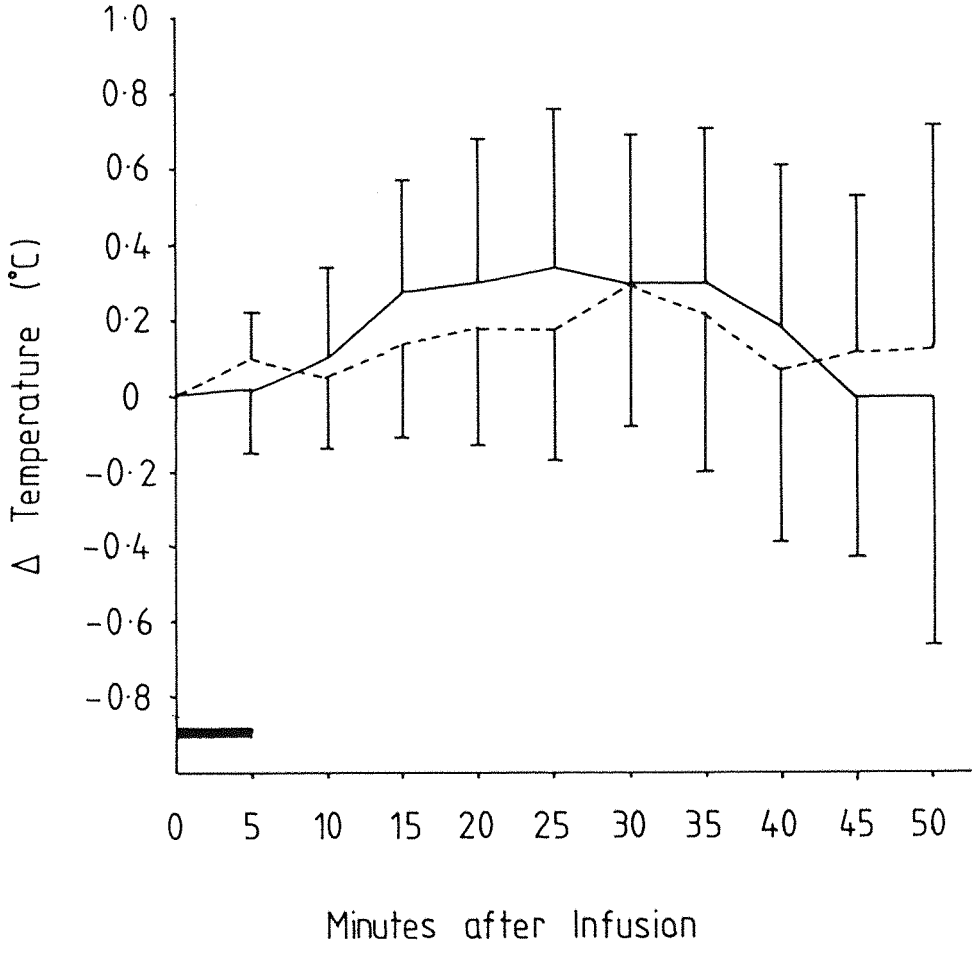
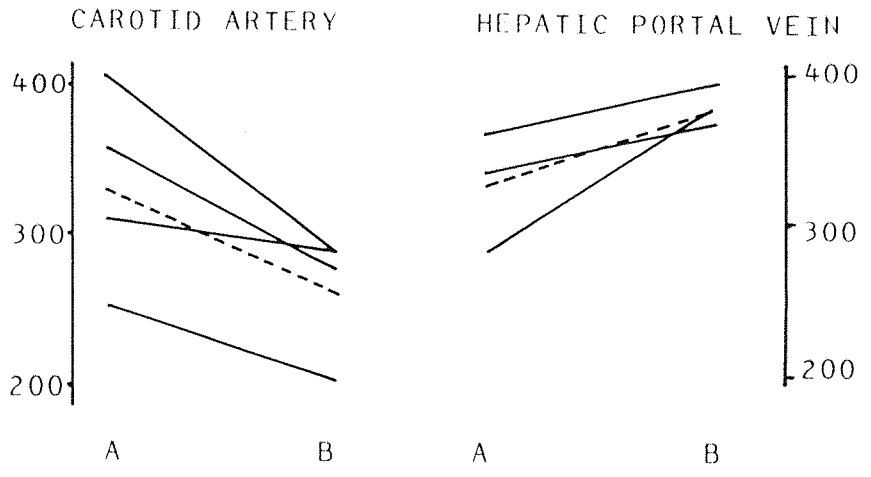

1·0
0·8
0·6
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
Δ Temperature (°C)
0
5
10
15
20
25
30
35
40
45
50
Minutes after Infusion

Figure 3.4.4.5 The Effects of 2DG Administered via the Carotid Artery or Hepatic Portal Vein on BAT Mitochondrial GDP binding in Anaesthetised Lean Zucker Rats.



——— joins pairs of animals infused with saline (A) or 2DG (B) in the same experiment.

------- joins means of each group

	BAT [^{3}H] GDP Binding			
	CAROTID ARTERY		HEPATIC PORTAL VEIN	
	SALINE	2DG	SALINE	2DG
	331 ± 33	261 ± 21	331 ± 23	381 ± 8
(n)	(4)	(4)	(3)	(3)

Values represent means ± S.E.M. of n animals in each group.

BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

3.4.5 Discussion.

The results from sections 3.2.2 and 3.4.1 show that 24 hour sucrose fed animals reduced BAT mitochondrial GDP binding in response to propranolol, but not 2DG. Conversely, 7 day sucrose feeding resulted in a sensitivity to 2DG but not propranolol. The implication from these observations is that prolonged overfeeding results in an activation of BAT, the maintenance of which is not dependent upon β-adrenergic mechanisms and that the change over period from propranolol-sensitive to propranolol-insensitive control, takes 1-7 days. The change in sympathetic activation could be due to an increase in the relative proportion of α_1 receptors in BAT, with α_1 receptor stimulation maintaining an increased tonic level of thermogenesis. This is supported by the observations that α_1 receptor number increases and β_1 receptors decrease on prolonged cold exposure (see Mohell, 1984) and on cafeteria feeding (see Cannon and Nedergaard, 1986). α_1 agonists have recently been shown to potentiate the effects of β_1-noradrenergic stimulation of BAT from cold acclimated animals (Foster, 1985), if the same effect occurred in overfed animals, this could account for the reduced effect of propranolol on chronically diet-stimulated BAT. Noradrenaline is a mixed $\alpha_1/\alpha_2/\beta_1$ agonist, so although the β-blockable component of BAT sympathetic activation apparently decreases after 7 days sucrose feeding, the α_1 component, which would not be sensitive to propranolol, could be increased. Tissue noradrenaline turnover is increased in 7-day sucrose fed lean rats (York et al., 1985b), so a reduced sympathetic drive to BAT is not responsible for the failure of propranolol to reduce BAT mitochondrial GDP binding. The reciprocal effect of 2DG and propranolol on BAT of 24 hour and 7 day sucrose fed animals could be explained thus: initially, overfeeding provokes powerful centrally mediated stimulation of BAT, acting through the relatively high proportion of β_1 receptors on the tissue. This powerful initial stimulus cannot be overridden by acute dietary regulators, such

as 2DG-induced changes in glucose metabolism, but β_1-blockade would lead to a large reduction in BAT mitochondrial GDP binding. As overfeeding persists, an increase in the relative proportions of α_1 receptors in BAT occurs resulting in potentiation of β_1 mediated effects, but no increase, or even a decrease, in direct β_1 stimulated effects in BAT. At the same time, the central mechanisms regulating DIT adjust to the new level of dietary stimulation and a change in sensitivity restores the ability of acute regulatory signals (such as 2DG induced glucoprivation) to affect BAT thermogenic function. So, after 7 days of sucrose feeding 2DG would be able again to reduce BAT mitochondrial GDP binding but propranolol would have a reduced effect due to the relatively low proportion of direct β_1-mediated stimulation of BAT. This hypothesis could be further investigated by the use of α_1-antagonists to investigate the magnitude of the α_1-adrenergic component in 7 day and 24 hour sucrose fed animals.

The investigations into the autonomic effector of the 2DG-mediated inhibition of BAT thermogenesis seemed to indicate that the effects were elicited through the sympathetic nervous system. Both surgical procedures, vagotomy and unilateral BAT denervation, resulted in a depression of BAT mitochondrial GDP binding relative to sham-operated control animals, eating similar amounts of food. Denervation of BAT has been shown to cause an involution of the tissue over 7-10 days (Rothwell and Stock, 1984b), so the experiment was performed as soon after surgery as possible (2 days) to avoid these changes. This was partially successful, as denervation caused only a 30% reduction in BAT mitochondrial GDP binding, compared to the 51% reduction of GDP binding over 10 days observed by Rothwell and Stock (1984b). A reduction in BAT GDP binding levels has also been previously observed after subdiaphragmatic vagotomy (Andrews et al., 1985a, b). The current study demonstrated a 20% decrease in BAT mitochondrial GDP binding after vagotomy, which is comparable to the reduction seen by Andrews et al., (1985a, b; 27% and 32% reductions respectively). Whereas the reduction in

BAT function after denervation (sympathectomy) is thought to be due to direct effects on the tissue (Minokoshi et al., 1986) the effects of subdiaphragmatic vagotomy on BAT activity are thought to be due to a reduction in circulating insulin levels and so reduced insulin-stimulated BAT activity, (Andrews et al., 1985a). The results of the current study showed that 2DG treatment reduced BAT mitochondrial GDP binding in vagotomised animals, but no reduction in binding was observed in the denervated BAT lobe of 2DG treated animals. These results suggest that the response of BAT to 2DG requires the presence of an intact sympathetic innervation, but the absence of abdominal vagal afferent or efferent activity has little effect on the inhibition of BAT mitochondrial GDP binding caused by peripherally administered 2DG. It appears, therefore, that the effects of 2DG on BAT GDP binding were due to a decrease in sympathetic activity to the tissue which implies that the actions of 2DG were mediated at the central site. This suggestion gains support from the recent observations by Niijima (1986) that glucose injected into the jugular vein enhances BAT nerve efferent activity, possibly through activation of the VMH gluco-receptors.

Carotid artery infusion of 2DG decreased BAT and rectal temperatures and tended to decrease BAT mitochondrial GDP binding (although this change was not significant). No such effects were seen when 2DG was infused into the hepatic portal vein. These observations also provide support that 2DG is acting centrally to inhibit BAT thermogenesis.

The results presented in this section suggest that the inhibition of BAT mitochondrial GDP binding caused by 2DG treatment is effected through the sympathetic nerve supply to BAT and is due to central actions of 2DG.

Section 3.5 Summary and Discussion.

The inability of the obese Zucker rat to activate BAT thermogenesis in response to dietary stimuli has been attributed to a lack of diet-related sympathetic activity in BAT (York et al., 1985a, b). The measurement of [^{3}H]GDP binding to BAT mitochondria has been widely used as an index of BAT thermogenic activity (Desautels et al., 1978; Nicholls, 1979; Sundin and Cannon, 1980; Brooks et al., 1980; Holt et al., 1982). Obese Zucker rats have low basal levels of BAT mitochondrial GDP binding (Holt and York, 1982) and BAT sympathetic activity (Levin et al., 1983b; York et al., 1985b), the former observation being confirmed in the present study. Young obese rats respond normally to cold exposure by increasing BAT GDP binding (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983) and BAT noradrenaline turnover (York et al., 1985b) but are unable to increase either BAT GDP binding or BAT sympathetic activity in response to overfeeding (Holt et al., 1983; York et al., 1985a, b). The results presented in section 3.2 also demonstrate that obese rats are able to increase BAT GDP binding in response to cold, but not dietary stimulation. Adrenalectomy improves the low levels of GDP binding and BAT noradrenaline turnover in obese rats to normal lean levels, and restores the ability of these obese animals to respond to dietary stimuli (Holt et al., 1983; York et al., 1985a, b) an effect that has been associated with the removal of corticosterone. Inhibition of BAT thermogenesis (GDP binding and noradrenaline turnover) occurs when normal animals are treated with glucocorticoids (Rothwell and Stock, 1984a, York et al., 1985a), and diet-induced increases in BAT thermogenesis and the thermic effect of feeding are enhanced by adrenalectomy (Marchington et al., 1986). Also, the age-related decrease in the capacity for DIT is ameliorated by adrenalectomy (Rothwell et al., 1984b). In spite of the apparent inhibitory effects of corticosterone on diet-induced thermogenesis, permissive amounts of this hormone are required for the survival of cold exposure in normal animals (Fellenz et al.,

1982). Other forms of obesity also show a sensitivity to glucocorticoids. VMH-lesioned animals do not become obese when they are adrenalectomised (Bruce et al., 1982) but replacement of corticosterone to these animals restores the development of hypothalamic obesity in rats (Bruce et al., 1982) and mice (Debons et al., 1982, 1986). Like Zucker rats, VMH-lesioned rats respond normally to cold exposure (Luboshitsky et al., 1983). Obese (ob/ob) mice also improve BAT function and tissue noradrenaline turnover after adrenalectomy (Holt and York, 1983; Vander Tuig et al., 1984, respectively). It appears, therefore, that glucocorticoids are extensively involved in the aetiology of several experimental obesities, and the results presented in this chapter give further confirmation to the observation that BAT function is restored by adrenalectomy in obese rats. The suggestion has been made from these collective observations that glucocorticoids can inhibit DIT through the inhibition of diet-related sympathetic activation of BAT (York et al., 1985b).

2DG has been demonstrated to produce a range of independent effects on the activity of the sympathetic nervous system, which are thought to be mediated through multiple brain sites (see LeMagnen, 1983). Adrenal nerve activity is enhanced in 2DG treated rats in the absence of renal nerve stimulation (Niijima, 1975), and cardiac noradrenaline turnover is decreased in the presence of striking adrenal medullary stimulation (Rappaport et al., 1982). Niijima (1986) has recently demonstrated that glucose administration via the jugular vein increases the efferent discharge rate of BAT nerves and suggested that this response to glucose is related to the regulation of BAT and DIT. As 2DG is known to oppose the effects of glucose in the VMH/LH glucose sensing neurones (Oomura et al., 1978) it could be inferred from the observations of Niijima (1986) that 2DG would inhibit BAT nerve activity.

2DG has been demonstrated to inhibit BAT mitochondrial GDP binding in lean rats. The ability to demonstrate normal noradrenaline stimulation of BAT mitochondrial GDP binding in 2DG treated rats suggests that 2DG was not acting

directly on the tissue to inhibit glucose utilisation. The major substrate for BAT thermogenesis is thought to be fatty acids either released from triglyceride stores within the cell or imported through the actions of lipoprotein lipase (Radomski and Orme, 1971; Carneheim et al., 1984). Recent evidence suggests, however, that glucose could be an important substrate for noradrenaline stimulated BAT thermogenesis (Gibbins et al., 1985). Noradrenaline stimulation of BAT leads to activation of glycolytic enzymes and an inhibition of acetyl CoA carboxylase, and hence a greater capacity for glucose utilisation (Gibbins et al., 1985; McCormack et al., 1986). However, the enhanced capacity for glucose utilisation exists only in noradrenaline stimulated tissue (Gibbins et al., 1985), so in situations of limited glucose availability, for instance starvation, high fat feeding or 2DG inhibition of glucose metabolism, it seems likely that the major thermogenic fuel would be fatty acids. Indeed, the observation that noradrenaline stimulation produces similar increases in BAT mitochondrial GDP binding in 2DG treated and control rats, suggests that even though the capacity for glucose utilisation is present in noradrenaline stimulated BAT, similar levels of 32K protein mediated uncoupled respiration are achieved in the presence or absence of glucose as a thermogenic substrate. It thus seems unlikely that the inhibition of BAT mitochondrial GDP binding caused by 2DG is due to inhibition of glucose utilisation. Further support for this suggestion is gained from the demonstration that 2DG is incapable of inhibiting cold stimulated BAT GDP binding in lean or obese rats, although diet stimulated BAT GDP binding was inhibited by 2DG. This suggests that 2DG preferentially inhibits diet-related thermogenesis. The differential effects of 2DG on BAT GDP binding in relation to cold and diet stimulation implies that 2DG is affecting a controlling system of BAT thermogenesis, rather than BAT itself.

The regulation of BAT thermogenesis is thought to occur primarily through the SNS (Landsberg et al., 1982;

Rothwell and Stock, 1984c), however, atropine injection and cervical vagotomy have both been shown to abolish the inhibitory effects of 2DG on metabolic rate in Sprague-Dawley and lean Zucker rats (Shiraishi and Mager, 1980b; Rothwell et al., 1981). This suggests that the PNS may be involved in these effects of 2DG. The investigation of the effects of 2DG on BAT mitochondrial GDP binding showed that 2DG was still able to inhibit GDP binding in vagotomised animals, suggesting that the vagus nerve plays no role in the BAT thermogenic effects of 2DG. Vagotomy itself caused a decrease in BAT GDP binding that was not dependent upon decreased food intake but which may have been related to reduced food absorption, as gastric motility and emptying is reduced in vagotomised animals (Roman and Gonella, 1981). In contrast, sympathetic denervation of BAT led to the abolition of the effects of 2DG on BAT mitochondrial GDP binding, suggesting that 2DG acts through inhibition of the sympathetic drive to BAT. It could be argued that the failure of cervical sympathectomy to alter the hypothermic effects of 2DG (Shiraishi and Mager, 1980b) provides evidence against the suggestion that 2DG inhibits BAT mitochondrial GDP binding through the depression of SNS activity to BAT. However, although cervical sympathectomy denevates the cervical BAT pads (Hull and Segall, 1965a) it has no effect on the innervation of interscapular depot (Hull and Segall, 1965b) so interscapular BAT would still be responsive to the SNS. BAT denervation in young animals abolishes the tissue hypertrophy and increased thermogenic capacity of BAT in response to either cold or diet (Rothwell and Stock, 1984b). Diet induced responses (i.e. inhibition of BAT thermogenesis by 2DG) have been demonstrated here as being absent in denervated tissue. The suggestion that variations in glucose availability or uptake can affect the sympathetic output to BAT is supported by the recent observation by Niijima(1986) that intravenous glucose administration resulted in an increase in the firing rate of the sympathetic nerves to BAT.

It is impossible in the studies so far mentioned to

distinguish between central and peripheral effects of intraperitoneally injected 2DG. Although 2DG seems to affect BAT through depression of the sympathetic drive to the tissue, whether the depression was effected directly by a central action of 2DG, or indirectly through peripheral modification of central sympathetic output could not be ascertained. The presence of a 2DG inhibitory effect on BAT mitochondrial GDP binding in the absence of an intact abdominal vagal system suggested that the hepatic glucoreceptor system was not involved in the regulation of BAT. However, the role of the hepatic glucoreceptors were further investigated by varying the route of administration of 2DG. This visceral sensory system has been widely implicated as an important regulator of food intake, the initiation of feeding and of blood glucose homeostasis (Hirschowitz and Sachs, 1965; Novin and VanderWeele, 1977; Kral,1981; Niijima, 1983). Infusion of glucose into the hepatic portal vein decreases the activity of hepatic and pancreatic vagal afferents. In contrast, 2DG administered via the same route increases hepatic and pancreatic vagal afferent activity (Niijima, 1981). Infusion of 2DG into the hepatic portal vein had no effect on body or rectal temperatures in anaesthetised lean Zucker rats. This suggests that vagal afferent signals have no regulatory effect on BAT thermogenesis, and this is supported by the observation that subdiaphragmatically vagotomised animals still responded to 2DG by decreasing BAT mitochondrial GDP binding. The failure of subdiaphragmatic vagotomy to alter the BAT thermogenic responses to 2DG and hepatic portal vein infusion of 2DG to decrease body or BAT temperatures (or to affect BAT GDP binding), suggests that the peripheral actions of 2DG on the abdominal vagal system are not involved in the thermogenic response to 2DG. This is in contrast to the suggestions of Shiraishi and Mager (1980b) and Rothwell et al (1981), who found that the 2DG-induced inhibition of metabolic rate and rectal temperature were inhibited in the presence of atropine or by cervical vagotomy. The suggestion was made that 2DG enhances parasympathetic activity which inhibits peripheral thermogenesis, however, BAT is not innervated by the PNS

(Bryant et al., 1983) so any effects of the PNS on BAT activity would have to be mediated indirectly. It has been demonstrated that the potentiating effects of atropine on the thermogenic effects of feeding on metabolic rate and BAT temperature are not mediated centrally, and it has been suggested that the parasympathetic involvement in these responses may be due to a change in hormone or metabolite levels (Bryant et al., 1983). Evidence against a major role for the PNS in the regulation of energy expenditure is that chronic treatment of lean and obese rats (fa/fa) or mice (ob/ob) with parasympathetic stimulators or inhibitors has no effect on energy expenditure or weight gain that could not be accounted for by alterations in food intake (Dulloo and Miller, 1986).

The observations that intracarotid infusion of 2DG ($6mg.100g^{-1}$) produced significant decreases in both BAT and rectal temperatures and tended to decrease BAT mitochondrial GDP binding, suggests that the actions of 2DG on BAT are mediated centrally. If this is so, then a likely site of action is the hypothalamus. The ventromedial and lateral hypothalamic areas have been constantly implicated in the regulation of energy balance (see Bray and York, 1979; Bray, 1984). The VMH has a functional neural link with BAT (Niijima et al., 1984; Perkins et al., 1981a, b; Holt et al., 1986) and also possesses an insulin sensitive glucoreceptor system (Anand et al., 1964; Oomura, 1976). Glucose stimulates the VMH glucoreceptors increasing VMH activity, an effect which is enhanced by insulin and attenuated by 2DG or free fatty acids (Oomura et al., 1978). Centrally acting 2DG would reduce the VMH output to BAT thereby decreasing its thermogenic activity. The observation that 2DG is not effective in denervated BAT adds support to this suggestion. It would seem from the evidence presented so far, that 2DG acts centrally in the lean rat, to inhibit BAT mitochondrial GDP binding, BAT temperature and rectal temperature through the depression of the sympathetic drive to BAT.

2DG failed to inhibit BAT mitochondrial GDP binding in the obese rat, or in situations in the lean rat which are known to depress SNS activity in BAT. 2DG inhibition of GDP binding did not occur in corticosterone treated lean rats, adrenalectomised corticosterone-replaced lean rats or in any obese group of animals, except after adrenalectomy. Corticosterone treatment depresses BAT sympathetic activity (York et al., 1985a) and it is possible that the replacement dose of corticosterone to adrenalectomised lean rats led to an increased central level of the hormone, as discussed previously in section 3.3.3. The elevation of BAT mitochondrial GDP binding in obese rats after ACTH treatment is not associated with an increase in BAT sympathetic activity (Marchington, 1985). Adrenalectomy, however, not only normalises BAT mitochondrial GDP binding in obese rats but also increases sympathetic activity in the tissue to a level equivalent to that seen in lean rats (York et al., 1985b). The observation that 2DG inhibits GDP binding only in adrenalectomised obese rats of the two situations where BAT function is apparently normal in these mutants, suggests that 2DG acts only in the presence of normal BAT sympathetic activity. It could be construed from this data, and the previous observation that 2DG preferentially inhibits DIT, that both corticosterone and 2DG inhibit the normal sympathetic drive to dietary stimuli. It is not clear if 2DG and corticosterone both inhibit BAT function through effects on glucose metabolism or even whether they act at the same site. However, their effects do not appear to be additive as 2DG does not suppress BAT mitochondrial GDP binding any further than corticosterone alone in corticosterone treated lean rats. This could be due to both corticosterone and 2DG eliciting maximal responses at the doses used (10 $mg.kg^{-1}$ and 360$mg.kg.^{-1}$ body weight, respectively), so it might prove interesting to investigate the effects of sub-maximal doses of both these agents on BAT mitochondrial GDP binding and their interaction. Although the major effect of glucocorticoids is to enhance hepatic gluconeogenesis through protein catabolism, peripheral glucose utilisation is reduced in the presence of glucocorticoids through non-competitive inhibition of glucse transport (Munck, 1971;

Fain, 1979). Glucocorticoids are also thought to contribute towards the insulin resistance of the fa/fa rat (Freedman et al., 1986b) as insulin stimulated glucose uptake from the circulation is normalised after adrenalectomy. Adrenalectomy also reverses the insulin resistance in muscle of the obese (ob/ob) mouse (Ohshima et al., 1984). Insulin resistance in this genetically obese mutant is characterised by a reduction in insulin receptor number in WAT, liver and muscle and a reduction in insulin stimulated glucose uptake, glucose metabolism and glycogen synthetase activity in muscle (Soll et al., 1975; LeMarchand-Brustel and Freychet, 1978; LeMarchand et al., 1978). Basal glucose transport and insulin-stimulated glucose transport are normalised in muscle of the obese mouse after adrenalectomy (Ohshima et al., 1984). The ob/ob mouse, unlike the fa/fa rat, has characteristically higher levels of circulating corticosterone compared to lean littermates (Dubuc, 1977), but treatment of adrenalectomised lean mice with comparable levels of corticosterone does not mimic the obese syndrome (Shimomura et al., 1981). It has, therefore, been suggested that obese mice are more sensitive to the effects of corticosterone and the decreased insulin-stimulated glucose transport in muscle is due to a direct effect of corticosterone on the tissue (Ohshima et al., 1984). An alternative explanation for the reduction in insulin resistance after adrenalectomy could be that the reduced insulin concentration seen in both ob/ob mice and fa/fa rats (Yukimura and Bray, 1978; Yukimura et al., 1978) results in a decrease in the rate of insulin receptor degradation and a normalisation of insulin receptor levels. It has been suggested that insulin reduces the number of its receptors by enhancing the receptor degradation rate (Kasuga et al., 1981). It is possible that corticosterone removal enhances insulin sensitivity by the reduction of peripheral inhibition of insulin stimulated glucose uptake, and by the removal of a glucocorticoid mediated enhancement of PNS activity, which results in a decrease in vagal stimulation of insulin release.

The effects on food intake elicited by 2DG were thought initially to be due to central release of noradrenaline stimulating α-adrenergic feeding mechanisms in the hypothalamus. Injection of 2DG into the IIIrd ventricle induces hyperphagia in rats that can be reduced by α-antagonists such as azapetine, yohimbine and phentolamine injected via the same route. β blockers such as propranolol had no effect on feeding (Muller et al., 1972). The PVN has been identified as the effector of a central α_2-noradrenergic feeding drive (Leibowitz, 1978). This feeding system is activated by noradrenaline injected into the PVN the effects of which are enhanced by corticosterone, possibly via modification of α_2-receptor density in the PVN, in direct proportion to circulating corticosterone concentration (Bhakthavatsalam and Leibowitz, 1986). Adrenalectomy attenuates the feeding response elicited by noradrenaline stimulation of the PVN, and corticosterone replacement restores noradrenaline induced feeding (Leibowitz et al., 1984). 2DG induced hyperphagia was still present in adrenalectomised lean rats and was only present in obese rats after adrenalectomy. The persistance of 2DG induced hyperphagia after adrenalectomy was also noted by Bhakthavatsalam and Leibowitz (1986). These observations tend to dissociate the 2DG-induced feeding response from the α_2-PVN corticosterone dependent mechanism. It is possible that the intact obese rat has an overly sensitive PVN-feeding system due to its suggested corticosteroid hyperreactivity (Yukimura et al., 1978; Holt et al., 1983) which would account for the hyperphagia of the obese rat and the restoration of normal food intake after adrenalectomy. The obese rat does respond to intraventricular injection of noradrenaline with an increase in food intake, although the magnitude of the eating response is much greater in the lean animal (Ikeda et al., 1980). It would appear from these observations that the feeding response elicited by 2DG is not mediated through the PVN-noradrenergic feeding mechanism and it is not clear whether the response is due to 2DG-stimulated noradrenaline release acting on other nuclei than the PVN or if 2DG is having a separate effect. It has been reported that lesions of the suprachiasmatic nucleus abolish the hyperphagic effects

of 2DG (Yamomoto et al., 1985). It is possible that the hyperphagic effects of 2DG are peripherally mediated. 2DG alters hepatic and pancreatic vagal afferent activity which then modifies vagal efferents to the liver and pancreas, and increases food intake (Novin and VanderWeele, 1977; Niijima, 1981, 1983). Activity of the PNS is thought to be elevated in the obese Zucker rat and this is thought to be at least partially responsible for hyperinsulinaemia and abnormal glucose tolerance in these animals (Rohner-Jeanrenaud and Jeanrenaud, 1985b). Adrenalectomy is thought to correct the overactivity of the PNS as well as the depressed SNS in the obese rat (Marchington, 1985). The absence of a hyperphagic response to 2DG in the intact obese rat could be due to insensitivity of the liver and pancreas to 2DG due to vagal hyperactivity. If adrenal-ectomy corrects the elevated PNS activity, then the sensitivity to the peripheral effects of 2DG would be restored and 2DG-induced hyperphagia would become apparent.

2DG has been shown to inhibit BAT mitochondrial GDP binding in a manner consistent with a reduction in the centrally mediated sympathetic drive to the tissue. The reduction in BAT mitochondrial GDP binding after 2DG does not appear to result from a peripheral effect of the drug, acting either directly on BAT or indirectly through the vagus nerve. The denervation of BAT abolishes the 2DG dependent decrease in BAT GDP binding and the effects of 2DG on BAT and rectal temperatures appear to be mediated centrally, possibly at the VMH/LH glucoreceptor level. The effects of 2DG on BAT GDP binding are inhibited in the lean rat by exogenous corticosterone, which may be as a result of increased central levels of the hormone. 2DG only inhibits BAT mitchondrial GDP binding in the obese rat in the complete absence of corticosterone.

2DG appears to preferentially inhibit diet related thermogenesis in the lean rat and is ineffective in cold-acclimated lean or obese animals. These findings lend further support to the hypothesis that the obesity of the fa/fa rat arises from an insensitivity to dietary stimuli at a central locus and suggest that a glucocorticoid

dependent inhibition of glucose sensitivity may be an important factor in the obesity of the fa/fa rat.

CHAPTER 4. THE EFFECTS OF HIGH FAT AND HIGH CARBOHYDRATE FEEDING ON THE RESPONSES OF LEAN AND OBESE ZUCKER RATS TO ADRENALECTOMY.

High energy density diets normally provoke an increase in energy expenditure and BAT function in lean, but not obese, Zucker rats (Rothwell and Stock, 1982, 1983; Triandafillou and Himms-Hagen, 1983). The high energy diets previously investigated with respect to Zucker rats have included cafeteria feeding, sucrose overfeeding and fat or carbohydrate intubation. Low protein diets will also stimulate an adaptive thermogenic response in lean Zucker rats (relative to animals pair-fed a normal protein diet to the same level of energy intake) although their food intake is actually reduced (Young et al., 1980). Obese Zucker rats maintain their ad lib. energy intake on low protein diets and do not show any changes in energy expenditure. High fat, low carbohydrate type cafeteria diets have a more potent effect on reducing net energetic efficiency than the normal self-selected cafeteria diet (Rothwell et al., 1983c). It has recently been demonstrated that 'normal composition' cafeteria diets (30% fat, 20% protein, 50% carbohydrate, by energy) still provoke a greater body energy gain and increase in metabolic efficiency in young Sprague-Dawley rats which are restricted to the energy intake of chow fed rats, than in the ad lib. chow fed animals (Rothwell et al., 1985c). In that report, Rothwell et al., (1985c) also showed that the ad lib. intake of a semi-synthetic high fat diet (of the same energy composition as the cafeteria diet) was similar to the energy intake of chow fed control animals, but that energetic efficiency was increased in these high fat fed animals. Comparison of high fat fed and cafeteria fed animals at the same level of energy intake showed that gross energetic efficiency was slightly higher in the cafeteria fed group, although energy expenditure was not significantly different. The increased efficiency seen on cafeteria or high-fat diets may be a result of the reduced energetic cost of fat synthesis and deposition. However, both dietary fat (Schwartz et al., 1983) and dietary sucrose (Young and Landsberg, 1977a; Landsberg and Young, 1978) independently stimulate general

SNS activity, and thus energy expenditure, through activation of BAT as well as increasing hepatic thermogenesis (Young and Landsberg, 1979; Berry et al., 1985). Noradrenaline was able to stimulate a greater increase in oxygen consumption in high fat-fed animals than cafeteria or chow fed animals at the same level of energy intake (72.5%, 65% and 59% increase, respectively) suggesting that sympathetic activation may be slightly higher in the high-fat fed group (Rothwell et al., 1985c). It seems that the same basic energy composition diet was capable of producing different effects upon adaptive thermogenesis, so it may be possible that the type of diet, as opposed to just energy consumption, may be an important factor in the regulation of energy balance.

Adrenalectomy of obese Zucker rats restores the ability of these animals to respond to sucrose overfeeding by increasing BAT thermogenic function, and restores the increase in metabolic rate that occurs in response to a single meal (Holt et al., 1983; Marchington et al., 1983). This procedure also enhances the adaptive thermogenic response of young and old Sprague-Dawley rats to ad lib. cafeteria feeding (Rothwell et al., 1984b). Basal BAT function is restored to lean levels in obese adrenalectomised Zucker rats, as is gross energetic efficiency and body weight gain in chow fed animals (Holt and York, 1982; Marchington et al., 1983). These effects of adrenalectomy are thought to reflect the removal of corticosterone, as replacement of this hormone to adrenalectomised animals causes BAT function(mitochondrial GDP binding and tissue noradrenaline turnover) and the development of obesity (body weight gain and body composition) to revert to pre-operative levels (York et al., 1985a; Freedman et al., 1986a). Hypothalamic obesity is also alleviated by adrenalectomy and again, the replacement of corticosterone either peripherally or centrally restores the obese state (Bruce et al., 1982;. Debons et al., 1982, 1986). Adrenalectomy also normalises growth (Saito and Bray, 1984) and restores BAT function (mitochondrial GDP binding and tissue noradrenaline turnover) to normal in obese (ob/ob) mice (Holt and York, 1984; Vander Tuig et al., 1984,

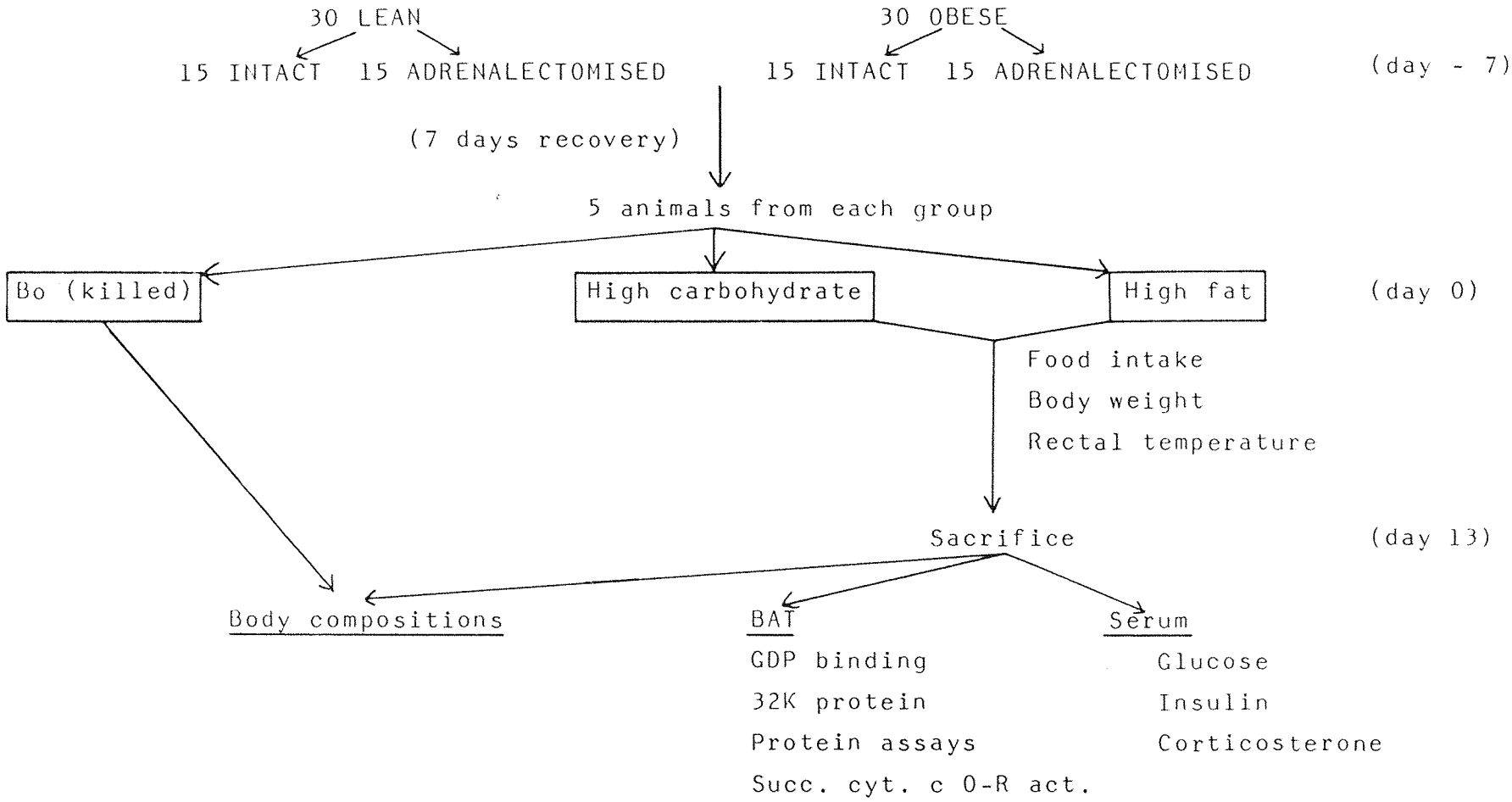
respectively). It has recently been suggested that this improvement in adaptive thermogenesis (and therefore energy balance) in obese mice is dependent upon dietary composition (Smith and Romsos, 1985). These workers found that adrenalectomised ob/ob mice maintained on a high fat (60% by energy) diet did not show the marked improvements in muscle protein gain, energy intake, energy gain or energetic efficiency that were observed in adrenalectomised animals fed a stock chow (high carbohydrate) diet. The suggestion was made that although glucocorticoids may substantially contribute towards the development of obesity in obese mice, they were not necessary to the development of the obese state resulting from a high fat diet in these animals, and that dietary composition may influence factors mediating the development of obesity which were independent of adrenal status. In this section I shall describe experiments which were designed to investigate the effects of dietary composition on the response of obese (fa/fa) rats to adrenalectomy. The impact of high fat feeding on changes in BAT function and composition, body weight gain, energy balance and energetic efficiency after adrenalectomy in lean and obese (fa/fa) rats was examined.

Experimental Procedure.

30 lean and 30 obese rats between 4 and 5 weeks of age were used in these experiments. 15 rats of each phenotype were adrenalectomised and maintained as described in section 2.6.2 for 7 days. During this time all animals (intact and adrenalectomised) were fed laboratory chow. 7 days after adrenalectomy, 5 rats from each group (lean intact, lean adrenalectomised, obese intact and obese adrenalectomised) were sacrificed for determination of initial body compositions (Bo group, see section 2.7). At this time the remaining rats were individually housed in wire mesh bottomed cages and 5 rats in each group were given ad lib. access to one of the two experimental diets, high fat (22%, 34% and 44% protein, fat and carbohydrate energy respectively) or high carbohydrate (24%, 8% and 68% protein, fat and carbohydrate energy respectively). These diets are described

fully in section 2.4.2. Food intake was measured daily for 13 days, with allowance being made for spillage which was collected beneath the hopper. Body weights were measured at 3 day intervals. After 13 days, animals were sacrificed by decapitation, trunk blood was collected and serum prepared, as outlined in section 2.9.1. Serum assays were later performed for the determination of glucose, insulin and corticosterone concentrations (sections 2.9.2, 2.9.4 and 2.9.5, respectively). Success of adrenalectomy was verified by visual inspection at sacrifice and by the determination of serum corticosterone levels as not detectable (2 obese adrenalectomised high carbohydrate rats were excluded on this evidence). Upon sacrifice, interscapular BAT was dissected out, weighed, BAT mitochondria were prepared and specific [^{3}H]-GDP binding assays were performed, as described in sections 2.12 and 2.13 respectively. Prepared mitochondria were frozen for later analysis of protein content (section 2.10.2), radioimmunoassay of 32K protein concentration (section 2.18), polyacrylamide gel electrophoresis and Western blotting (sections 2.19 and 2.20). Depot protein content was determined from a sample of BAT homogenate and activity of succinate-cytochrome c oxidoreductase (succ.cyt.c O-R) assayed, as described in section 2.14, for determination of mitochondrial population. Carcasses were eviscerated and weighed, and liver and inguinal fat pad weights were noted. Liver, fat pad and head were then returned to the body cavity and carcasses frozen for later analysis of body compositions (section 2.7). Energy expenditure was calculated by difference between body energy gain (final energy content - initial energy content calculated from Bo group) and cumulative energy intake (from food intake). Gross energetic efficiency was calculated from body energy gain as a percentage of cumulative energy intake. Experimental procedure is summarised in scheme 4.1.

Scheme 4.1


30 LEAN
30 OBESE
15 INTACT
15 ADRENALECTOMISED
15 INTACT
15 ADRENALECTOMISED
(day - 7)
(7 days recovery)
5 animals from each group
Bo (killed)
High carbohydrate
High fat
(day 0)
Food intake
Body weight
Rectal temperature
Sacrifice
(day 13)
Body compositions
BAT
GDP binding
32K protein
Protein assays
Succ. cyt. c O-R act.
Serum
Glucose
Insulin
Corticosterone

4.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on Body Weight Gain and Food Intake in Lean and Obese Zucker Rats.

The effects of adrenalectomy on body weight and food intake of lean and obese rats fed a semi-synthetic high carbohydrate (HC) diet for 13 days are shown in table 4.1.1 and figure 4.1.1. Figure 4.1.1 shows the time course of weight gain for all 4 groups. There were no significant differences in body weight between any of the groups until day 7, when intact animals of both phenotypes were significantly heavier than their adrenalectomised counterparts. A significant difference in body weight between lean and obese animals was not apparent until day 9. At no time was there any difference between the body weights of the two adrenalectomised groups. The intact obese group gained more weight than the equivalent lean animals, but the amount of weight gained was similar in both adrenalectomised groups (table 4.1.1). No significant differences were apparent between weight gains of intact and adrenalectomised animals of the same phenotype but this was probably due, in the case of obese animals, to the large standard error in the smaller adrenalectomised group. Daily food and energy intakes were higher in intact obese animals than in the equivalent lean group, but adrenalectomy reduced food intake (and energy intake) in both phenotypes to a similar level.

4.2 The Effects of Adrenalectomy and High Carbohydrate Feeding on the Body Compositions of Lean and Obese Zucker Rats.

Body compositions are presented on a percentage wet body weight basis in figure 4.2.1. All lean animals had significantly higher amounts of water and protein and less fat than obese animals of the same adrenal status. Adrenalectomy significantly increased water and decreased fat content in lean animals, and decreased fat content in obese animals.

Figure 4.1.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on Body Weight Gain in Lean and Obese Zucker Rats.

The body weights of each group of animals over the 13 day period are illustrated. Early error bars are omitted for clarity.

Points represent means $\pm$ S.E.M. of 5 animals in each group (except obese adrenalectomised, n=3).

* $P < 0.05$, ** $P < 0.01$ compared to obese animals of the same adrenal status.

\+ $P < 0.05$, ++ $P < 0.01$ compared to adrenalectomised animals of the same phenotype.

□ lean intact group

■ obese intact group

○ lean adrenalectomised group

● obese adrenalectomised group

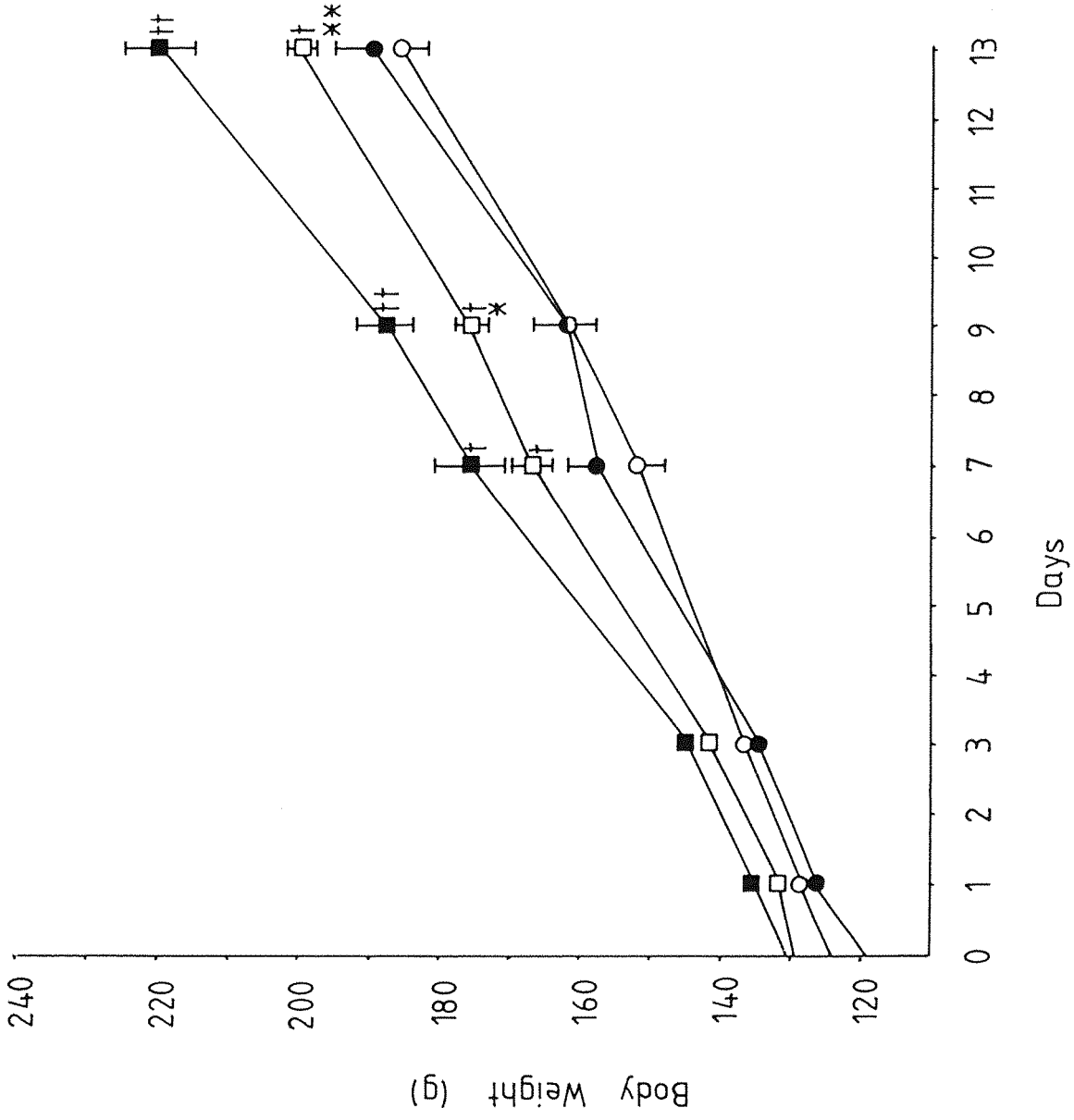

240
220
200
180
160
140
120
Body Weight (g)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Days
++
+
*
**

Table 4.1.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on Body Weight Gain and Energy Intake of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Eviscerated Carcass Weight (g)	177.8 ± 1.7**	196.7 ± 4.0	166.7 ± 4.0+	169.7 ± 7.5+
Weight Gain (g)	71.6 ± 2.0***	91.1 ± 2.2	62.4 ± 3.9	68.5 ± 11.0
Daily Food Intake (g)	16.9 ± 0.2***	20.2 ± 0.4	14.4 ± 0.2+++	16.1 ± 1.0++
Daily Energy Intake (kJ)	259 ± 3**	308 ± 11	219 ± 3+++	247 ± 15+

Values represent means ± S.E.M. of 5 animals per group (except obese adrenalectomised, n=3).
** $P < 0.01$, *** $P < 0.002$ compared to obese animals of the same adrenal status.
+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of the same phenotype.

Food intake was measured daily. Energy intake was calculated from the theoretical energy content of the diet (section 2.4.2).

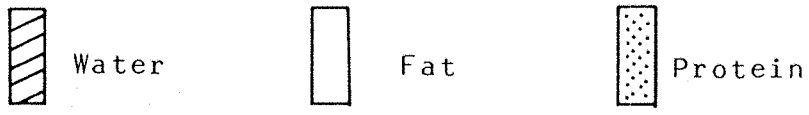
Figure 4.2.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on the Body Composition of Lean and Obese Zucker Rats.

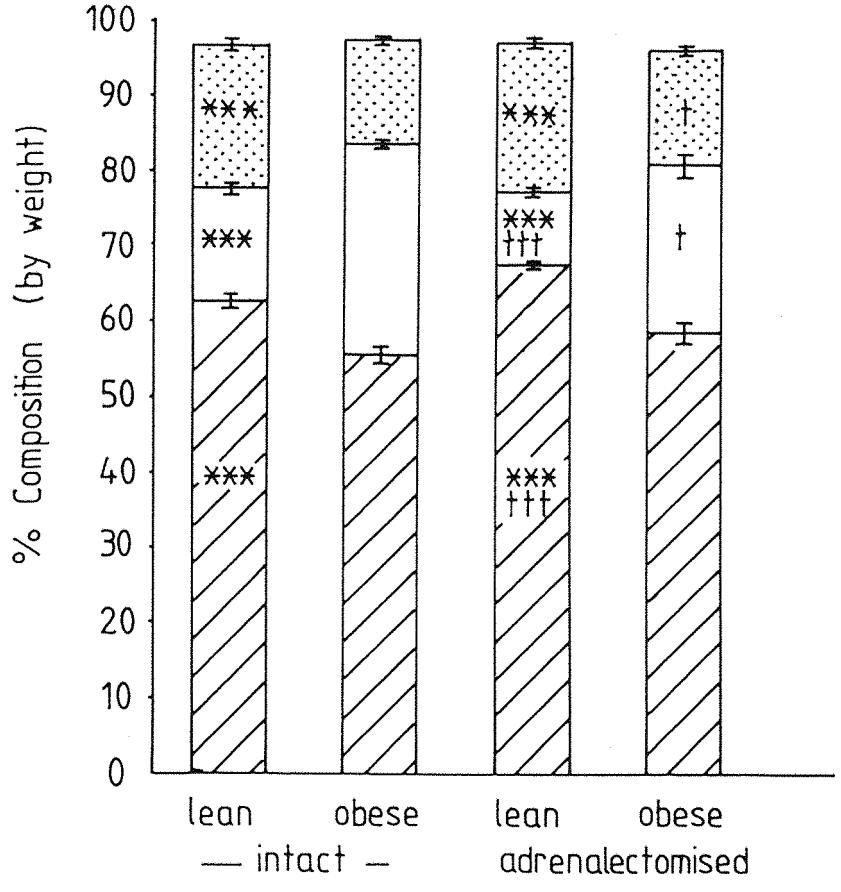
Dried, homogenised carcasses were analysed as described in sections 2.7.1, 2.7.3 and 2.7.4 for water, fat and nitrogen content, respectively. The body compositions presented are expressed as a percentage of wet carcass weight.

Bars represent means $\pm$ S.E.M. for each constituent for 5 animals in each group (except obese adrenalectomised, n=3).

*** $P < 0.002$ compared to obese animals of the same adrenal status.

+ $P < 0.05$. +++ $P < 0.002$ compared to intact animals of the same phenotype.




100
90
80
70
60
50
40
30
20
10
0
% Composition (by weight)
***
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†††
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lean
obese
lean
obese
— intact —
adrenalectomised

4.3 The Effects of Adrenalectomy and High Carbohydrate Feeding on the Energetic Efficiency of Lean and Obese Zucker Rats.

Data pertaining to energy balance is shown in table 4.3.1. Cumulative energy intake reflects the summation of food and energy intakes shown in table 4.1.1. Final carcass energy content was significantly reduced by adrenalectomy in both phenotypes, however, obese animals of both treatment groups had 1½ fold higher carcass energy contents than the corresponding lean animals. Energy gain was significantly higher in intact obese than intact lean rats, and was corrected by adrenalectomy. Energy expenditure was similar for all groups, although it was significantly reduced in lean adrenalectomised rats compared to the lean intact group. Gross energetic efficiency was significantly higher in intact obese animals with respect to both intact lean and obese adrenalectomised rats. Lean animals' energetic efficiency was unaffected by adrenalectomy Table 4.3.2 shows the energy cost of growth (energy expenditure per gram body weight gain). There were no significant differences in this factor, but the obese intact value did tend to be lower than all other groups. Final carcass energy density was higher in both obese groups, but was decreased after adrenalectomy in both phenotypes. Final energy density was greater in all groups except the obese adrenalectomised group .

4.4. The Effects of Adrenalectomy and High Carbohydrate Feeding on Tissue Weight and Serum Metabolites of Lean and Obese Zucker Rats.

Table 4.4.1 shows the inguinal fat pad and liver weights and serum metabolite levels in HC fed rats. Inguinal fat pad weight was elevated in obese animals compared to lean animals of the same adrenal status. Adrenalectomy reduced fat pad weight significantly in both phenotypes. Obese intact animals had heavier livers than the equivalent lean group, a difference that was abolished by adrenalectomy. Lean liver weight was unaffected by adrenalectomy. Serum glucose and corti-

Table 4.3.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on the Energetic Efficiency of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Cumulative Energy Intake (kJ)	3364 ± 44**	4011 ± 138	2854 ± 40^{+++}	3208 ± 197^{+}
Carcass Energy Content (kJ)	1564 ± 45***	2444 ± 58	1175 ± 59^{***+++}	1836 ± 85^{+++}
Energy Gain (kJ)	883 ± 45***	1303 ± 58	678 ± 59^{+}	771 ± 85^{+++}
Energy Expenditure (kJ)	2481 ± 53	2708 ± 182	2176 ± 59^{++}	2434 ± 117
Gross Efficiency (%)	26.1 ± 1.3*	32.9 ± 2.5	23.7 ± 1.9	23.9 ± 1.3^{+}

Values represent the means ± S.E.M. of 5 animals in each group (except obese adrenalectomised, n=3).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

\+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of the same phenotype.

Carcass energy contents were determined from body compositions as described in section 2.7.

Gross energetic efficiency was calculated as $\frac{\text{Body energy gain}}{\text{Cumulative Energy Intake}} \times 100$.

Table 4.3.2 The Effects of Adrenalectomy and High Carbohydrate Feeding on the Energy Cost of Growth and Body Energy Density of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Energy Expenditure per Weight Gain ($kJ.g^{-1}$)	38.7 ± 0.9	32.7 ± 2.6	38.1 ± 2.5	38.1 ± 4.0
Final Carcass Energy Density ($kJ.\bar{W}^{0.75}$)	$31.98 \pm 0.93^{***}$	46.52 ± 0.63	$25.31 \pm 1.02^{***+++}$	$39.07 \pm 1.90^{++}$
Initial Carcass Energy Density ($kJ.\bar{W}^{0.75}$)	$22.02 \pm 0.45^{\phi\phi\phi}$	$38.44 \pm 1.29^{\phi\phi\phi}$	$17.28 \pm 0.34^{\phi\phi\phi}$	36.65 ± 1.75

Values represent means $\pm$ S.E.M. of 5 animals in each group (except obese adrenalectomised, n=3).

*** $P < 0.002$ compared to obese animals of the same adrenal status.

++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of the same phenotype.

$\phi\phi\phi$ $P < 0.002$ compared to final body energy density of the same group.

Table 4.4.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on Tissue Weights and Serum Metabolites of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Inguinal fat pad weight (g)	$0.98 \pm 0.14^{**}$	1.68 ± 0.11	$0.45 \pm 0.08^{***++}$	$1.21 \pm 0.09^{+}$
Liver weight (g)	$9.56 \pm 0.09^{*}$	10.79 ± 0.48	8.91 ± 0.70	9.85 ± 0.41
Serum Glucose ($mg.100ml^{-1}$)	118 ± 4	119 ± 3	122 ± 5	127 ± 7
Insulin ($ng.ml^{-1}$)	$12.69 \pm 1.22^{***}$	48.66 ± 6.73	$7.30 \pm 1.16^{***+}$	$19.49 \pm 0.79^{++}$
Corticosterone ($ng.ml^{-1}$)	114 ± 13	107 ± 27	n/d	n/d

Values represent means $\pm$ S.E.M. of 5 animals per group (except obese adrenalectomised, n=3).
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$ compared to obese animals of the same adrenal status.
+ $P < 0.05$, ++ $P < 0.01$ compared to intact animals of the same phenotype.

Serum assays were performed as described in sections 2.9.2, 2.9.4 and 2.9.5. n/d denotes not detectable.

-costerone levels were not affected by phenotype, and serum glucose was not affected by adrenalectomy. Serum insulin levels in all obese rats were significantly higher than in their lean counterparts. Adrenalectomy reduced insulin levels in both lean and obese rats.

4.5 The Effects of Adrenalectomy and High Carbohydrate Feeding on BAT of Lean and Obese Zucker Rats.

Table 4.5.1 shows the data relating to the gross compositions of BAT. Interscapular BAT weight was greater in both obese groups and was not significantly changed by adrenalectomy. Total depot protein was depressed in intact obese rats but was increased to a similar value to lean animals by adrenalectomy. Protein content in lean animals was not affected by adrenalectomy. Depot mitochondrial protein content (calculated on the basis of 100% mitochondrial recovery as assessed from succinate cytochrome c oxidoreductase activities) was depressed in intact obese rats, but was normalised by adrenalectomy. Table 4.5.2 shows the effects of adrenalectomy and HC feeding on BAT mitochondrial GDP binding and 32K protein content in lean and obese Zucker rats. BAT mitochondrial GDP binding, interscapular BAT depot GDP binding, BAT mitochondrial 32K protein content and interscapular depot 32K protein content were all significantly depressed in obese intact rats, but were normalised by adrenalectomy. Adrenalectomy had no significant effects on these parameters in lean animals. The molar binding ratio of GDP to 32K protein was similar for all groups.

Figure 4.5.1 shows the visualised mitochondrial proteins and mitochondrial 32K protein by polyacrylamide gel electrophoresis and Western blotting respectively. The upper photograph depicts all the solubilised mitochondrial proteins. Lanes 0 and 1 represent molecular weight markers and purified 32K protein respectively. Lanes 2 and 3 show solubilised mitochondria from intact lean and obese animals respectively and Lanes 3 and 4, the same for adrenalectomised lean and obese animals. The lower photograph shows the Western blots for the same samples and is numbered in the same manner. The 32K protein band on the gels was difficult to pick out, but the

Table 4.5.1 The Effects of Adrenalectomy and High Carbohydrate Feeding on BAT Composition of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
BAT Depot weight (g)	$0.46 \pm 0.03^{***}$	0.78 ± 0.02	$0.44 \pm 0.07^{*}$	0.84 ± 0.09
BAT Depot protein content (mg/depot)	$52.8 \pm 2.7^{***}$	33.6 ± 2.5	51.6 ± 5.8	$56.6 \pm 3.9^{+++}$
BAT Mitochondrial prot. content (mg/depot)	$31.7 \pm 3.8^{**}$	14.8 ± 1.7	37.1 ± 3.4	$39.5 \pm 1.1^{+++}$

Values represent means $\pm$ S.E.M. of 5 animals per group (except obese adrenalectomised, n=3).
* $P < 0.05$, ** $P < 0.01$, *** $P < 002$ compared to obese animals of the same adrenal status.
+++ $P < 0.002$ compared with intact animals of the same phenotype.

Protein assays were performed as described in section 2.10.2. Depot mitochondrial protein content was calculated from succinate cytochrome c oxidoreductase activities measured as described in section 2.14.

Table 4.5.2 The Effects of Adrenalectomy and High Carbohydrate Feeding on BAT Mitochondrial GDP Binding and 32K Protein Concentration in Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
$[^3H]$-GDP Binding (pmols.mg protein^{-1})	358 ± 22*	270 ± 21	439 ± 43	420 ± 45+
BAT Depot $[^3H]$-GDP Binding (pmols.depot^{-1})	11262 ± 1389**	3916 ± 514	15730 ± 1451	15891 ± 1682+++
Mitochondrial 32K protein concentration (µg.mg protein^{-1})	34.5 ± 4.8*	19.3 ± 2.8	34.5 ± 3.0	35.2 ± 3.2++
BAT depot 32K protein (µg.depot^{-1})	1121 ± 184***	280 ± 48	1276 ± 123	1315 ± 54+++
GDP:32K molar ratio	0.37 ± 0.07	0.49 ± 0.11	0.42 ± 0.05	0.38 ± 0.04

Values represent means ± S.E.M. of 5 animals per group (except obese adrenalectomised, n=3).

* $P < 0.05$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

\+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of same phenotype.

BAT mitochondria were prepared, GDP binding assays performed and 32K protein concentration determined as described in sections 2.12, 2.13 and 2.18 respectively. Depot GDP binding and 32K protein concentrations were calculated on the basis of 100% mitochondrial recovery as assessed from succinate cytochrome c oxidoreductase activities, as described in section 2.14.

Figure 4.5.1 Visualisation of Mitochondrial Protein and 32K Protein of Solubilised BAT Mitochondria from Adrenalectomised High Carbohydrate Fed Lean and Obese Zucker Rats by Polyacrylamide Gel Electrophoresis and Western Blotting.

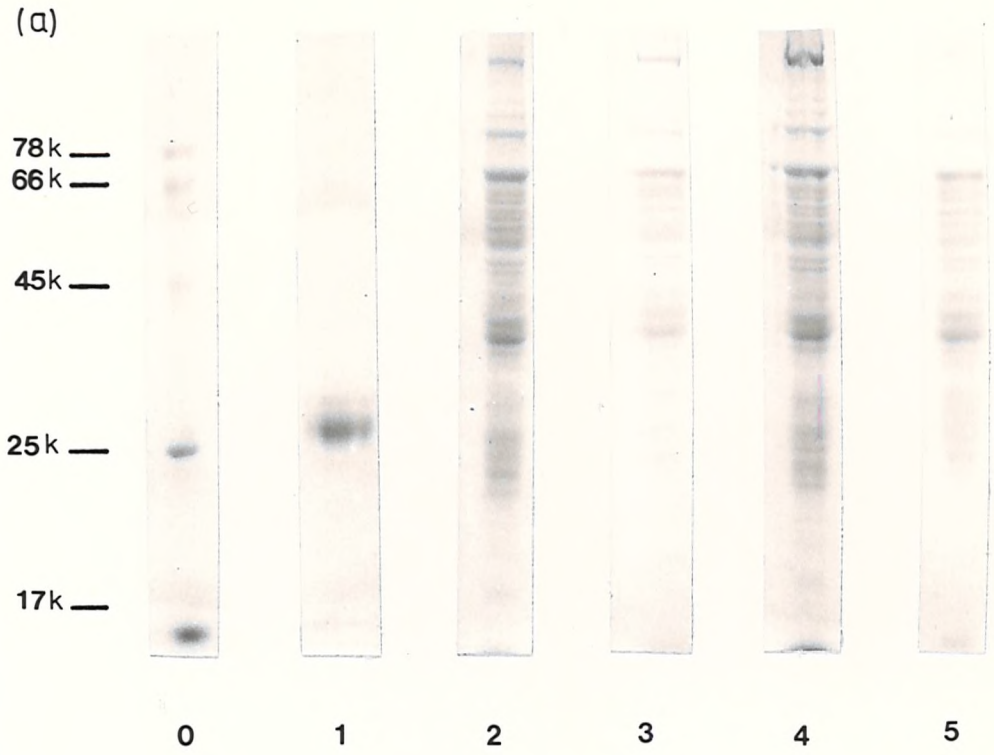
Solubilised BAT mitochondria from intact and adrenalectomised lean and obese Zucker rats fed a high carbohydrate diet were applied to two 12% polyacrylamide slab gels and run as described in section 2.19. One of each gel pair was stained with Coomassie blue R for the visualisation of all mitochondrial proteins. Proteins on the second gel were transferred to nitrocellulose paper and 32K protein was visualised by specific antibody binding, as described in section 2.20. This is shown in plate (b).

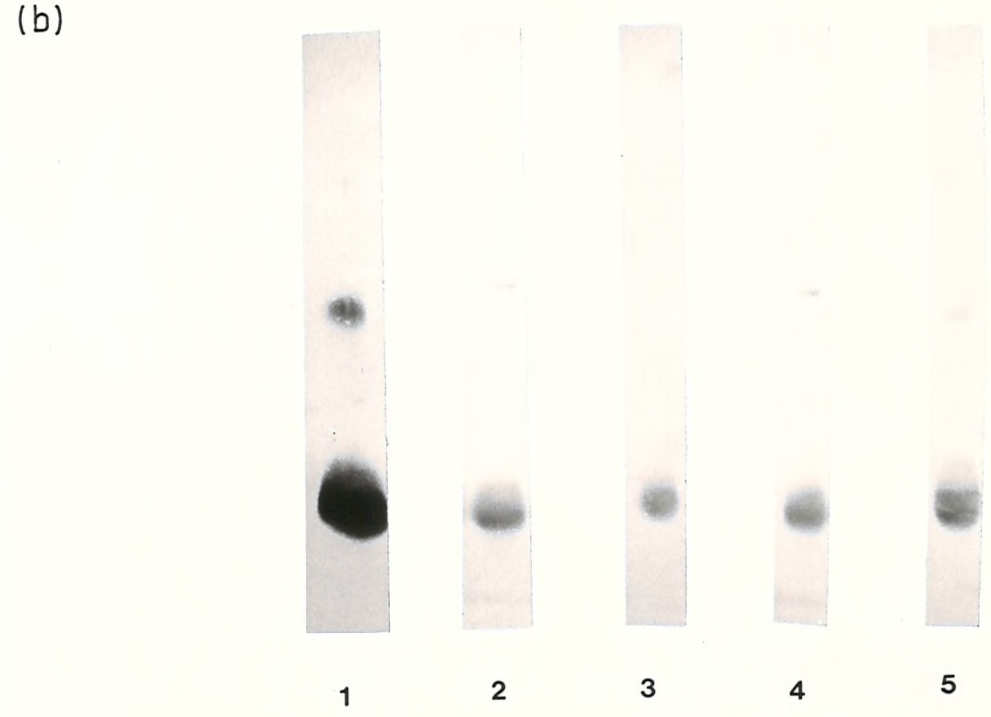
Lanes are as follows:-

0. -Electran molecular weight markers (12,800-78,000 M.W.)
1. -Purified 32K protein (see section 2.16).
2. -Solubilised BAT mitochondria from lean intact animals.
3. -Solubilised BAT mitochondria from obese intact animals.
4. -Solubilised BAT mitochondria from lean adrenalectomised animals.
5. -Solubilised BAT mitochondria from obese adrenalectomised animals.

a. Polyacrylamide Gel.

b. Western Blot.


(a)
78k
66k
45k
25k
17k
0
1
2
3
4
5


(b)
1
2
3
4
5

blot clearly showed the presence of 32K protein in all tracks. The lower band on the blot represented 32K protein and the upper faint band probably represented the dimeric form of the uncoupling protein. There appeared to be less of the 32K uncoupling protein present in mitochondria from obese intact rats (lane 3) compared to lean intact (lane2) or obese adrenalectomised (lane 5) animals, an observation confirmed by radioimmunoassay (see table 4.5.2).

4.6 The Effect of Adrenalectomy and High Fat Feeding on Body Weight Gain and Energy Intakes of Lean and Obese Zucker Rats.

Figure 4.6.1 shows the increase in body weight for intact and adrenalectomised rats of both phenotypes throughout the 13 day period of high fat (HF) feeding. As seen in the HC fed animals there were no differences in body weight between and groups until day 6, when both lean and obese intact rats became significantly heavier than their equivalent adrenalectomised counterparts. By day 9, obese intact rats were significantly heavier than the intact lean group. At no time were the body weights of the two adrenalectomised groups' different, again as seen in the HC fed animals. Table 4.6.1 shows the details of body weight gain and food intake in HF fed animals. Adrenalectomy significantly reduced final body weights in both lean and obese animals, and obese animals were no longer heavier than their lean counterparts after adrenalectomy. The amount of weight gained remained greater in obese rats compared to lean, irrespective of adrenal status, but again both adrenalectomised groups gained significantly less weight than their intact control groups. Daily food and energy intakes were significantly lower in both lean groups compared to obese and in both adrenalectomised groups compared to intact.

Figure 4.6.1 The Effects of Adrenalectomy and High Fat Feeing on Body Weight Gains in Lean and Obese Zucker Rats.

The body weights of each group of animals over the 13 days period are illustrated. Early error bars are omitted for clarity.

Points represent means $\pm$ S.E.M. of 5 animals in each group.

+ $P < 0.05$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

* $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to adrenalectomised animals of the same phenotype.

□ lean intact group

■ obese intact group

○ lean adrenalectomised group

● obese adrenalectomised group

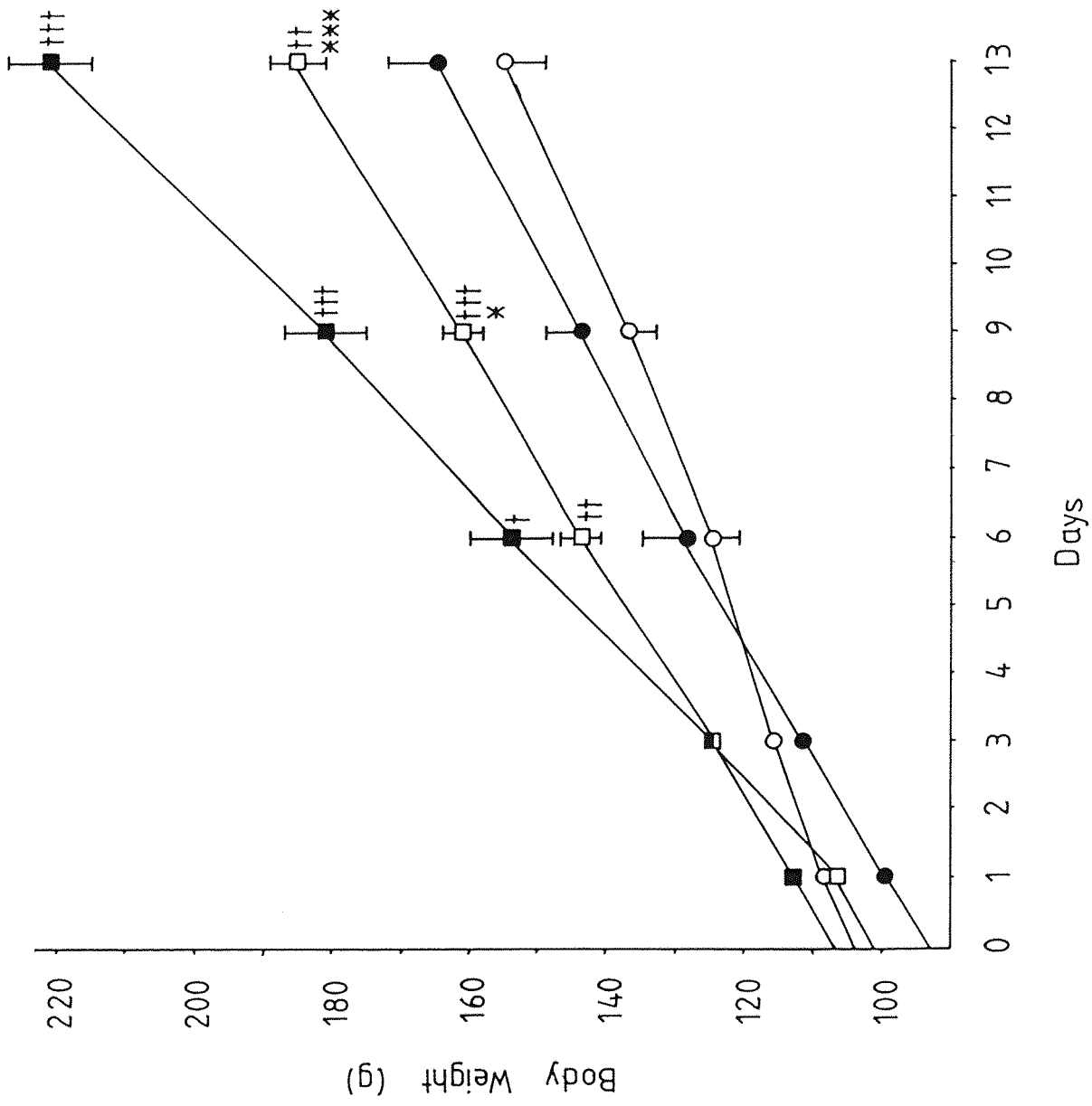

Body Weight (g)
220
200
180
160
140
120
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Days
+++
++
***
+++
*
++
+

Table 4.6.1 The Effects of Adrenalectomy and High Fat Feeding on Body Weight Gain and Energy Intake of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Eviscerated Carcass Weight (g)	163.6 ± 3.0***	192.7 ± 4.3	137.2 ± 4.3^{+++}	142.1 ± 5.7^{+++}
Weight Gain (g)	78.1 ± 2.3***	119.6 ± 2.0	50.8 ± 2.7^{***+++}	72.2 ± 3.1^{+++}
Daily Food Intake (g)	13.1 ± 0.3***	19.4 ± 0.7	10.4 ± 0.4^{***+++}	12.8 ± 0.3^{+++}
Daily Energy Intake (kJ)	235 ± 5***	349 ± 13	187 ± 6^{***+++}	230 ± 6^{+++}

Values represent means ± S.E.M. of 5 animals per group.

*** $P < 0.002$ compared to obese animals of the same adrenal status.

+++ $P < 0.002$ compared to intact animals of the same phenotype.

Food intake was measured daily. Energy intake was calculated from the theoretical energy content of the diet (section 2.4.2).

4.7 The Effect of Adrenalectomy and High Fat Feeding on the Body Compositions of Lean and Obese Zucker Rats.

Figure 4.7 illustrates the changes in body composition in lean and obese rats which occurred after adrenalectomy. Both groups of lean animals had higher protein and water and lower fat contents than the equivalent obese groups. Adrenalectomy had no effect on body composition in lean animals, but significantly increased protein content in obese rats.

4.8 The Effects of Adrenalectomy and High Fat Feeding on the Energetic Efficiency of Lean and Obese Zucker Rats.

Table 4.8.1 shows the energetics of HF fed intact and adrenalectomised animals. Cumulative energy intake was greater in both obese groups compared to the equivalent lean group and was reduced by adrenalectomy in both lean and obese animals. These observations reflect the summation of the daily energy intakes show in table 4.6.1. Carcass energy contents at the end of the experiment were greater in both obese groups, but adrenalectomy significantly reduced obese carcass energy content. Body energy gain was elevated in obese intact animals but was normalised in this phenotype by adrenalectomy. Energy gain in lean animals was unaffected by adrenalectomy. Energy expenditure was greater in both obese groups and was significantly reduced by adrenalectomy in both phenotypes. Gross energetic efficiency was significantly greater in obese intact animals compared to lean animals of the same adrenal status and was significantly reduced by adrenalectomy to a similar level seen in lean adrenalectomised animals. Adrenalectomy did not affect energetic efficiency in lean animals.

Table 4.8.2 outlines the energy cost of growth (energy expenditure per weight gain) and the changes in energy density in HF fed animals. The energy cost of growth was low in intact animals compared to the intact lean group, but was elevated to lean levels by adrenalectomy. Carcass

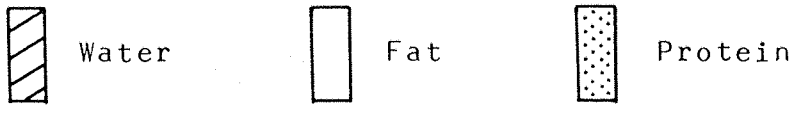
Figure 4.7.1 The Effects of Adrenalectomy and High Fat Feeding on the Body Composition of Lean and Obese Zucker Rats.

Dried, homogenised carcasses were analysed as described in sections 2.7.1, 2.7.3 and 2.7.4 for water, fat and nitrogen content, respectively. The body compositions presented are expressed as a percentage of wet carcass weight.

Bars represent means $\pm$ S.E.M. for each constituent for 5 animals in each group.

** $P < 0.01$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

+ $P < 0.05$ compared to intact animals of the same phenotype.



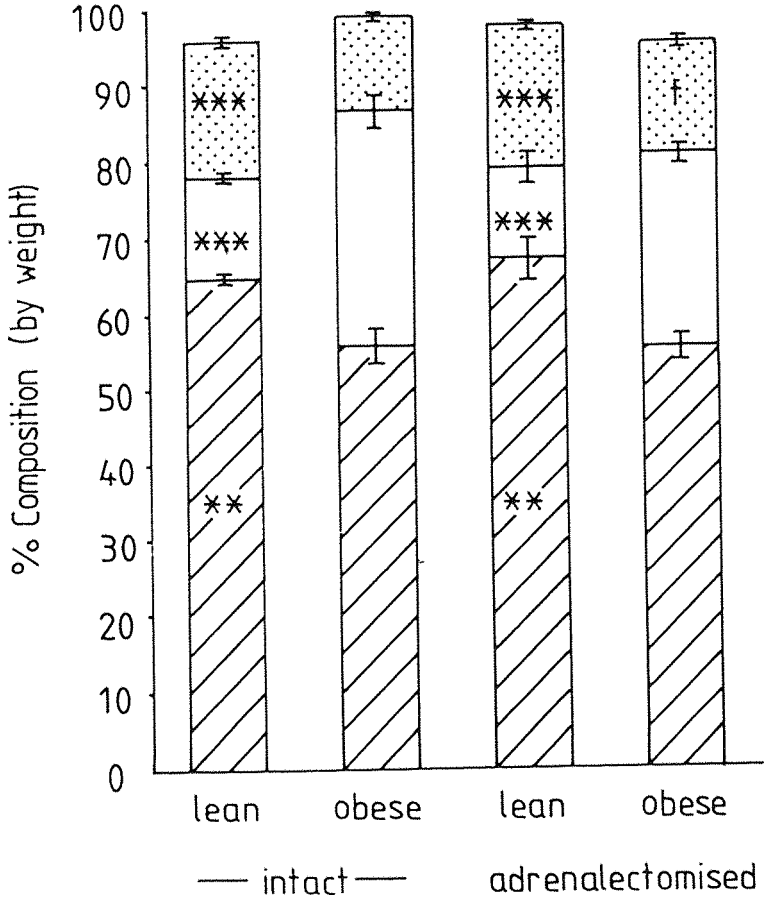

100
90
80
70
60
50
40
30
20
10
0
% Composition (by weight)
***
***
**
***
***
**
lean
obese
lean
obese
intact
adrenalectomised

Table 4.S.1 The Effects of Adrenalectomy and High Fat Feeding on the Energetic Efficiency of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Cumulative Energy Intake (kJ)	$3070 \pm 47^{***}$	4535 ± 45	$2435 \pm 70^{+++}_{***}$	$2991 \pm 71^{+++}$
Final Carcass Energy Content (kJ)	$1300 \pm 51^{***}$	2621 ± 196	$1049 \pm 121^{***}$	$1681 \pm 52^{+++}$
Energy Gain (kJ)	$619 \pm 51^{**}$	1480 ± 196	552 ± 121	$617 \pm 52^{++}$
Energy Expenditure (kJ)	$2450 \pm 39^{**}$	3059 ± 156	$1883 \pm 57^{***}_{+++}$	$2374 \pm 56^{++}$
Gross Efficiency (%)	$20.1 \pm 1.5^{*}$	32.5 ± 4.0	22.2 ± 4.4	$20.6 \pm 1.5^{+}$

Values represent means $\pm$ S.E.M. of 5 animals per group.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

\+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of the same phenotype.

Carcass energy contents were determined from body compositions as described in section 2.7. Gross energetic efficiency was calculated as $\frac{\text{Body energy gain}}{\text{Cumulative Energy Intake}} \times 100$

Table 4.8.2 The Effects of Adrenalectomy and High Fat Feeding on the Energy Cost of Growth and Body Energy Density of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	INTACT	LEAN	INTACT
Energy Expenditure per Weight Gain ($kJ.g^{-1}$)	$35.6 \pm 1.0^{*}$	29.5 ± 1.9	42.5 ± 3.3	$38.6 \pm 1.5^{++}$
Final Carcass Energy Density ($kJ.W^{-0.75}$)	$28.43 \pm 1.06^{***}$	50.12 ± 3.12	$25.98 \pm 2.5^{***}$	$40.95 \pm 1.22^{+}$
Initial Carcass Energy Density ($kJ.W^{-0.75}$)	$22.02 \pm 0.45^{\phi\phi\phi}$	$38.44 \pm 1.29^{\phi\phi}$	$17.28 \pm 0.34^{\phi\phi}$	36.65 ± 1.75

Values represent means $\pm$ S.E.M. of 5 animals in each group.

* $P < 0.05$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

\+ $P < 0.05$, ++ $P < 0.01$ compared to intact animals of the same phenotype.

$\phi\phi$ $P < 0.01$, $\phi\phi\phi$ $P < 0.002$ compared to final body energy density of the same group.

energy density at the end of the experiment was significantly lower in both lean groups compared to the respective obese group, but adrenalectomy lowered final carcass energy density in obese animals only. All groups, except the obese adrenalectomised animals significantly increased their energy density over the 13 days experimental period.

4.9 The Effects of Adrenalectomy and High Fat Feeding on Tissue Weights and Serum Metabolites in Lean and Obese Zucker Rats.

Inguinal fat pad weights, liver weights and serum glucose insulin and corticosterone concentrations of HF fed rats are shown in table 4.9.1. Inguinal fat pad weight was significantly higher in both obese groups compared to the equivalent lean groups, but adrenalectomy reduced fat pad weight in both phenotypes. Liver weight in intact obese rats was greater than that of lean intact rats, as was seen in HC fed rats, but unlike HC fed rats, HF fed lean and obese rats' liver weights were reduced by adrenalectomy. Glucose levels in the serum were unaffected by either phenotype or adrenal status, but the elevated insulin levels seen in obese intact rats were reduced by adrenalectomy but were still elevated compared to lean adrenalectomised rats. Adrenalectomy did not significantly reduce insulin levels in lean rats. Corticosterone levels were significantly higher in obese intact rats compared to lean rats.

4.10 The Effects of Adrenalectomy and High Fat Feeding on BAT of Lean and Obese Zucker Rats.

Table 4.10.1 shows the effects of adrenalectomy and high fat feeding on BAT composition in lean and obese rats. Intact obese rats had heavier interscapular BAT depots than their lean counterparts, but adrenalectomy reduced BAT depot weights in obese animals to a level not significantly different from adrenalectomised lean animals. Lean BAT depot weight was unaffected by adrenalectomy. The reduced depot protein and mitochondrial protein content seen in intact obese rats compared to the intact lean group was normalised by adrenalectomy. Depot and mitochondrial

Table 4.9.1 The Effects of Adrenalectomy and High Fat Feeding on Tissue Weights and Serum Metabolites of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
Inguinal fat pad weight (g)	$0.69 \pm 0.06^{***}$	2.00 ± 0.23	$0.28 \pm 0.02^{***+++}$	$0.81 \pm 0.09^{+++}$
Liver weight (g)	$9.50 \pm 0.36^{*}$	11.68 ± 0.65	$6.90 \pm 0.37^{+++}$	$6.18 \pm 0.74^{+++}$
Serum Glucose ($mg.100^{-1}$)	118 ± 3	135 ± 9	124 ± 2	128 ± 1
Insulin ($ng.ml^{-1}$)	$6.54 \pm 1.59^{***}$	41.26 ± 6.32	$4.11 \pm 0.72^{***}$	$15.40 \pm 1.37^{++}$
Corticosterone ($ng.ml^{-1}$)	$97 \pm 18^{*}$	150 ± 13	n/d	n/d

Values represent means $\pm$ S.E.M. of 5 animals per group.

* $P < 0.05$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of the same phenotype.

Serum assays were performed as described in sections 2.9.2, 2.9.4 and 2.9.5. n/d denotes not detectable.

Table 4.10.1 The Effects of Adrenalectomy and High Fat Feeding on BAT Composition of Lean and Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
BAT depot weight (g)	$0.55 \pm 0.04^{***}$	1.13 ± 0.10	0.48 ± 0.04	$0.72 \pm 0.10^{+}$
BAT Depot protein content ($mg.depot^{-1}$)	$49.9 \pm 2.3^{*}$	36.7 ± 5.3	54.4 ± 3.8	$53.8 \pm 1.8^{++}$
BAT Mitochondrial protein content ($mg.depot^{-1}$)	$20.1 \pm 1.4^{***}$	12.5 ± 0.6	21.5 ± 1.3	$20.8 \pm 1.3^{++}$

Values represent means $\pm$ S.E.M. of 5 animals per group.

* $P < 0.05$, *** $P < 0.002$ compared to obese animals of the same adrenal status.

\+ $P < 0.05$, ++ $P < 0.01$ compared to intact animals of the same phenotype.

Protein assays were performed as described in section 2.10.2. Depot mitochondrial protein content was calculated from succinate cytochrome c oxidoreductase activities as described in section 2.14.

protein contents were unaffected by adrenalectomy in lean animals.

Table 4.10.2 shows the effects of HF feeding and adrenalectomy on BAT thermogenic capacity in lean and obese rats. Intact lean animals had significantly higher specific BAT mitochondrial GDP binding, depot GDP binding, mitochondrial 32K protein concentration and depot 32K protein content compared to intact obese rats. Adrenalectomy significantly increased mitochondrial and depot GDP binding in both lean and obese rats, so although BAT mitochondrial GDP binding was improved in obese adrenalectomised animals it was still lower than that of lean adrenalectomised animals. Mitochondrial 32K concentrations and depot 32K protein content were both increased by adrenalectomy in obese, but not lean animals. Post-adrenalectomy levels of mitochondrial 32K protein concentration and depot 32K protein content were similar for both phenotypes. The molar binding ratio of GDP to 32K protein was unaffected by either phenotype or adrenalectomy.

Figure 4.10.1 shows the mitochondrial proteins and mitochondrial 32K protein visualised by polyacrylamide gel electrophoresis and Western blotting respectively. The upper photograph (a) shows the polyacrylamide gel visualising all the mitochondrial proteins from solubilised BAT mitochondria from lean intact, obese intact, lean and adrenalectomised and obese adrenalectomised rats (Lanes 2-4 respectively). Lanes 0 and 1 show molecular weight markers and purified 32K protein respectively. The lower photograph (b) shows the Western blots of the same samples and is numbered in the same manner. As seen with the HC fed animals, the 32K band on the gel was virtually impossible to pick out. The Western blot showed clearly the presence of 32K uncoupling protein in all lanes (lower band). As mentioned previously, the upper band was probably dimerised uncoupling protein. There again seemed to be less 32K protein present in the intact obese animals (lane 3) compared to intact lean (lane 2) and adrenalectomised obese (lane 4) animals. This observation was again confirmed bu the radioimmunoassay of mitochondrial 32K protein concentration (table 4.10.2).

Table 4.10.2 The Effects of Adrenalectomy and High Fat Feeding on BAT Mitochondrial GDP Binding and 32K Protein Concentration.

	INTACT		ADRENALECTOMISED	
	LEAN	OBESE	LEAN	OBESE
[^{3}H]-GDP Binding (pmols.mg protein^{-1})	305 ± 18*	225 ± 20	409 ± 23*++	326 ± 25+
BAT depot [^{3}H]-GDP binding (pmols.depot^{-1})	6542 ± 839**	3134 ± 274	8665 ± 410**	6700 ± 608+++
Mitochondrial 32K protein concentration (µg.mg protein^{-1})	33.1 ± 2.1*	24.2 ± 1.6	41.9 ± 4.4	46.4 ± 3.9+++
BAT depot 32K protein (µg.depot^{-1})	757 ± 164*	303 ± 25	912 ± 142	966 ± 123+++
GDP:32K Molar ratio	0.30 ± 0.21	0.30 ± 0.03	0.33 ± 0.04	0.23 ± 0.02

Values represent means ± S.E.M. of 5 animals per group.

* $P < 0.05$, ** $P < 0.01$ compared to obese animals of the same adrenal status.

+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to intact animals of same phenotype.

BAT mitochondria were prepared, GDP binding assays performed and 32K protein concentration determined as described in sections 2.12, 2.13 and 2.18 respectively. Depot GDP binding and 32K protein concentrations were calculated on the basis of 100% mitochondrial recovery as assessed from succinate cytochrome c oxidoreductase activities, as described in section 2.14.

Figure 4.10.1 Visualisation of Mitochondrial Protein and 32K Protein of Solubilised BAT Mitochondria from Adrenalectomised High Fat Fed Lean and Obese Zucker Rats by Polyacrylamide Gel Electrophoresis and Western Blotting.

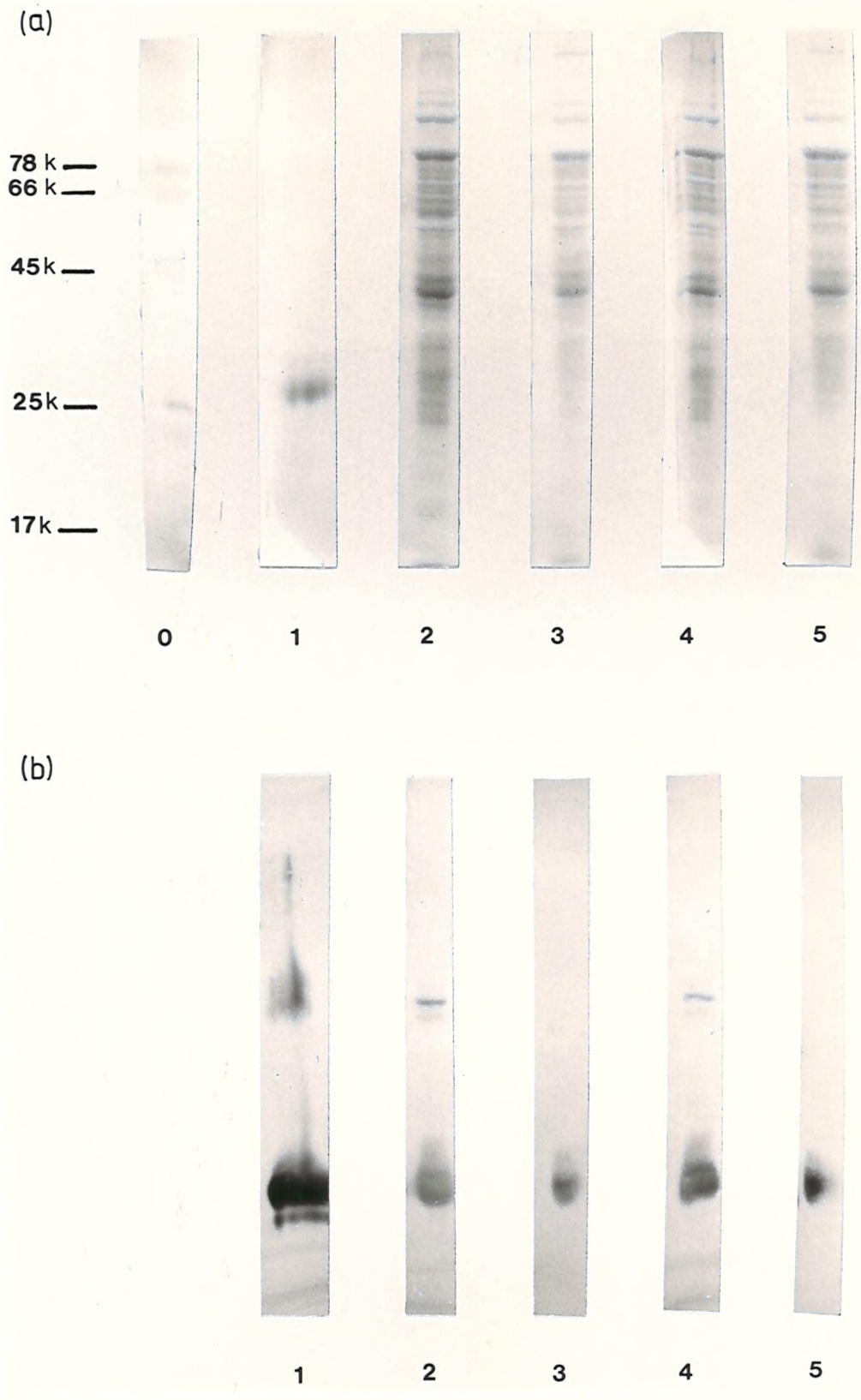
Solubilised BAT mitochondria from intact and adrenalectomised lean and obese Zucker rats fed a high fat diet were applied to two 12% polyacrylamide slab gels and run as described in section 2.19. One of each gel pair was stained with Coomassie blue R for the visualisation of all mitochondrial proteins. Proteins on the second gel were transferred to nitrocellulose paper and 32K protein was visualised by specific antibody binding, as described in section 2.20. This is shown in plate (b)

Lanes are as follows:-

0. -Electran molecular weight markers (12,800-78,000 M.W.)
1. -Purified 32K protein (see section 2.16).
2. -Solubilised BAT mitochondria from lean intact animals.
3. -Solubilised BAT mitochondria from obese intact animals.
4. -Solubilised BAT mitochondria from lean adrenalectomised animals.
5. -Solubilised BAT mitochondria from obese adrenalectomised animals.

a. Polyacrylamide Gel.

b. Western Blot.


(a)
78 k
66 k
45k
25k
17k
0
1
2
3
4
5
(b)
1
2
3
4
5

4.11 Summary and Discussion.

The changes which occurred in body weight gain and energy intake due to adrenalectomy were similar on either diet for both phenotypes. Intact obese animals tended towards a greater weight gain on the HF diet, but direct comparison between the dietary regimes is difficult as the animals were of different ages at the beginning of the experiment, HF animals being 1 week younger (the Bo group was of an intermediate age). The greater weight gain of the HF fed obese intact animals was associated with a greater inguinal fat pad weight and a slightly greater carcass fat content. The daily energy intakes of each group were similar for each diet, allowing for body weight differences. Adrenalectomy significantly reduced energy intake in both phenotypes and on both diets. It is an interesting point that intact obese animals on each diet ate similar amounts of food (by weight) resulting in a greater energy intake in the HF group, but after adrenalectomy energy intakes on each diet were similar in obese animals due to a greater reduction in the weight of the HF diet consumed. This suggests that the normal regulation of energy intake is restored in fa/fa rats after adrenalectomy. The reduction in food intake seen in all adrenalectomised groups of both phenotypes fed on either diet could be due to the impairment of the corticosterone dependent α_2-noradrenergic feeding drive (Leibowitz et al., 1984). The decrease in body weight, inguinal fat pad weight, and body fat (the latter in the HC fed animals only) seen in lean adrenalectomised animals was probably due to reduced food intake. Adrenalectomy has previously been shown to normalise food intake, weight gain and body composition of fa/fa rats fed on a normal laboratory chow (high carbohydrate) diet (Yukimura et al, 1978; Freedman et al., 1985, 1986a), and it has been demonstrated in the present study that similar changes occur on a high fat diet. Body fat in adrenalectomised obese animals was reduced by a similar percentage on the HC and HF feeding regimes (4.9% and 5.6% reduction respectively) but only the HC decrease was statistically significant. Body protein content was significantly increased by adrenalectomy of obese rats on both diets.

A significant improvement in carcass energy content, energy gain and gross energetic efficiency was seen after adrenalectomy in obese rats on both diets, as was also seen by Marchington et al., (1983). Carcass energy contents, energy expenditure, energy density and the energy cost of growth were similar for equivalent groups of animals on either diet. This suggests that the type of diet did not affect the ability of the animals to respond to adrenalectomy. The energy cost of growth (energy expenditure per gram weight gain) was increased by adrenalectomy of obese animals in comparison to their intact controls, but the increase was only significant in HC fed animals. Adrenalectomy significantly reduced energy expenditure in all groups except HC fed obese animals (reduction not significant). This finding is again similar to that seen by Marchington et al. (1983).

The changes observed in inguinal fat pad weights mirrored those changes seen in body fat in all groups and on both diets, except the reduction in WAT mass due to adrenalectomy, was significant in both obese groups. Liver weight was significantly greater in intact obese groups compared to lean animals on the same diet, but this weight difference was removed by adrenalectomy. Liver weight was significantly reduced by adrenalectomy in both phenotypes only on the HF diet. Liver weights were reduced by adrenalectomy by 27% and 47% for lean and obese HF fed rats respectively. Hepatic fatty acid synthesis is markedly reduced in lean and obese Zucker rats fed a high fat diet (Lemonnier et al., 1974). The reduction in liver weight may be partially due to a reduction in steroid-dependent glycogen synthesis (Friedman et al., 1967) and a depletion of glycogen stores, which might be more pronounced in the HF fed animals with a lower dietary carbohydrate intake.

Overall, it appears that energy balance is improved by adrenalectomy in obese rats in a manner independent of dietary composition. This is in contrast to the findings of Smith and Romsos (1985) who found that adrenalectomy did not affect the development of high-fat fed diet-induced obesity in ob/ob mice. It should, however, be noted that

the composition of the diets on the two studies varied. The diet used by Smith and Romsos was 60% fat by energy, and the one used here was 34% fat. It is difficult to compare HC and HF fed animals directly, as the HF group were about one week younger and so proportionaly lighter than the HC animals, but it appears that the post-adrenalectomy energy intakes were similar in obese rats although intact obese animals tended to eat more of the high fat diet (intact HC, 4011 ± 138 vs HF, 4535 ± 45; adrenalectomised HC, 3208 ± 197 vs HF, 2911 ± 71 MJ). Smith and Romsos (1985) observed only slight reductions in food intake after adrenalectomy of ob/ob mice fed the high fat diet. Although this reduction in intake was not significant at 6-9 weeks of age it was significant at 3-6 weeks. It has been demonstrated that adrenalectomy in obese mice is more effective in younger animals (maintained on a stock diet), but even then the procedure does not entirely reverse the abnormal metabolism or body composition of ob/ob mice (Debuc and Wilden, 1986). These results suggest that although the effects of adrenalectomy in ob/ob mice may be diet dependent, the fa/fa rats seems to respond equally well to adrenalectomy on both HC and HF diets, and the differences observed in the responses of these two models of genetic obesity may be due to differences in the aetiology of their syndromes.

Serum glucose levels were unaffected by phenotype, adrenalectomy or diet. The elevated insulin levels seen in obese animals on both diets were reduced by adrenalectomy, but were still higher than lean adrenalectomised levels on both diets. Adrenalectomy significantly reduced insulin levels in lean rats only on the HC diet. The insulin levels of all groups were significantly lower in the HF fed animals possibly due to the greater effect of dietary carbohydrate compared to fat on stimulating insulin release (Mayhew et al., 1969).

The response of BAT to adrenalectomy in lean and obese rats was similar on both diets. BAT depot weight was unaffected by adrenalectomy or diet in lean animals, but

BAT weight of obese animals showed slightly different responses to adrenalectomy on the two diets, in that BAT depot weight was unchanged by adrenalectomy in obese HC fed animals, but was significantly reduced in the HF fed group. BAT depot weight in the HF fed intact obese group was apparently heavier than all other obese groups, probably due to excess dietary lipid deposition in the inactive tissue. Total BAT depot protein was similar in equivalent groups on either diet, with the total protein content of the obese intact animals reduced compared to intact lean values. Adrenalectomy improved BAT depot protein content significantly in obese rats on both diets. These observations are similar to the changes normally seen in BAT composition of Zucker rats after adrenalectomy (Holt and York, 1982; Marchington et al., 1983). The mitochondrial protein content of BAT depots reflected the levels of total protein in each treatment group, but mitochondrial content in the HF fed animals tended to represent a smaller percentage of the tissue protein (HC - 60%, 44%, 72% and 70%; HF - 40%, 34%, 39% and 39% for intact lean, intact obese, adrenalectomised lean and adrenalectomised obese groups respectively). This seems to represent a somewhat larger proportion of BAT protein than reported by Marchington et al. (1983) (14%; lean intact chow-fed Zucker rats, 8 weeks old at sacrifice) but is similar to that reported by Nedergaard et al (1983) (44% Sprague-Dawley rats, 20% fat diet by energy, 6 weeks old), and so could possibly be an effect of the higher energy density diets. BAT mitochondrial GDP binding was reduced in both intact obese groups compared to intact lean animals, but was increased by adrenalectomy on both diets. Adrenalectomy also increased GDP binding in lean animals, although the increase was only significant in HF fed rats. The increase in BAT GDP binding after adrenalectomy in lean rats is normally masked by the reduction in food intake and concomitant decrease in BAT activity. Pair-feeding studies revealed that sham adrenalectomised lean animals pair fed to the level of adrenalectomised rats showed a 24% decrease in BAT mitochondrial GDP binding (Holt, 1984). Basal

levels of BAT mitochondrial GDP binding appear to be elevated in both lean and obese animals on both diets. However, BAT mitochondrial GDP binding values for chow fed animals from other experiments performed during the same time were also elevated ($344^{+}33$ and $212^{+}15$ for lean and obese rats respectively). This apparent elevation may have been due to a seasonal variation, or more likely a batch of defatted BSA more potent than usual, leading to higher GDP binding levels.

A previously unobserved effect of adrenalectomy was the change in BAT mitochondrial 32K protein concentration in obese rats. Intact obese animals had lower concentrations of mitochondrial 32K protein than the respective lean groups on both diets, and a reduced depot 32K protein content. Depressed 32K protein levels in obese Zucker rats have only previously been observed at 12 weeks of age (Ashwell et al., 1985). At the end of this study, animals were between 7 and 8 weeks old (HF fed animals younger) which could suggest that a decrease in 32K protein levels occurs between 5 and 7 weeks of age, as normal 32K protein levels have been observed in 5 week old obese Zucker rats (Ashwell et al., 1985). The effect of adrenalectomy on 4-5 week old obese Zucker rats was to apparently normalise 32K protein levels within 20 days. If 32K protein levels were normal at 4-5 weeks, then the effect of adrenalectomy was to prevent a decrease in the concentration of the protein that normally occurs with age. Alternatively, the higher levels of protein seen in lean animals and adrenalectomised obese animals may reflect an effect of the high energy density diets (15.29 $MJ.kg^{-1}$ and 17.94 $MJ.kg^{-1}$ for HC and HF respectively) compared to the effect of chow feeding (10.79 $MJ.kg^{-1}$) to which obese intact animals were unable to respond. Obese Zucker rats at 4 weeks of age increased mitochondrial 32K protein levels in response to cold (Ashwell et al., 1985) and it appears that they may be unable to do this in response to diet. The increase in 32K protein concentration observed in adrenalectomised obese rats is consistent with the suggestion that sympathetic activity is responsible for increasing the amount of the protein in

BAT (Mory et al., 1984), as adrenalectomy is known to also increase SNS activity in BAT (York et al., 1985b). It has been proposed that the increased levels of 32K protein and 32K protein mRNA that are observed after 7 day cold exposure or 9 day noradrenaline infusion, are triggered by noradrenaline induction of the mRNA encoding the 32K protein (Bouillaud et al., 1984). It is possible, therefore, that the increase in SNS activity in BAT associated with adrenalectomy is sufficient to increase mitochondrial 32K protein levels in obese rats to lean levels.

The observation that 5-week old Zucker rats have depressed levels of BAT mitochondrial GDP binding in the presence of apparently normal levels of 32K protein (Ashwell et al., 1985), but that by 7-8 weeks of age BAT mitochondrial GDP binding and 32K protein concentrations are both depressed, suggests that an alteration in the degree of masking of 32K protein occurs. At 7-8 weeks of age there was no significant difference between lean and obese or intact and adrenalectomised groups in the molar binding ratio of GDP to 32K protein on each diet, suggesting that the degree of masking was similar for all groups. The decrease in mitochondrial 32K protein concentration in obese rats with age, accompanied by a decrease in masking, may account for the impaired ability of older fa/fa rats to respond to cold stimulation.

Although the degree of masking was not changed by either phenotype or adrenal status, HC fed rats had a consistently higher molar binding ratio of GDP to 32K protein compared to each equivalent HF fed group (pooled data: HC, 0.41 ± 0.03; HF, 0.29 ± 0.02: $P < 0.01$). This suggests that diet, but not phenotype, affects the degree of masking of 32K protein, such that HC fed animals utilised a greater proportion of the available 32K protein than HF fed rat. Mitochondrial 32K protein concentration was similar on both diets, but GDP binding levels were consistently (although not significantly) higher in HC fed rats (15%, 17%, 7% and 21% for intact lean, intact obese, adrenalectomised lean and adrenalectomised obese rats respectively). It is unlikely

that this is an effect of age, as the HF rats were younger and if anything would thus be expected to have a slightly higher GDP binding level (York et al., 1984). The reduced mitochondrial protein content of HF fed rats accounts for the lower depot GDP binding and 32K protein concentrations in these animals. It has been suggested that the availability of dietary essential fatty acids can affect BAT function. High fat diets (20% fat energy) containing excess essential fatty acids stimulate BAT mitochondrial GDP binding to a greater extent than in animals maintained on a similar energy dense diet with normal essential fatty acid content (Nedergaard et al., 1983). No measurements of 32K protein concentrations were made in these studies, so it is impossible to say whether availability of excess essential fatty acids also caused an increase in 32K protein content. It is possible that a high availability of essential fatty acids may lead to an increase in 32K protein unmasking and hence increased mitochondrial GDP binding. The observation that unmasking was depressed in the HF fed animals in this study in the presence of similar levels of mitochondrial 32K protein suggests that any differences which may have been caused by essential fatty acids could have been masked by the effects of a high fat diet per se. Both diets used in this study contained corn oil, which is not considered as being essential fatty acid deficient (Morgan et al., 1981), however, the increased content of oil in the high fat diet could be interpreted as giving an excess of essential fatty acids. The effects of high fat diets on BAT mitochondrial GDP binding may simply be due to effects of fat upon the activity of the sympathetic nervous system (Schwartz et al., 1983), however, if the type of fat also plays a role in masking and unmasking of 32K protein within the mitochondria then this may account for the difference in response to cafeteria or semisynthetic high fat diets of the same basic energy composition, observed by Rothwell et al. (1985c).

CHAPTER 5 THE EFFECTS OF NALOXONE ON BAT THERMOGENESIS AND FOOD INTAKE IN LEAN AND OBESE ZUCKER RATS.

Naloxone is an opiate antagonist with potent actions against morphine and β-endorphin stimulated events. It acts primarily through the blockade of μ-type opiate receptors (see Clark 1981) and as such has been widely used as a β-endorphin antagonist in investigation of the effects of this endogenous opioid on physiological systems. The effects of naloxone are wide ranging. Naloxone is clinically regarded as a pure opiate antagonist and as such is used in the treatment of narcotic overdose. Unlike other opiate antagonists it does not depress respiratory function. Naloxone administered to opiate addicts can cause severe withdrawal symptoms (Martin 1976). Naloxone has been used to treat hyperphagic patients with Prader-Willi syndrome and obese patients, although there are conflicting reports of the effectiveness of naloxone to reduce food intake in humans (Kyriakides et al., 1980; O'Brien and Stunkard, 1982). Naloxone blocks the effects of a variety of antinociceptive agents in rodents, possibly as an effect of drug displacement by naloxone from opiate receptors (see Sawynok et al., 1979). Naloxone has been shown to suppress food and water intake in rats (Holtzmann 1974; Frenk and Rogers, 1979), opposite effects to those caused by centrally administered β-endorphin (Grandison and Guidotti, 1977). Naloxone (and therefore β-endorphin) is thought to exert its effects on food intake at a central site by activation of the vagus nerve, as subdiaphragmatic vagotomy and methylatropine (an atropine derivative with mainly peripheral effects) both abolish naloxone induced hyp o phagia (Jones and Richter, 1981). Hypothalamic obese rats reduce their food intake in response to naloxone (King et al., 1979) so it appears that opiate receptors located in the VMH are not essential to the opioid effects of feeding. Morley (1980) suggests that the endogenous opioids are part of a system generating a tonic stimulus for feeding located in the lateral hypothalamus, so naloxone would be expected to reduce the feeding drive associated with this mechanism.

Feeding elicited by electrical stimulation of the LH is reduced by vagotomy, so a common effector in the apparent mechanism of action of naloxone and that of the LH on food intake exists.

Naloxone depresses food intake in lean and obese Zucker rats, but the effect is accentuated in obese animals (Thornhill et al., 1982; McLaughlin and Baile, 1984a). The reduction in food intake stems from a reduced meal size rather than meal frequency, suggesting that naloxone may increase satiety rather than reduce hunger (McLaughlin and Baile, 1984a). Most evidence, however, suggests that β-endorphins increase hunger in satiated rats (see Morley and Levine, 1983), and in studies where naloxone has been shown to reduce food intake in humans, subjects reported decreased hunger sensations before meals (Krotiewski et al., 1983). It has been shown that levels of β-endorphin are higher in the pituitaries and plasma of genetically obese (ob/ob) mice and rats (fa/fa) compared to their lean littermates (Margules et al., 1978; Recant et al., 1983) and the suggestion has been made that the elevated levels of β-endorphin may be responsible for the hyperphagia of these obese rodents. Castonguay and Stern (1983) suggested that the decrease in food intake after adrenalectomy of obese Zucker rats was dependent upon the inhibition of β-endorphin activity by ACTH, the levels of which are increased after adrenalectomy.

The endogenous opioids may also influence energy balance through their effects on thermoregulation. Administration of morphine and β-endorphin, as well as other opioids such as met- and leu-enkephalin and γ-endorphin, has been reported to affect body temperature. The effects are variable, according to dose, site of injection (peripheral, intrahypothalamic or intracerebroventricular) and the ambient temperature at which the experiment was carried out. Hyperthermic effects of morphine and βendorphin (Cox et al., 1976; Lin et al., 1979, respectively) occur at low doses and at a wide range of temperatures (2-35^{o}C, Holaday et al., 1978a; Tache et al., 1979). Higher doses

of morphine (Clark and Clark, 1980) or β-endorphin (Tache et al., 1979) at cool (2-10°C) or normal (20-27°C) temperatures induce hypothermia in rats. The majority of evidence suggests that opiates and opioids have a hyperthermic effect at ambient temperatures (see Clark 1981). Naloxone will inhibit the hypo- and hyperthermic effects of morphine (Clark and Clark, 1980) and the hyperthermic effects of β-endorphin. In addition, it has an independent hypothermic effect (Stewart and Eikelboom, 1979), however, naloxone can cause hyperthermic in rats at high ambient temperatures (Holaday et al., 1978b; Thornhill et al., 1980).

The experiments described in this section were carried out to clarify the effect of naloxone on thermogenesis in lean and obese rats, and to investigate the possible role of BAT in this response. The acute and chronic effects of naloxone on food intake were also investigated in lean and obese Zucker rats.

5.1 The Time Course of the Response to Naloxone in Lean and Obese Zucker Rats.

The effect of a single dose of naloxone (1mg.kg^{-1} body weight,s.c., in 0.1ml saline) on BAT mitochondrial GDP binding, and food intake were measured over a 6 hour period. The changes in rectal temperature, serum glucose, insulin and corticosterone were measured over the first two hours of the experimental period. Measurements were made at 0, 40, 80 minutes and 2, 4 and 6 hours after injection of naloxone.

Results.

Figure 5.1.1 illustrates the changes in BAT mitochondrial GDP binding throughout the 6 hour period. GDP binding was elevated in lean animals at the 80 minute time point, and remained increased over zero time throughout the remainder of the experiment, however, only at 80 minutes and 2 hours was the elevation significant. There were no significant changes in mitochondrial GDP binding in naloxone treated obese rats at any time point of the experimental period. Figure 5.1.2 shows the changes in rectal temperature that occurred over the first 2 hours. Both lean and obese rats show a slight depression in rectal temperature at 40 minutes, but in neither phenotype was the depression significant. Only at zero time were the rectal temperatures of the obese rats lower than the temperatures seen in the lean group. Figure 5.1.3 shows the changes in serum levels of glucose, insulin and corticosterone over the initial 2 hour period of naloxone administration. Neither glucose nor insulin levels were affected by naloxone in either phenotype over this period. There were, however, significant changes in corticosterone levels. Lean animals showed a depression in corticosterone at 80 minutes, coincident with the rise in mitochondrial GDP binding levels. This depression in corticosterone levels in lean animals had returned to normal levels by 2 hours post-injection. Obese animals had elevated serum corticosterone concentrations at 40 minutes, but this too was a transient change as normal serum corticosterone

Figure 5.1.1 The Time Course of the Effects of Naloxone on BAT Mitochondrial GDP Binding in Lean and Obese Zucker Rats.

Lean and obese rats were injected with $1mg.kg^{-1}$ naloxone in 0.1ml saline, s.c., and killed at the times indicated. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively.

Values represent means $\pm$ S.E.M. of 7-10 animals in each group.

o Lean • Obese

* $P < 0.05$, ** $P < 0.01$ compared to zero time controls.

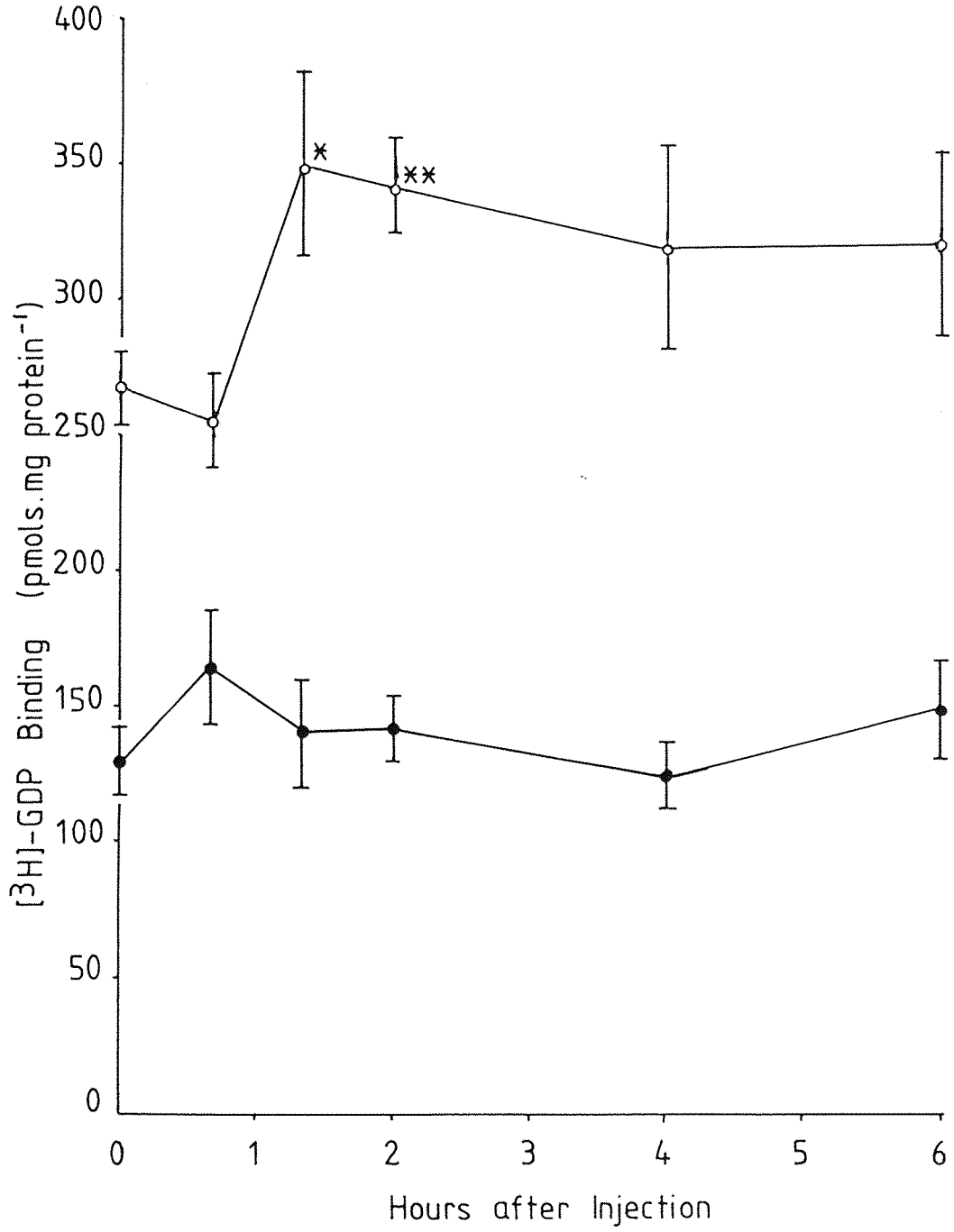

400
350
300
250
200
150
100
50
0
[3H]-GDP Binding (pmols.mg protein-1)
*
**
0
1
2
3
4
5
6
Hours after Injection

Figure 5.1.2 The Time Course of the Effect of Naloxone on Rectal Temperature in Lean and Obese Zucker Rats.

Lean and obese animals were injected with $1mg.kg^{-1}$ naloxone in 0.1ml saline, s.c., and killed at the times indicated. Rectal temperature was measured as described in section 2.3.2 (conscious animals) just before sacrifice.

Dotted line denotes zero time temperature for lean (L) and obese (O) animals.

Values represent means $\pm$ S.E.M. of 4 animals in each group.

o Lean • Obese

++ $P < 0.01$ compared to equivalent lean group.

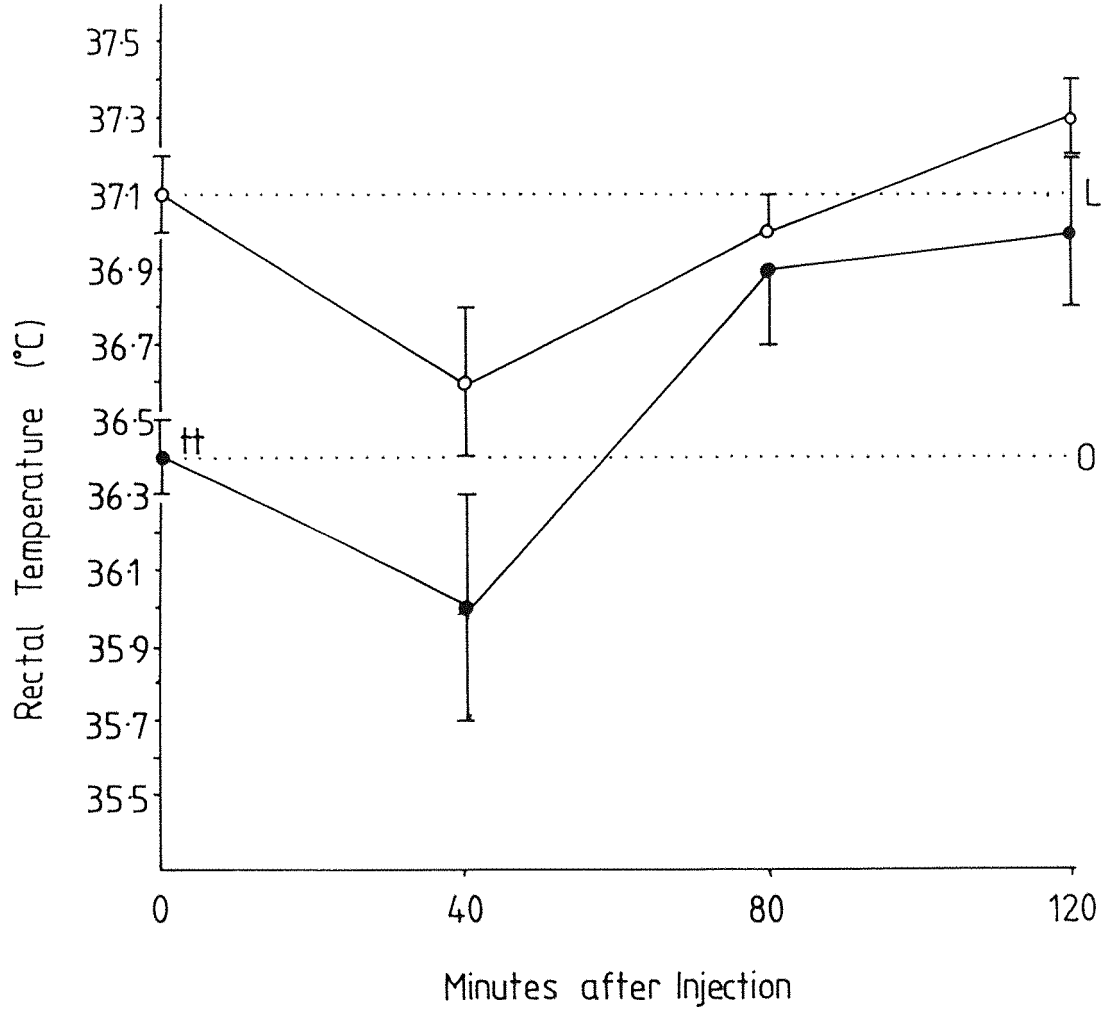

37·5
37·3
37·1
36·9
36·7
36·5
36·3
36·1
35·9
35·7
35·5
Rectal Temperature (°C)
tt
L
0
0
40
80
120
Minutes after Injection

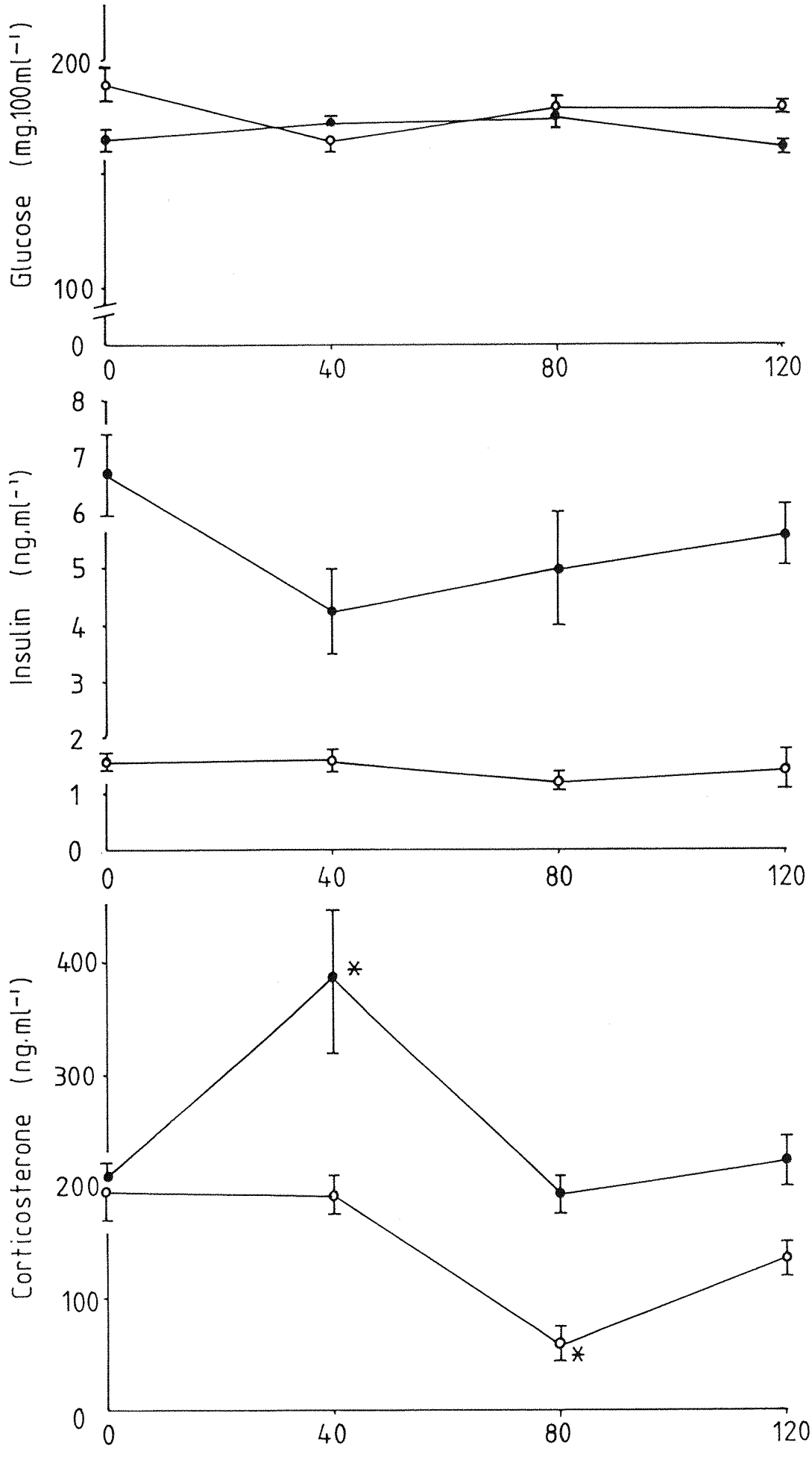
Figure 5.1.3 The Time Course of the Effects of Naloxone on Serum Metabolites of Lean and Obese Zucker Rats.

Lean and obese rats were injected with 0.1mg.kg^{-1} naloxone in 0.1ml saline, s.c., and killed at the times indicated. Animals were sacrificed by decapitation, trunk blood collected and serum prepared, as described in section 2.9.1. Serum concentrations of glucose, insulin and corticosterone were determined as described in sections 2.9.2, 2.9.4 and 2.9.5, respectively.

Values represent means $\pm$ S.E.M. of 3-6 animals in each group.

o Lean • Obese

* $P < 0.05$ compared to zero time group.


Glucose (mg.100ml⁻¹)
200
100
0
0
40
80
120
Insulin (ng.ml⁻¹)
8
7
6
5
4
3
2
1
0
0
40
80
120
Corticosterone (ng.ml⁻¹)
400
300
200
100
0
*
*
0
40
80
120

concentration in obese rats was observed at 80 minutes after naloxone injection.

Changes in food intake over the 6 hour experimental period are illustrated in figure 5.1.4. Food intake over any of the time periods indicated was not significantly affected by naloxone injection in lean animals, although there was a slight decrease in food intake in naloxone injected animals over the 0-40 and 0-80 minute periods. Obese animals' food intake was significantly lower in naloxone injected animals over the 0-80 and 0-120 minute periods. There was no significant difference between the food intakes of naloxone injected groups over the first three time blocks (0-40, 0-80 and 0-120 minutes).

5.2 The Effects of Adrenalectomy on the Acute Response to Naloxone in Lean and Obese Zucker Rats.

Adrenalectomy corrects many of the defects of the obese Zucker rat. Having demonstrated that adrenalectomy normalised the responses of obese rats to 2DG and diet, it was of interest to examine the effect of adrenalectomy on the response of BAT to naloxone.

5-6 week old lean and obese animals were adrenalectomised and maintained as described in section 2.6.2. 1 week after surgery, half the animals were injected with $1mg.kg^{-1}$ naloxone in 0.1ml saline, or 0.1ml saline, s.c., and sacrificed 80 minutes later. An 80 minute experimental period was chosen as it was the time point of maximal BAT stimulation in intact lean animals and was within the plasma half-life of naloxone (80-100 minutes).

Results.

Table 5.2.1 shows the effects of adrenalectomy on the responses of lean animals to naloxone. Adrenalectomy abolished the stimulatory effect of naloxone on BAT mitochondrial GDP binding in lean animals and had no effect on the responses of serum glucose or insulin to the drug. Table 5.2.2 shows the results of the experiment in obese

Figure 5.1.4 The Time Course of the Effect of Naloxone on Food Intake in Lean and Obese Zucker Rats.

Lean and obese animals were individually housed and injected with $1mg.kg^{-1}$ naloxone in 0.1ml saline, or o.1ml saline, s.c. Food intakes were measured over the periods indicated. (0-40 minutes; 0-80 minutes; 0-120 minutes; 0-240 minutes and 0-360 minutes).

Values represent means $\pm$ S.E.M. of 7-9 animals in each group.

 Saline treated animals.

 Naloxone treated animals.

* $P < 0.05$, *** $P < 0.002$ compared to saline injected controls of the same phenotype.

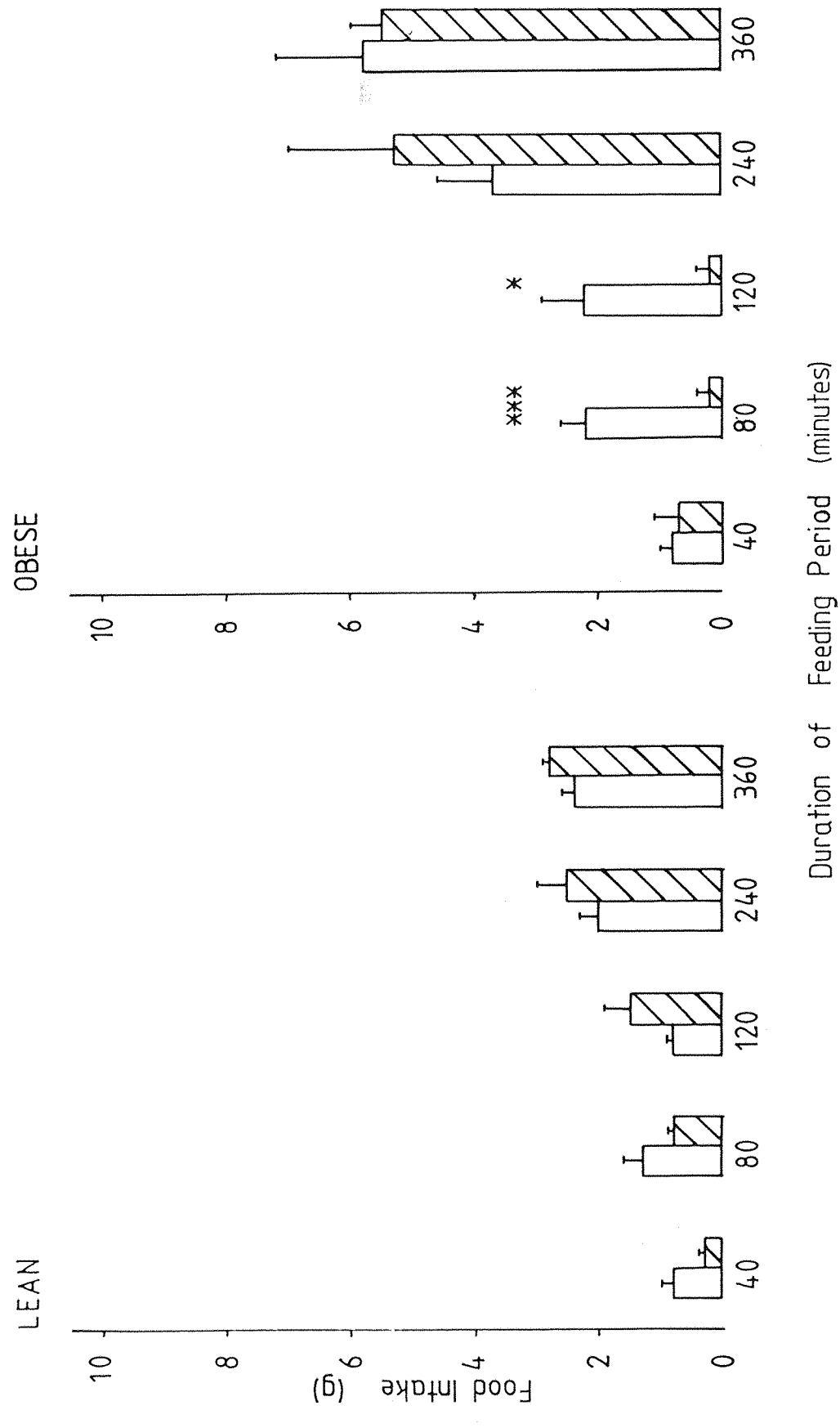

LEAN
OBESE
Food Intake (g)
0
2
4
6
8
10
40
80
120
240
360
***
*
Duration of Feeding Period (minutes)

Table 5.2.1 The Acute Effects of Naloxone on BAT and Serum Metabolites in Intact and Adrenalectomised Lean Zucker Rats.

	INTACT		ADRENALECTOMISED	
	SALINE	NALOXONE	SALINE	NALOXONE
[^{3}H] -GDP binding (pmols.mg protein^{-1})	267 ± 14 (10)	351 ± 34* (7)	257 ± 15 (7)	268 ± 14 (7)
Serum Glucose (mg.100ml^{-1})	184 ± 8 (6)	178 ± 4 (6)	187 ± 5 (7)	170 ± 7 (7)
Serum Insulin (ng.ml^{-1})	1.62 ± 0.12 (3)	1.20 ± 0.11 (3)	1.04 ± 0.15+ (7)	1.02 ± 0.16(7)
Serum Corticosterone (ng.ml^{-1})	195 ± 27 (3)	60 ± 15* (3)	n/d	n/d

Values represent the means ± S.E.M. for the number of animals shown in parenthesis.
n/d - not detectable.
* $P < 0.05$ compared to saline injected animals of the same adrenal status.
+ $P < 0.05$ compared to intact animals in the same treatment group.

Animals were adrenalectomised and maintained as described in section 2.6.2 and used 7 days later. Naloxone (1mg.kg^{-1}) or saline was injected s.c. in a 0.1ml volume 80 minutes prior to sacrifice. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Serum assays were performed as described in sections 2.9.2, 2.9.4 and 2.9.5.

Table 5.2.2 The Acute Effects of Naloxone on BAT and Serum Metabolites in Intact and Adrenalectomised Obese Zucker Rats.

	INTACT		ADRENALECTOMISED	
	SALINE	NALOXONE	SALINE	NALOXONE
$[^3H]$ GDP binding (pmols.mg protein^{-1})	130 ± 12 (9)	140 ± 18 (9)	258 ± 19^{+++} (9)	289 ± 22^{+++} (9)
Serum Glucose (mg.100ml^{-1})	164 ± 4 (3)	175 ± 4 (3)	189 ± 7^{+} (5)	199 ± 7^{+} (5)
Serum Insulin (ng.ml^{-1})	6.68 ± 0.73 (3)	4.96 ± 1.0 (3)	5.06 ± 0.77 (5)	3.46 ± 0.48 (5)
Serum Corticosterone (ng.ml^{-1})	208 ± 9 (3)	192 ± 17 (3)	n/d	n/d

Values represent means ± S.E.M. for the number of animals shown in parenthesis.
+ $P < 0.05$, +++ $P < 0.002$ compared to intact animals in the same treatment group.

Animals were adrenalectomised and maintained as described in section 2.6.2 and used 7 days later. Naloxone (1mg.kg^{-1}) or saline was injected s.c. in a 0.1ml volume 80 minutes prior to sacrifice. BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Serum assays were performed as described in sections 2.9.2, 2.9.4 and 2.9.5.

animals. Although adrenalectomy increased GDP binding levels in obese rats, no effect of naloxone was observed on GDP binding in these animals. No effect of naloxone was observed in either intact or adrenalectomised animals on serum glucose or insulin concentrations.

5.3 The Chronic Effects of Naloxone on 5 Month Old Lean and Obese Zucker Rats.

Acute naloxone treatment has been shown to reduce food intake in young obese, but not lean, Zucker Rats (section 5.1). The chronic effects of naloxone were investigated in adult (5-month old) lean and obese rats, to examine the effects of prolonged opiate blockade on food intake and body weight.

5-month old animals were individually housed and injected at 8 hourly intervals (at 10.00 hours, 18.00 hours and 02.00 hours) with $1mg.kg^{-1}$ body weight naloxone in $1ml.kg^{-1}$ body weight saline, or $1ml.kg^{-1}$ saline, s.c., for 20 days. Food intake, body weight and rectal temperature were measured daily. After 20 days, animals were killed by decapitation with 30 minutes of the 10.00 hours naloxone injection. Trunk blood was collected and serum prepared as described in section 2.9.1. Serum glucose, insulin and corticosterone concentrations were assayed as described in sections 2.9.2, 2.9.4 and 2.9.5 respectively. Upon sacrifice BAT mitochondria were prepared and GDP binding assays performed, as described in sections 2.12 and 2.13 respectively. Protein concentrations and succinate cytochrome c oxidoreductase activities were assayed as outlined in sections 2.10.2 and 2.14 respectively.

Results.

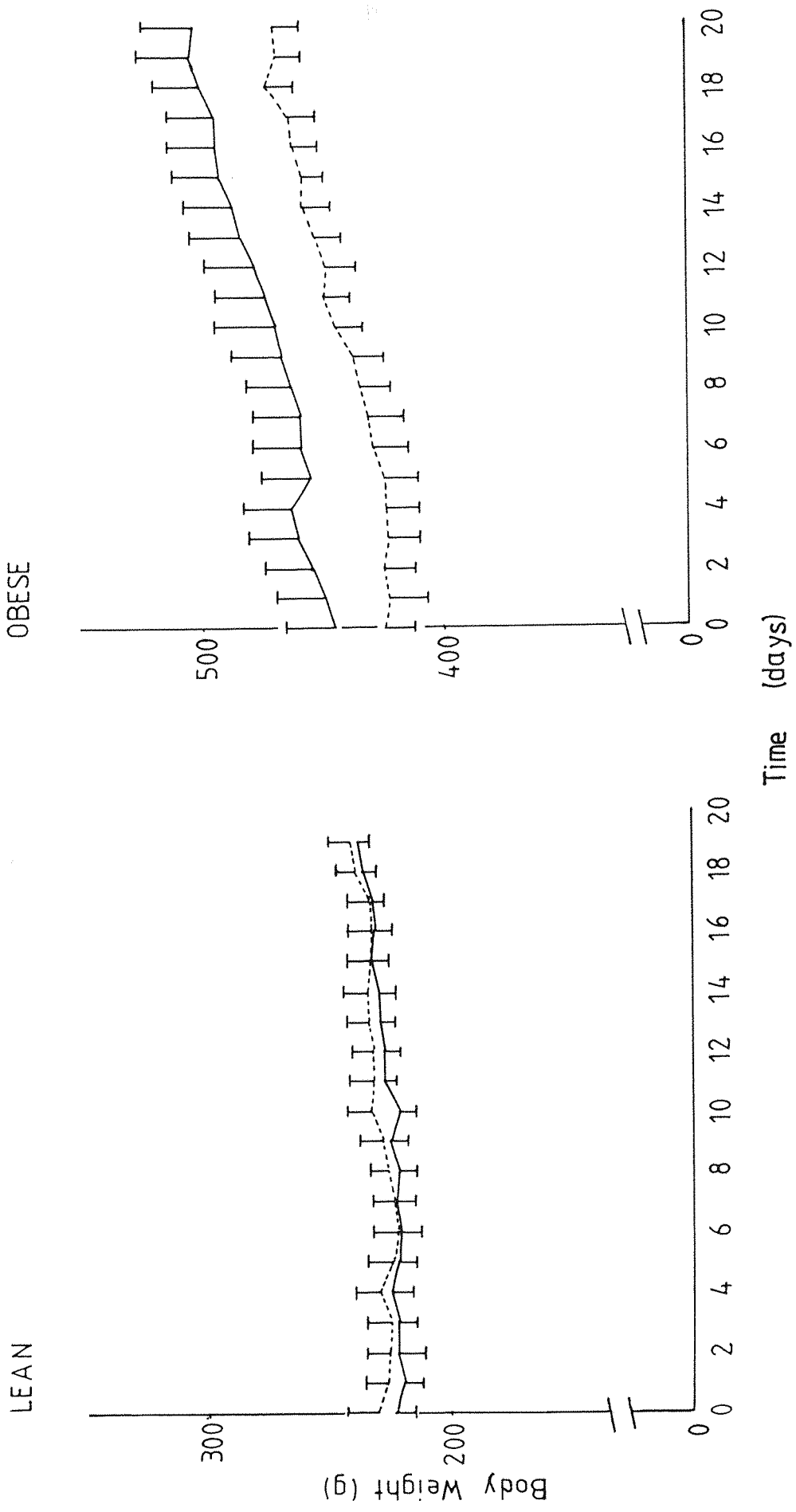
Figure 5.3.1 shows the daily body weights of all four groups. At no point were there any significant differences between saline treated and naloxone treated animals of the same phenotype. Table 5.3.1 shows the effects of chronic naloxone administration on food intake, weight gain, rectal temperature and serum metabolites in lean and obese adult

Figure 5.3.1 The Effects of Chronic Naloxone Treatment on Body Weight Gain In Adult Lean and Obese Zucker Rats.

Lean and obese rats were injected with $1mg\text{-}kg^{-1}$ naloxone in $1ml\text{-}kg^{-1}$ body weight saline, or $1ml\text{-}kg^{-1}$ body weight saline, s.c., 8 hourly for 20 days. Animals were weighed daily.

Values represent means $\pm$ S.E.M. of 4 animals in each group.

_____ Saline injected animals. ------ Naloxone injected animals.


LEAN
OBESE
Body Weight (g)
300
200
500
400
0
2
4
6
8
10
12
14
16
18
20
Time (days)

Zucker rats. Food intake was greater in both saline and naloxone injected obese rats in comparison to lean animals, but naloxone had no effect on food intake in either phenotype. Neither percentage increase in body weight nor rectal temperature was significantly changed by naloxone administration in either phenotype. Rectal temperatures were only measured over the first week of the experiment, as no changes were seen by this time and the procedure was distressing to the animals. Lean animals treated with naloxone had higher blood glucose and corticosterone levels compared to their saline injected controls, but no significant effects of naloxone were observed on these parameters in obese animals. Serum insulin levels were unaffected by naloxone in either phenotype.

Table 5.3.2 shows the effects of chronic naloxone treatment on BAT of adult lean and obese rats. The normal phenotype differences in BAT were all present; obese rats had lower mitochondrial GDP binding, depot GDP binding, lower BAT protein content and higher tissue wet weight in comparison to lean animals. Mitochondrial population (depot succinate cytochrome c oxidoreductase activity) was only slightly reduced in obese animals. Naloxone treatment had no effect on any aspect of BAT examined in either lean or obese animals.

Table 5.3.1 The Chronic Effects of Naloxone on Adult Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	NALOXONE	SALINE	NALOXONE
Food Intake (kJ/day)	165 ± 5	163 ± 9	318 ± 13^{+++}	290 ± 9^{+++}
% increase in body weight	7.4 ± 0.8	5.2 ± 1.1	12.8 ± 0.7^{++}	10.6 ± 0.9^{++}
Rectal temperature (^{o}C, first week only)	38.4 ± 0.1	38.6 ± 0.1	37.9 ± 0.1^{++}	37.7 ± 0.1^{+++}
Serum Glucose (mg.100ml^{-1})	164 ± 5	182 ± 2*	182 ± 7	185 ± 3
Serum Insulin (ng.ml^{-1})	3.49 ± 0.60	2.13 ± 0.24	15.43 ± 2.37^{++}	14.28 ± 0.98^{+++}
Serum Corticosterone (ng.ml^{-1})	217 ± 23	495 ± 42***	409 ± 84	385 ± 83

Values represent means ± S.E.M. of 4 animals in each group.

* $P < 0.05$, *** $P < 0.002$ compared to saline injected animals of the same phenotype.

++ $P < 0.01$, +++ $P < 0.002$ compared to lean animals in same treatment group.

5 month old lean and obese rats were individually housed and injected 8 hourly with naloxone (1mg.kg^{-1}) or saline (0.1ml) for 20 days. Food intake and body weight was measured daily. Serum assays were performed as described in sections 2.9.2, 2.9.4 and 2.9.5. Rectal temperature was measured as described in section 2.3.2 (conscious animals) for the first week only.

Table 5.3.2 The Chronic Effects of Naloxone on BAT of Adult Lean and Obese Zucker Rats.

	LEAN		OBESE	
	SALINE	NALOXONE	SALINE	NALOXONE
[^{3}H] GDP binding (pmols.mg^{-1})	248 ± 14	299 ± 34	138 ± 9^{+++}	162 ± 8^{++}
BAT Depot wet weight (g)	0.40 ± 0.03	0.31 ± 0.03	1.92 ± 0.02^{+++}	1.94 ± 0.25^{+++}
Total BAT depot protein (mg)	47.6 ± 8.9	52.5 ± 5.2	31.0 ± 4.1	31.8 ± 2.9^{+}
Total succ. cyt c O-R act. (μmols.min^{-1}.depot^{-1})	11.0 ± 1.5	9.9 ± 0.7	9.3 ± 1.4	9.1 ± 1.6
Total GDP binding (pmols. depot^{-1})	3290 ± 343	3281 ± 227	1449 ± 268^{++}	1227 ± 141^{+++}

Values represent means ± S.E.M. of 4 animals per group.

No naloxone dependent significant differences occurred.

+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.002$ compared to equivalent lean group.

BAT mitochondria were prepared and GDP binding assays performed as described in sections 2.12 and 2.13 respectively. Protein concentrations were assayed as outlined in section 2.10.2 and succinate cytochrome c oxidoreductase activities (succ.cyt.c O-R Act.) were measured as described in section 2.14. Total depot GDP binding was calculated on the basis of 100% mitochondrial recovery.

5.4 Summary and Discussion.

It has been suggested that the elevated levels of β-endorphin found in the plasma and pituitaries of genetically obese rodents (ob/ob mice and fa/fa rats) may be responsible for the hyperphagia seen in these animals and contribute towards their obesity (Margules et al., 1978; Recant et al., 1983). In support of this suggestion, naloxone has been shown to be more effective at reducing food intake in obese animals than in their lean counterparts (Margules et al., 1978; McLaughlin and Baile, 1984a).

The results presented in section 5.1 suggest that naloxone acutely reduces food intake in young obese rats for the first two hours post injection. This period of hypophagia was followed by a mild rebound hyperphagia up to 4 hours, resulting in a normalisation of 6-hour food intake. The food intake of lean animals seemed to be unaffected by naloxone at any time. The short period of hypophagia in obese animals is coincident with the plasma half-life of naloxone as 80-100 minutes (Fishman et al., 1973). The effects of different doses of naloxone on food intake during the 'day' of a 12 hour light/dark cycle in adult Zucker rats were examined by McLaughlin and Baile, (1984a). They claimed that at all 3 doses of naloxone used (0.5, 1 and 2mg.kg^{-1}), naloxone suppressed food intake over the 12-hour 'daylight' period in both lean and obese rats, although to a greater extent in obese animals. However, examination of their data for 1mg.kg^{-1} naloxone revealed that for obese rats, food intake was reduced only over the first 3 hour period, followed by a compensatory hyperphagia which resulted in the cumulative 12-hour food intake being unaffected by naloxone. The initial hypophagic period supports the observations of the acute effects of naloxone seen here. Margules et al. (1978) reported that 4 hours after naloxone administration (1mg.kg^{-1} body weight) to obese (ob/ob) mice, food intake was only minimally reduced (obese mice, intake reduced from 3.3g to 3.2g by naloxone; lean mice, intake reduced from 2.3g to 2.2g by

naloxone over a 4 hour period). These results suggest that opiate blockade with naloxone acutely reduces food intake in obese animals, but that the deficit is made up by an ensuing compensatory hyperphagia which results in no overall change in food intake.

Naloxone had no significant effects on body temperature in either young acutely treated or adult chronically treated rats of either phenotype, except for an insignificant transitory hypothermia at 40 mintes post-injection in young acutely treated rats. BAT mitochondrial GDP binding was unaffected by acute naloxone injection in obese animals, but lean animals exhibited an increase in GDP binding at 80 and 120 minutes after naloxone injection. This increase in GDP binding was not accompanied by a significant increase in rectal temperature over basal levels. The hyperthermic effect of morphine, which can be blocked by naloxone, is not associated with the activation of BAT thermogenesis. No increase in BAT mitochondrial GDP binding was seen after a $10mg.kg^{-1}$, i.p., injection of morphine, despite a $1^{o}C$ rise in rectal temperature (Thornhill and Desautels, 1984). The increase in rectal and BAT temperature observed by these workers upon morphine injection still occurred after bilateral denervation of the interscapular BAT depot. The results reported in this section support these observations and the suggestion that it is unlikely that BAT is involved in the temperature changes caused by opiates or opiate blockade. As naloxone is a morphine/β-endorphin antagonist, it would be expected that naloxone would depress core temperature at the ambient temperature at which the experiment was carried out ($22-24^{o}C$). The observed increase in BAT mitochondrial GDP binding caused by naloxone in young lean rats is difficult to reconcile with this expectation. It is possible that naloxone has an effect on BAT which is independent of its anti-opiate properties. Naloxone is known to potentiate the steroidogenic properties of ACTH (Lymangrover et al., 1981) and has been demonstrated to cause ACTH release in humans (Morley et al., 1980). It may be that naloxone potentiates the effects of ACTH in its properties as a humoral activator of BAT, or that it

stimulates sufficient ACTH release to independently activate BAT, or that naloxone directly stimulates BAT through the same cellular mechanism as ACTH. Similarities in binding properties between naloxone and ACTH could be inferred from the observation that ACTH will also antagonise opiate effects by interaction with opiate receptors (Terenius, 1976; Smock and Fields, 1981). It is possible, therefore, that naloxone may stimulate BAT directly at the tissue level in the same manner as ACTH. The transient depression in corticosterone levels seen 80 minutes after naloxone injection suggests that circulating ACTH was not involved in the BAT response, a suggestion reinforced by the observation that elevated corticosterone levels, and presumably ACTH levels, occurred in obese rats and were unaccompanied by an increase in BAT GDP binding. It has been suggested that opiates may act by inhibiting CRF release (Gaillard et al., 1981), if so, it is possible that naloxone releases CRF which then independently stimulates BAT mitochondrial GDP binding (Arase, York and Bray: unpublished observations).

Adrenalectomy abolished the acute naloxone-dependent increase in BAT mitochondrial GDP binding in lean animals. This could be due to the high circulating levels of ACTH which are present after adrenalectomy competing with, or masking the effects of naloxone at its site of activation of BAT. This may explain the failure of naloxone to increase GDP binding in obese rats. Adrenalectomy corrected basal GDP binding levels in obese animals, but no effect of naloxone on BAT was seen. Observations of ACTH and β-endorphin levels of young lean and obese adrenalectomised 4-5 week old Zucker rats, which were made in our laboratory, reveal that plasma β-endorphin and ACTH levels in obese rats are consistently lower than those seen in lean animals. Adrenalectomy increases plasma levels of both peptides in both phenotypes, but the ratio of ACTH:β-endorphin remains similar and obese plasma concentrations are still lower than in lean animals (Holt: unpublished observations). This is in contrast to the findings of Margules et al. (1978), who observed increased plasma β-endorphin levels in fa/fa rats, however, the animals used were older (5 months).

Increased levels of β-endorphin in the pituitary gland of obese rats was observed only at 8 weeks of age (Holt: unpublished observations). Pituitary levels of β-endorphin are thought to reflect the plasma concentration of the peptide, and hypothalamic levels, the cerebrospinal fluid β-endorphin concentration (Baile et al., 1986). This would be consistent with our observations of elevated pituitary β-endorphin levels in obese rats only at 8 weeks of age, and the observations of Margules et al. (1978) that plasma β-endorphin levels were elevated at 3-5 months of age. Recant et al. (1983) observed increased hypothalamic and pituitary levels of β-endorphin in animals of approximately 10-12 weeks of age (205g and 400g body weight for lean and obese animals respectively). These observations suggest that in animals of the age used in these experiments (5-6 weeks) plasma and pituitary levels of β-endorphin were probably normal, or slightly elevated. Adrenalectomy does not alter the hyperthermic effect of morphine, which suggests that the centrally activated thermogenic mechanisms which are responsible for the increase in body temperature are not dependent upon the hormones of the pituitary-adrenal axis (Thornhill and Saunders, 1985). The abolition of the naloxone stimulation of BAT mitochondrial GDP binding after adrenalectomy implies that this effect is dependent upon the pituitary-adrenal axis, and viewed in the light of the findings of Thornhill and Saunders (1985) of the pituitary-adrenal independence of opiate effects, it seems unlikely that the naloxone stimulated increase in GDP binding is a centrally mediated effect operating via opiate receptors.

Immunoreactive β-endorphin like activity has been found in the pancreas (Grube et al., 1979) and it is thought that circulating β-endorphin may play a role in the regulation of insulin and glucagon release. Naloxone inhibits morphine-stimulated insulin and glucagon release from cultured pancreatic islet cells, but naloxone has no independent effects on the release of these hormones (Kanter et al., 1980). Naloxone treatment was not observed to significantly depress

insulin levels when given either acutely to young or chronically to adult rats of either phenotype. This is in accord with the *in vitro* observations of Kanter et al. (1980), that naloxone had no independent effects on tonic insulin release.

Chronic naloxone treatment was not observed to have any effects on food intake, body weight gain on BAT thermogenesis in either lean or obese adult Zucker rats. This is in complete contrast with the findings of Wexler and McMurtry (1985), who demonstrated marked effects on food intake and body weight gain in another strain of genetically obese rodent, the obese spontaneously hypertensive rat (obese/SHR). These animals are an inbred substrain of the corpulent, or Koletsky, rat (fa^k) (Koletsky, 1973) inbred to exaggerate the Cushingoid features of these animals (Wexler et al., 1980). These animals exhibit many of the features shown by Cushings Syndrome sufferers such as obesity, fatty liver, hyperlipidaemia, hyperglyceamia, hyperinsulanaemia, hypertension, infertility, muscle wasting and skin fragility (Wexler et al., 1980). These animals differ from the Zucker obese rat in that they are hypertensive and have very high serum corticosterone levels. Adrenalectomy largely alleviates hypertension and obesity in these obese/SHR rats (Wexler and McMurtry, 1981). When these rats were injected chronically with naloxone (8 month old rats injected 3 times daily for 81 days) their hyperphagia was abolished towards normal non-obese/SHR levels and they lost weight precipitously (Webster and McMurtry, 1985). These effects were less pronounced in young animals (5 weeks old at the initiation of 10 weeks of treatment). Serum insulin, corticosterone and β-endorphin levels were significantly reduced by naloxone treatment in the obese and non-obese/SHR, and again, the effects were more pronounced in older rats. ACTH levels were only reduced by naloxone in obese/SHR rats. Growth hormone levels were also increased by naloxone treatment.

The differential effects of naloxone on obese Zucker and obese/SHR animals could be due to a number of differences

in the aetiology of the obesities, and in the experimental protocol. Unlike normal rats and humans, naloxone inhibited ACTH secretion and reduced corticosterone levels. Corticosterone levels were elevated in 5 month old Zucker rats and were not reduced by naloxone treatment, in fact lean animals treated with naloxone showed elevated serum corticosterone levels. Insulin levels were also unaffected by naloxone in either phenotype in the study described here. A major difference in the protocol was that Wexler and McMurtry injected their animals 3 times during the day, when naloxone has been shown to have a greater effect on food intake. (McLaughlin and Baile, 1984a), and the injections were timed so as to coincide with the periods of compensatory hyperphagia following the previous injection (0700 hours, 1200 hours and 1530 hours). This treatment regime significantly reduced food intake in the obese animals, but not the non-obese animals. In this study, the 3 injections of naloxone were spaced evenly throughout the 24 hour period, which resulted in no significant reduction in food intake, but did result in an elevated corticosterone level in lean rats, suggesting that the evenly spaced injections were effective at producing a chronic response of some sort.

It is possible that the massive weight loss seen in the naloxone treated obese/SHR was due to a combination of the effects of a marked reduction in food intake, accompanied by the reduction in corticosterone levels which alone would reduce the obesity of the obese/SHR (Wexler and McMurtry, 1981). The differential effects of naloxone on the pituitary-adrenal axis of the two genetic models of obesity was probably instrumental in the diversity of the response to naloxone.

In summary, naloxone caused an acute increase in BAT mitochondrial GDP binding in young lean rats that was not associated with an increase in rectal temperature and which was abolished by adrenalectomy. GDP binding was not increased by naloxone in obese animals under any conditions. The observation that the naloxone dependent increase in BAT GDP

binding was abolished by adrenalectomy in lean animals suggests that the response may have been masked by elevated ACTH levels which occurred after adrenalectomy. BAT mitochondrial GDP binding was unaffected by chronically injected naloxone in adult rats of either phenotype, it seems, therefore, unlikely that naloxone would significantly affect energy expenditure via BAT in either lean or obese rats.

Food intake was acutely depressed by naloxone over a 2 hour period in young rats which was coincident with the half-life of the drug. Over a longer 6 hour period, food intake was not significantly affected in lean or obese rats. Chronic naloxone treatment had no effect on food intake of adult animals of either phenotype. It would be of interest to examine the effects of naloxone administered continuously by a mini-osmotic pump, and to investigate the changes in food intake and body weight, having bypassed the problem of the relatively short half-life of the drug. From the results presented in this chapter it seems unlikely that opioid peptides play a significant role in the development of the obesity of the Zucker rat, despite the apparent effects on the short term regulation of feeding.

CHAPTER 6. GENERAL DISCUSSION.

The obesity of the fa/fa rat is thought to result from an inability to activate DIT in BAT in response to dietary signals. Low basal levels of BAT sympathetic activity and BAT mitochondrial GDP binding (an index of BAT thermogenic status) are characteristic of the obese Zucker rat (York et al., 1985a) as well as the obese (ob/ob) mouse (Himms-Hagen and Desautels, 1978; Young and Landsberg, 1983) and the VMH-lesioned rat (Seydoux et al., 1982; Vander Tuig et al., 1982). Hyperinsulinaemia is also a characteristic of these 3 obese models (Bray and York, 1979). Adrenalectomy reverses the obesity of the fa/fa rat, the ob/ob mouse and the VMH-lesioned rat (Yukimura et al., 1978; Holt and York, 1982; Bruce et al., 1982, respectively) suggesting that some common glucocorticoid-mediated factor or system is involved in the maintenance of obesity. The obesity of the VMH-lesioned rat, but not that of the fa/fa rat, is abolished by subdiaphragmatic vagotomy (Powley and Opsahl, 1974; Opsahl and Powley, 1976).

It has been demonstrated that the effects of adrenalectomy on energy balance in obese rats are not dependent upon dietary composition. The improvement in energy balance, body weight gain and BAT function in the obese rat, which occurred after adrenalectomy, were independent of the energy composition of the diet, and animals responded equally well to adrenalectomy on a high fat (34% and 44% fat and carbohydrate energy respectively) or a high carbohydrate (8% and 68% fat and carbohydrate energy respectively) diet. This is in contrast to the effects seen in the ob/ob mouse, where obese animals fed a high fat diet do not apparently respond to the ameliorating effects of adrenalectomy (Smith and Romsos, 1985). The obese mouse has several dissimilarities with the obese rat. Obese mice are more intolerant to cold exposure (Trayhurn and James, 1978) and fail to increase BAT mitochondrial GDP binding in response to cold unless first acclimated to an intermediate temperature (Himms-Hagen and Desautels, 1978; Zahror-Behrens and Himms-Hagen, 1982). ob/ob mice are less sensitive to the effects

of noradrenaline on metabolic rate (Trayhurn and James, 1978; Thurlby and Trayhurn, 1980) and it has recently been shown that dietary fat and dietary sucrose can elicit increases in cardiac and BAT noradrenaline turnover in ob/ob mice with no concomitant increase in energy expenditure (Knehans and Romsos, 1984). Cafeteria feeding increases BAT mitochondrial GDP binding in obese mice (Trayhurn et al., 1982; Himms-Hagen, 1985; Himms-Hagen et al., 1986) with no improvement in body weight gain or energetic efficiency, and it has been suggested that the increased energy expenditure in cafeteria fed ob/ob mice contributes towards the maintenance of normal body temperature, rather than the disposal of excess dietary energy (Himms-Hagen et al., 1986). It seems that a further dissimilarity between these two models of genetic obesity - the ob/ob mouse and the fa/fa rat - exists, in that the effects of adrenalectomy on BAT of obese mice are diet dependent, whilst the effects on fa/fa rats are not. The 2DG-mediated inhibition of BAT mitochondrial GDP binding in lean Zucker rats was independent of diet, in that 7-10 days overfeeding with either sucrose or high fat diets resulted in similar responsiveness to 2DG.

It has been demonstrated that BAT mitochondrial GDP binding of fa/fa rats is unresponsive to the central glucoprivic actions of 2DG unless normal sympathetic activity has been restored to the tissue by adrenalectomy. It has also been demonstrated that corticosterone inhibits the response of BAT of lean animals to 2DG. The restorative effects of adrenalectomy on BAT function in obese animals (either genetic or experimental) has been widely demonstrated (Yukimura et al., 1978; Holt and York, 1982, 1984; Bruce et al., 1982; King et al., 1983; Marchington et al., 1983). BAT sympathetic activity and basal GDP binding levels are increased, insulin levels are depressed towards normal, longitudinal growth is restored and hyperphagia and deranged feeding patterns are abolished. It is possible that corticosterone removal has a direct role in many of these changes. It has been suggested that the obese (fa/fa) rat and the obese (ob/ob) mouse are hypersensitive to the

effects of corticosterone. Obesity is restored in fa/fa adrenalectomised rats with low replacement doses of the steroid that have little effect in lean animals (Freedman et al., 1986a). The hyperphagia and the abnormal diurnal rhythm of food intake could be explained by a hypersensitivity of the corticosterone stimulated noradrenergic feeding mechanism in the PVN in obese animals. Noradrenaline injected centrally stimulates feeding in both lean and obese Zucker rats so the noradrenergic feeding mechanism appears to be intact in the fa/fa rat (Ikeda et al., 1980). Adrenalectomised animals do not respond to central noradrenaline by increasing their food intake (Bhakthavatsalam and Leibowitz, 1986), so it is possible that the removal of corticosterone in obese animals leads to an inactivation of this PVN feeding mechanism which is normally hyperactive in intact animals, leading to an abolition of genetic hyperphagia.

The restoration of sensitivity to central glucoprivation after adrenalectomy could also be explained by corticosterone removal, as could the reduction in circulating insulin levels. The obesity of the fa/fa rat is thought to arise from an imbalance in autonomic nervous system function, which results in a reduced sympathetic activity in BAT (York et al., 1985b) and an increased vagal drive to the pancreas leading to hyperinsulinaemia (Rohner-Jeanrenaud et al., 1983). The reduced sympathetic activity in BAT could be related to an inability of the VMH to couple dietary afferent inputs to efferent activation of the tissue. Efferent VMH activity in response to electrical stimulation of the VMH in fa/fa rats has been shown to be normal (Holt et al., 1985, 1986). The increased parasympathetic activity may result from elevated LH activity. Electrical stimulation of the LH is associated with increased vagal activity (Oomura and Kita, 1981), and 2DG applied electroosmotically to the LH increases gastric acid secretion (Shiraishi and Simpson, 1982). The elevation of PNS and depression of the SNS in relation to LH and VMH outputs could be explained in terms of a glucocorticoid mediated inhibition of glucose uptake into the LH glucose-sensitive or VMH glucoreceptor neurones. If corticosterone inhibits the uptake of glucose

into peripheral cells by inhibition of the transporter system (Munck, 1971; Fain, 1979) it is possible that such an effect also occurs in the central glucoreceptors. Inhibition of glucose uptake in the glucoreceptors of the VMH would result in a decrease in neuronal firing rate and a reduction in sympathetic output. Reduction of glucose uptake due to corticosterone in the LH glucose sensitive cells would increase their firing rate and lead to an increase in PNS activity, possibly acting through the DMN of the vagus, and an enhancement of feeding activity and insulin secretion. Adrenalectomy would remove corticosterone (and thus glucocorticoid effects in the LH and VMH) resulting in a normalisation of glucoreceptor function and a restoration of the balance between the hypothalamic outputs to the sympathetic and parasympathetic limbs of the autonomic nervous system. The recent observation by Brown (1986) that the formation of GTG lesions in mice was prevented by pretreatment with hydrocortisone, provides evidence to suggest that glucocorticoids have a similar specificity for VMH neurones as a neurotoxic agent responsible for the generation of VMH obesity.

Figure 6.1 illustrates the intrahypothalamic connections and hypothalamic efferents which might be involved in these events. The VMH and LH both have sympathetic projections to the dorsomedial nucleus of the hypothalamus (DMH) which in turn has autonomic and neuroendocrine connections with the PVN. The PVN has direct neural connections with the DMN of the vagus, which is considered the primary parasympathetic outflow area, and eventually to the intermediolateral column and thus the thoracolumbar sympathetic preganglionic neurones. The PVN also exerts neuroendocrine control over the median eminence and pituitary, and serves as the main hypothalamic-pituitary link. The LH has sympathetic connections to the DMH, and parasympathetic connections direct to the DMN and the reticular formation thus influencing the PNS efferent areas. The VMH exerts only sympathetic influence on the DMH and the periaqueductal grey, which is the main sympathetic outflow area, equivalent to the DMN of the PNS (Luiten et al., 1986).

Figure 6.1 Possible Intrahypothalamic Connections and Hypothalamic Efferents Involved in the Regulation of Diet Induced Thermogenesis and Food Intake.

Sympathetic pathway.

Parasympathetic pathway.

Mixed autonomic pathway.

Neuroendocrine pathway.

A - pancreatic glucagon secreting cells.

β - pancreatic insulin secreting cells.

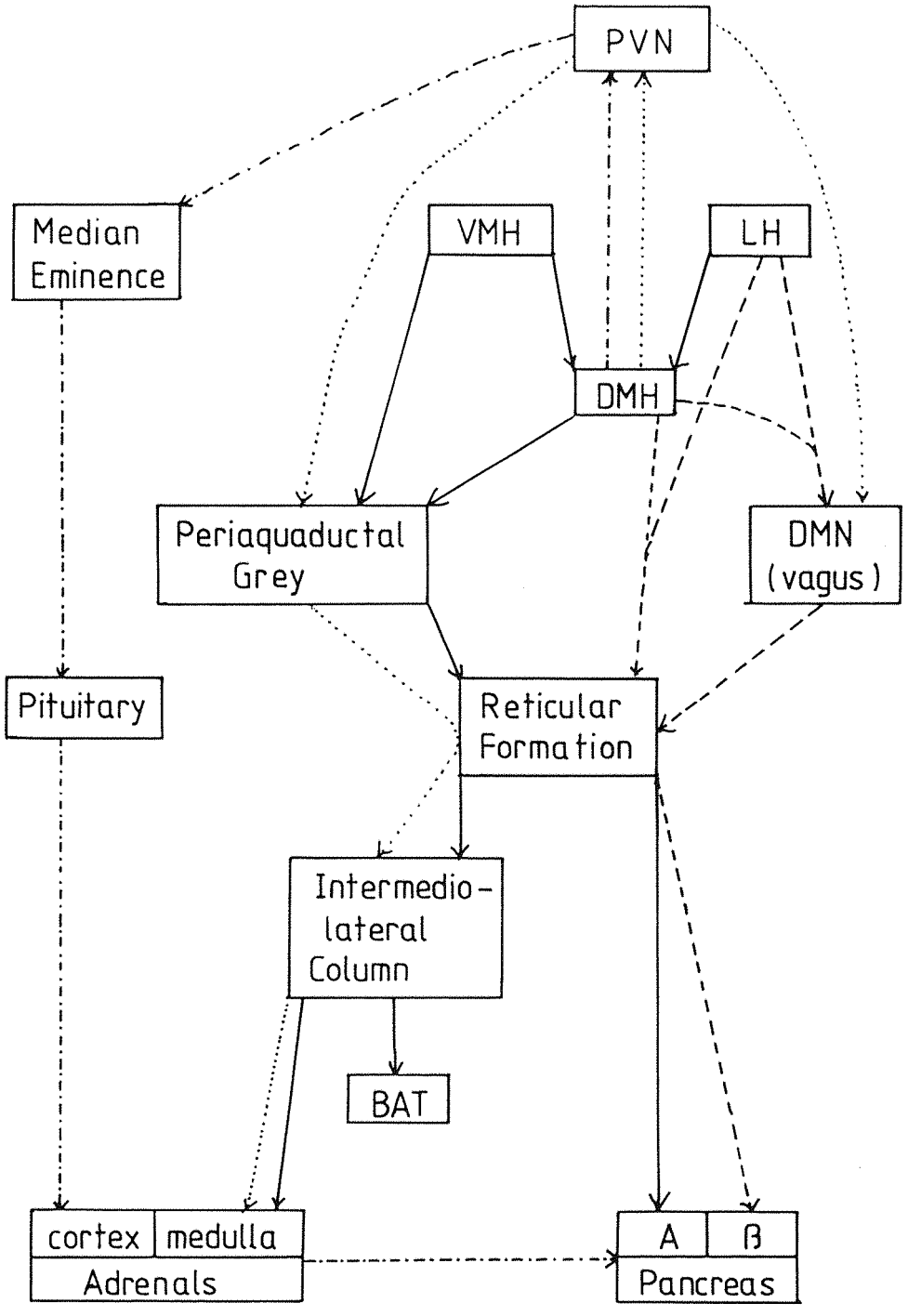
DMH - dorsomedial nucleus of the hypothalamus.

DMN - dorsal motor nucleus of the vagus.

LH - lateral hypothalamus.

PVN - paraventricular nucleus.

VMH - ventromedial hypothalamus.


PVN
Median Eminence
VMH
LH
DMH
Periaquaductal Grey
DMN (vagus)
Pituitary
Reticular Formation
Intermedio-lateral Column
BAT
cortex
medulla
Adrenals
A
β
Pancreas

This glucocorticoid sensitive hypothesis seems to fit the evidence for genetically obese rats and mice, and VMH obesity can also be at least partially explained this way. If glucocorticoids were involved in the normal regulation of dietary signals, which is possible, since corticosterone levels increase after feeding (Brindley et al., 1979),it could be that a normal tonic inhibitory/excitatory effect of glucocorticoids exists in the VMH/LH glucoreceptor system. The central level of glucocorticoids would then be able to determine the level of sensitivity to dietary signals, such as glucose availability. Ablation of the VMH has been observed to lead to increases in basal corticosterone levels (Coover et al., 1980). Elevated corticosterone levels would normally inhibit VMH (SNS) activity and enhance LH (PNS) activity, but in the absence of a functional VMH, only the elevation in LH activity would occur, leading to hyperinsulinaemia and hyperphagia. Adrenalectomy would reduce LH stimulation of insulin secretion and food intake and alleviate the obesity. The effects of corticosterone on the glucoreceptors of the VMH and LH could be investigated by examining the effects of centrally administered corticosterone, alone, or in combination with 2DG, glucose, free fatty acids or insulin on the firing rate of the efferent nerves to BAT, or the vagus nerve. It would also prove interesting to investigate changes in central glucocorticoid concentrations in response to various dietary states such as starvation and diet-induced hyperphagia, to examine the possible role of corticosterone as a regulator of the level of dietary sensitivity in the hypothalamus.

The 2DG induced hyperphagia could be explained by the inhibition of glucose metabolism in the glucosensitive neurones of the LH and VMH leading to an enhancement of PNS (LH) activity and an increase in feeding activity. The attenuation of the 2DG-inhibition of BAT mitochondrial GDP binding could reflect reduced 2DG uptake into the VMH due to increased dietary glucose availability. Alternatively, a postprandial elevation in corticosterone levels or even a 2DG stimulation of corticosterone release, could reduce the tonic firing rate of the VMH glucoreceptors and reduce

SNS activity to BAT. The hyperphagic effects of 2DG are not thought to be mediated through the corticosterone dependent $\alpha_2$noradrenergic feeding drive in the PVN, as 2DG still elicits feeding responses in adrenalectomised animals. This was shown in the present study and by Bhakthavatsalam and Leibowitz (1986). Hyperphagia induced by centrally injected 2DG does cause noradrenaline release, as its effects on food intake are blocked by pretreatment with phentolamine, azapetine or yohimbine (Muller et al., 1972). It is possible that 2DG-released noradrenaline acts on sites other than the PVN to increase feeding, such as the suprachiasmatic nucleus (Yamomoto et al., 1985) but it is also possible that the release of peptide hormones by 2DG is involved in 2DG elicited feeding. 2DG causes ACTH release, presumably through the actions of CRF, (Weidenfeld et al., 1984). It is known that ACTH secretion is accompanied by β-endorphin release (Schally et al., 1978; Young and Akil, 1985) and β-endorphin, as well as other opioid peptides, seem to be involved in the regulation of food intake (Morley, 1980; Morley and Levine, 1982; Baile et al., 1986). Penicaud and Thompson (1984) demonstrated that central or peripheral administration of naloxone reduced 2DG-dependent hyperphagia in Wistar rats. The results presented in the present study on the effects of naloxone on food intake suggest that although naloxone treatment did not affect food intake or weight gain in lean or obese Zucker rats, there was an acute inhibitory effect of naloxone on food intake in obese rats. It is possible, therefore, that β-endorphin stimulated hunger is involved in the acute hyperphagia caused by 2DG, and the failure of 2DG to evoke a feeding response in the obese rat may have been due to pre-existing high levels of hypothalamic β-endorphin preventing further elevations of food intake. Glucoprivation can cause release of several neurotransmitters from isolated mouse hypothalami, including dopamine serotonin and GABA, as well as noradrenaline (O'Fallon and Ritter, 1982). The central peptidergic neurotransmitter system is undoubtedly involved in the regulation of feeding (Morley, 1980; Baile et al., 1986) and recent evidence suggests that the hypothalamic peptide, CRF, has a stimulatory

effect upon BAT mitochondrial GDP binding (Arase, York and Bray: unpublished observations), which is mediated by an increase in SNS activity. Whether this has a physiological role in the regulation of energy expenditure remains to be seen. The anorectic drug fenfluramine increases central concentrations of β-endorphin through serotininergic mechanisms (Harsing et al., 1984), but the non-serotininergic anorectic drug amphetamine did not affect β-endorphin levels (Harsing et al., 1982). It seems rather odd that anorectic drugs such as fenfluramine should increase central β-endorphin levels, as increased central opioid activity is associated with increased ingestion (Baile et al., 1986). It has been suggested, however, that fenfluramine acts to inhibit endorphinergic transmission in the hypothalamus, leading to an intracellular accumulation of opioids and a reduction in putative hunger signals (Harsing et al., 1982, 1984). It seems, therefore, that serotoninergic mechanisms may be important in the control of feeding, possibly through the inhibition of opiate activity. Both fenfluramine and amphetamine also stimulate BAT mitchondrial GDP binding in rats (Lupien and Bray; Abdul-Karim and York, respectively: unpublished observations), so the investigation of the mechanisms and effects of these drugs may provide important information as to the integration of feeding and energy expenditure regulation within the hypothalamus.

In summary, it is possible that glucocorticoids play a normal regulatory role in setting the sensitivity of the VMH/LH glucoreceptor system to central glucose metabolism. Inhibition of glucose uptake by glucocorticoids into the VMH would result in decreased neuronal firing and a reduction in central sympathetic output, conversely inhibition by glucocorticoids of the LH glucosensitive neurones would enhance LH firing and increase parasympathetic output. The postulated interactions of these pathways are outlined in figure 6.2. In this way it is possible that glucocorticoids play a role in regulating the balance between the sympathetic and parasympathetic limbs of the autonomic nervous system. Inhibition of central glucose metabolism with 2DG has been shown to inhibit BAT mitochondrial GDP binding and increase

Figure 6.2 The Possible Involvement of Corticosterone in the Regulation of the Balance Between the Sympathetic and Parasympathetic Limbs of the Autonomic Nervous System.

Above the dotted line illustrates the effect of corticosterone on the glucoreceptor system of the VMH and LH (-,inhibitory; +,excitatory).

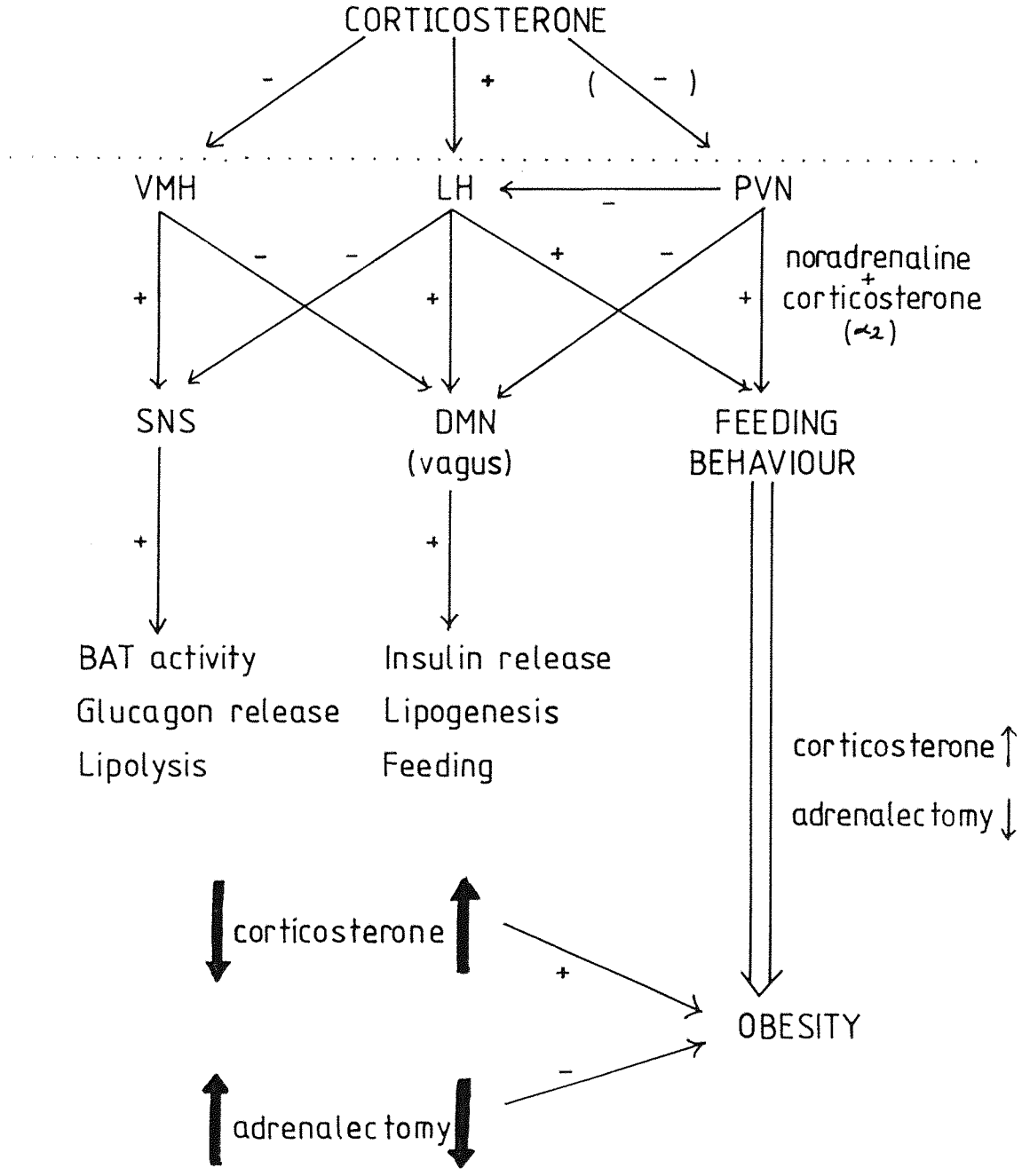
Below the dotted line illustrates the interactive effects of the hypothalamic nuclei, and efferent autonomic effects elicited by these centres. Corticosterone is envisaged as reducing SNS activity and increasing PNS activity with the result that BAT activity, glucagon release and lipolysis are reduced, and insulin release, lipogenesis and feeding behaviour are enhanced. Opposite effects are seen after adrenalectomy.

- inhibition

+ stimulation

↑ increased activity

↓ decreased activity


CORTICOSTERONE
-
+
(-)
VMH
LH
PVN
noradrenaline
+
corticosterone
(α2)
SNS
DMN
(vagus)
FEEDING
BEHAVIOUR
BAT activity
Glucagon release
Lipolysis
Insulin release
Lipogenesis
Feeding
corticosterone ↑
adrenalectomy ↓
corticosterone
adrenalectomy
OBESITY

food intake in lean, but not obese, Zucker rats. Restoration of normal sympathetic activity in BAT of obese rats by adrenalectomy results in a normal sensitivity of BAT mitochondrial GDP binding to 2DG inhibition, and a 2DG-induced increase in food intake. These observations suggest that the imbalance in the autonomic nervous system in the fa/fa rat may result from a hypersensitivity of these animals to the effects of glucocorticoids acting in the glucose sensing nuclei of the hypothalamus.

APPENDIX.

Publications.

Some of the work presented in this thesis has appeared in published form.

CHAPTER 3.

Allars, J.M. and D.A. York (1986).
'The Effects of 2-deoxy-D-glucose on Brown Adipose Tissue in Lean and Obese Zucker Rats.'
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CHAPTER 4.

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