University of
Southampton

University of Southampton Research Repository

Copyright © and Moral Rights for this thesis and, where applicable, any accompanying data are retained by the author and/or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This thesis and the accompanying data cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder/s. The content of the thesis and accompanying research data (where applicable) must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holder/s.

When referring to this thesis and any accompanying data, full bibliographic details must be given, e.g.

Thesis: Author (Year of Submission) "Full thesis title", University of Southampton, name of the University Faculty or School or Department, PhD Thesis, pagination.

Data: Author (Year) Title. URI [dataset]

THE CONTROL OF BROWN ADIPOSE TISSUE IN THE GENETICALLY OBESE ZUCKER (fa/fa) RAT

by

Susan Jayne Holt

A thesis presented for the degree of

Doctor of Philosophy

at the

University of Southampton

Department of Nutrition
University of Southampton

March 1984

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

DEPARTMENT OF NUTRITION

Doctor of Philosophy

THE CONTROL OF BROWN ADIPOSE TISSUE IN THE GENETICALLY OBESE ZUCKER (fa/fa) RAT

by Susan Jayne Holt

The binding of [^{3}H]- GDP to brown adipose tissue (BAT) mitochondria was used as a measure of the thermogenic state of the tissue. A reduced level of GDP binding was observed in obese (fa/fa) rats, consistent with their impaired thermogenesis. The reduced GDP binding resulted from a reduction in the number of binding sites, and not from a change in affinity.

BAT mitochondrial GDP binding was normalised in adrenalectomised fa/fa rats. Corticosterone replacement to adrenalectomised fa/fa rats restored the defect. The thermogenic response of BAT in fa/fa rats to changes in environmental temperature was normal in young (5 week old) rats, but attenuated in older (10 week old) rats. At thermoneutrality, GDP binding in fa/fa rats was reduced when compared to lean rats. The BAT thermogenic response to sucrose overfeeding was absent in fa/fa rats, but was restored after adrenalectomy. Restriction of food intake reduced GDP binding in lean rats, but not in fa/fa rats. These results suggested that the reduced thermogenic capacity of fa/fa rats resulted from corticosterone inhibition of diet-related, but not cold-related BAT thermogenesis.

Further experiments demonstrate that propranalol, a β antagonist, suppressed the level of BAT mitochondrial GDP binding of lean rats down to the levels of fa/fa rats and prevented the normalisation of GDP binding after adrenalectomy of fa/fa rats. These data have been interpreted as showing that the defect in diet-related BAT thermogenesis of fa/fa rats may reflect a corticosterone-dependent suppression of sympathetic stimulation to that tissue.

The decrease in GDP binding in fa/fa rats was detected as early as 10 days of age, and was closely related to the fa gene concentration. The fa gene dependency was still apparent in the adult rat.

Serum T_3 levels were found to be related to the thermogenic state of BAT, in that they were reduced in fa/fa rats, raised after adrenalectomy, and suppressed again after corticosterone replacement. These levels were also raised on cold acclimation in lean and fa/fa rats, and by sucrose overfeeding in lean rats. However, T_3 levels were not increased in sucrose-fed fa/fa rats. It is suggested that changes in serum T_3 levels may be secondary to changes in sympathetic activity in BAT.

Possible mechanisms for the control of diet-related sympathetic activity and BAT thermogenesis by glucocorticoids are discussed.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. David York for his expert guidance, and continuous motivation in production of this thesis. I am grateful to Professor T. G. Taylor for providing the excellent research facilities, and to the Science and Engineering Research Council for financial support. Thanks also to Dr. J. T. R. Fitzsimons and Mr. N. Orson for electron microscopy studies, and Mrs. J. Harris for performing the serum insulin assays. I would like to thank Colin Bunce and his staff for providing the animals. I would also like to thank Mary Campbell for the typing of this manuscript.

I would especially like to thank all my friends and associates in Boldrewood for providing such an enjoyable atmosphere in which to work.

Finally, my special thanks to all my family, particularly my mother and sister for their constant support and encouragement throughout my studies.

DEPARTMENT OF NUTRITION
SCHOOL OF BIOCHEMICAL AND
PHYSIOLOGICAL SCIENCES,
UNIVERSITY OF SOUTHAMPTON,
SOUTHAMPTON. SO9 5NH.

GAN TACÍOCHT FEARGUS NÍ BHEADH SÉ

DE MHISNEACH AGAM TABHAIRT FAOÍ

AN OBAIR SEO

ABBREVIATIONS

ABTS	2,2' azino-di(3-ethyl benz-thiazoline sulphonic acid)
ACTH	adrenocorticotrophic hormone
BAT	brown adipose tissue
B_{max}	maximum binding
BSA	bovine serum albumen
5'D II	Type II 5'-deiodinase
DIT	diet induced thermogenesis
EDTA	ethylenediaminetetraacetic acid
GDP	guanosine diphosphate
HEPES	2-(N-2-hydroxyethylpiperazine-N'-2) ethane sulphonic acid
HSTL	hormone sensitive triglyceride lipase
ip	intra peritoneal
Kd	dissociation constant
LH	lateral hypothalamus
LPL	lipoprotein lipase
NST	non shivering thermogenesis
PAGE	polyacrylamide gel electrophoresis
PRD	purina rat diet
sc	subcutaneous
SDS	sodium dodecyl sulphate
SNS	sympathetic nervous system
Succ.cyt.c O-R	succinate cytochrome c oxidoreductase
TRH	thyrotropic releasing hormone
TSH	thyroid stimulating hormone
T_4	thyroxine
T_3	triiodothyronine
TRIS	tris (hydroxymethyl) aminomethane
VMH	ventromedial hypothalamus
WAT	white adipose tissue

CONTENTS

Page

CHAPTER 1 INTRODUCTION 1

1.1 Obesity 1

1.2 Use of animal models in the study of obesity 1

1.2.1 Non-genetic models of obesity 2

a) Chemical or surgically induced obesity 2

b) Endocrine-induced obesity 2

c) Dietary-induced obesity 3

1.2.2 Genetic models of obesity 4

1.3 The Zucker 'fatty' (fa/fa) rat 4

1.3.1 Development of obesity in the fa/fa rat 4

1.3.2 Endocrine status in the obese (fa/fa) rat 7

a) Insulin 7

b) Thyroid function 8

c) Adrenal corticosteroids 8

1.3.3 Energy balance in the obese (fa/fa) rat 9

1.4 Thermogenesis 13

1.4.1 Non-shivering thermogenesis (NST) 13

1.4.2 Brown adipose tissue (BAT) 15

a) Morphology 16

b) Mitochondria 16

c) Blood supply 17

d) Innervation 17

1.4.3 Development of BAT in the rat 17

1.4.4 Evidence for involvement of BAT in NST 18

1.4.5 Mechanism of heat production in BAT 20

1.4.6 The substrate for BAT thermogenesis 23

1.4.7 Evidence for sympathetic regulation of BAT 23

1.4.8 Acute regulation of thermogenesis in BAT 24

1.4.9 The adaptive response 27

1.4.10 Diet-induced thermogenesis (DIT) 29

1.4.11 Central control of thermogenesis 31

1.4.12 Endocrine control of thermogenesis 32

a) Thyroid hormones 33

b) Adrenal corticosteroids and ACTH 35

1.5 Aim of the project 36

Page

CHAPTER 2 MATERIALS AND METHODS

2.1	Materials	37
2.2	Animals	39
2.2.1	Sacrifice of animals	39
2.2.2	Animal maintenance	39
2.3	Sucrose supplemented diet	39
2.4	Pair feeding	39
2.5	Identification of pre-obese rats	41
2.6	Regulation of environmental housing temperature	41
2.7	Adrenalectomy	41
2.8	Hormone streptozotocin and propranalol treatments	42
2.8.1	Aldosterone	42
2.8.2	Corticosterone	42
2.8.3	Addition of thyroid powder to the diet	42
2.8.4	Induction of diabetes with streptozotocin	43
2.8.5	Noradrenaline	43
2.8.6	Propranalol	43
2.9	Preparation of interscapular brown adipose tissue (BAT) mitochondria	43
2.10	Measurement of specific binding of [^{3}H]-guanosine diphosphate (GDP) to BAT mitochondria	43
2.11	Protein determination using the Lowry assay	45
2.12	Assay of succinate cytochrome c oxidoreductase (succ.cyt.c O-R)	46
2.13	Removal of fatty acids from bovine serum albumen (BSA) by charcoal treatment	46
2.14	Serum assays	47
2.14.1	Determination of serum free fatty acid concentration	47
2.14.2	Measurement of serum triglycerides concentration	48
2.14.3	Measurement of serum glucose concentration	49
2.14.4	Measurement of serum insulin using radio-immunoassay	49
2.14.5	Measurement of serum triiodothyronine (T_3) using radioimmunoassay	51
2.14.6	Measurement of total serum corticosterone concentration using radioimmunoassay	52
2.15	Preparation of BAT for electron microscopy	53
2.16	Determination of radioactivity	54
2.17	Statistics	54

Page

CHAPTER 3 RESULTS AND DISCUSSION 55

Section 3.1 BAT Function in the Obese (fa/fa) Rat 55

3.1.1 Effect of protein concentration on BAT mitochondrial GDP binding in lean and fa/fa rats 55

3.1.2 Effect of pH on BAT mitochondrial GDP binding in lean and fa/fa rats 57

3.1.3 Time course of association of GDP with BAT mitochondria from lean and fa/fa rats 57

3.1.4 Effect of the temperature of incubation on GDP binding in BAT mitochondria from lean and fa/fa rats 57

3.1.5 Treatment of BAT mitochondria from lean and fa/fa rats with 2% (w/v) BSA (fatty acid free) 61

3.1.6 Characteristics of BAT in lean and fa/fa rats 61

3.1.7 Scatchard analysis of BAT mitochondrial GDP binding in lean and fa/fa rats 65

3.1.8 Effect of age on BAT thermogenesis in lean and fa/fa rats 68

3.1.9 Discussion 68

a) BAT in the 5-6 week old fa/fa rat and the characteristics of the GDP binding sites 68

b) BAT and development in the fa/fa rat 72

Section 3.2 The Effect of Adrenalectomy on BAT in the Obese (fa/fa) Rat 74

3.2.1 Effect of adrenalectomy on BAT in lean and fa/fa rats 74

3.2.2 Scatchard analysis of BAT mitochondrial GDP binding in adrenalectomised lean and fa/fa rats 76

3.2.3 Effect of food intake on BAT function 79

3.2.4 Effect of replacing drinking water with saline on BAT mitochondrial GDP binding in lean and fa/fa rats 79

3.2.5 Time course of the changes in BAT mitochondrial GDP binding after adrenalectomy of lean and fa/fa rats 83

3.2.6 Effect of adrenalectomy on BAT mitochondrial GDP binding in lean and fa/fa rats of different ages 83

3.2.7 Effect of aldosterone and corticosterone replacement to adrenalectomised lean and fa/fa rats 83

Page

	3.2.8	Effect of adrenalectomy on serum triiodothyronine (T_3) concentration in lean and fa/fa rats	89
	3.2.9	Effect of thyroid hormone treatment on lean and fa/fa rats	89
	3.2.10	Effect of induction of diabetes with streptozotocin on BAT of lean and fa/fa rats	93
	3.2.11	Discussion	95
Section	3.3	The Effect of Temperature and Overfeeding on BAT Thermogenesis in the Obese (fa/fa) Rat	103
	3.3.1	Effect of environmental temperature on BAT of lean and fa/fa rats	103
	3.3.2	Effect of acclimation to 4°C on BAT mitochondrial GDP binding in lean and fa/fa rats of 5 and 10 weeks of age	109
	3.3.3	Effect of overfeeding with sucrose in lean and fa/fa rats	112
	3.3.4	Effect of cold and diet on serum T_3 and insulin concentration in lean and fa/fa rats	115
	3.3.5	Effect of sucrose feeding in adrenalecomised lean and fa/fa rats	118
	3.3.6	Discussion	120
Section	3.4	Sympathetic Regulation of BAT in the Obese (fa/fa) Rat	129
	3.4.1	Effect of noradrenaline on BAT of lean and fa/fa rats	129
	3.4.2	Effect of noradrenaline of BAT mitochondrial GDP binding in corticosterone treated lean rats	132
	3.4.3	Effect of the β antagonist propranalol on BAT mitochondrial GDP binding in lean and fa/fa rats	135
	3.4.4	Effect of β blockade with propranalol on BAT mitochondrial GDP binding in adrenalectomised lean and fa/fa rats	135
	3.4.5	Effect of β blockade with propranalol to adrenalectomised lean and fa/fa rats fed with additional sucrose	135
	3.4.6	Discussion	139
Section	3.5	Relationship of Defective BAT Function to the Recessive fa Gene	144
	3.5.1	Effect of fa gene dosage on BAT of the Zucker rat	144

Page

3.5.2	Effect of genotype on 10 day old pre-obese (fa/fa) rats	147
3.5.3	Discussion	147
CHAPTER 4	SUMMARY AND DISCUSSION	153
REFERENCES		157

CHAPTER 1

INTRODUCTION

1.1 Obesity

The term obesity is used to describe an excessive accumulation of adipose tissue. In affluent societies approximately 25% of the population between the ages of 30 and 40 are 20%, or more, above their ideal body weight, and can therefore be considered obese *(Renold, 1981)*. Obesity represents one of the major health problems in western societies. Few disease states have drawn so much of the publics attention, and both the public and the medical community generally assume that a reduction in the prevalence of obesity will result in a drastic improvement of the population's morbidity and mortality *(Bray, 1976)*.

Obesity is a problem of energy balance and results from an excessive energy intake as food, over energy expenditure as metabolic and physical activity. The situation may arise from either an excessive energy intake, a depressed energy expenditure, or from a failure to increase energy expenditure in line with energy intake.

Conventionally, obesity has been ascribed to a high level of energy intake, and there has been much evidence to support this finding *(Schachter & Rodin, 1974; Hashim, 1981)*. Recently, the importance of food intake in determining energy balance has been questioned *(James & Trayhurn, 1981; Miller & Parsonage, 1975; Garrow, 1978; Sims et al. 1973)*. Evidence for a reduced energy expenditure contributing to the accumulation of body fat has been demonstrated *(James & Trayhurn, 1976; Jung et al., 1979)* which cannot be explained as due simply to variations in physical activity *(Garrow, 1978)*. Due to the difficulties associated with long term energy balance studies in man, many workers have turned to the study of obese animals.

1.2 Use of animal models in the study of obesity

Detailed studies of the biochemical, physiological, endocrinological and pathological changes associated with the obese state have been facilitated by the availability of a wide range of animal models *(York, 1979; Cawthorne, 1979)*. Since human obesity undoubtedly represents the common end result of many differing pathologies, the

study of animal models should enhance our understanding of the many possible metabolic causes of obesity. The most commonly studied animal models have been the genetic obesities, the obesity which results from hypothalamic damage, and dietary induced obesity *(Bray & York, 1979; Rothwell & Stock, 1979b, 1980b)*.

1.2.1 Non-genetic models of obesity

a) Chemically or surgically induced obesity

It has been known for many years that lesions of the hypothalamus can produce obesity *(Smith, 1927; Hetherington & Ranson, 1939; Frohman, 1978)*. Lesions to the ventromedial part of the hypothalamus (VMH) produced by electrolytic injury, radiofrequency lesions, knife cuts transecting the longitudinal fibre tracts at the level of the paraventricular nucleus, or injection of goldthioglucose or monosodium glutamate, all produce ventromedial hypothalamic (VMH) obesity (for reviews see *Assimacopoulos - Jeannet & Jeanrenaud, 1976; Bray & York, 1979)*.

In the adult rat, bilateral ventromedial lesions are followed by increased food intake and a gradual increase in body weight and body fat. Pair-feeding does not prevent the rise in body fat. In the weanling rat, bilateral ventromedial lesions are accompanied by an increase in the percentage of body fat, but there is no increase in food intake and no increase in body weight (for review see *Bray & York, 1979)*.

b) Endocrine-induced obesity

Various hormones have a marked effect on metabolic rate and lipogenesis, and some have been used to increase the rate of fat deposition. Insulin lowers blood glucose and, by way of compensation, increases voluntary food intake *(Hoebel & Teitelbaum, 1966, Macdonald et al., 1976)*. There is an increase in lipogenesis, and the positive energy balance results not only from hyperphagia, but also from an increased efficiency of food utilization *(Chan et al., 1982)*. Progesterone tends to increase body fat *(Miller, 1979; Tarttelin & Gorski, 1973)*, and corticosteroid administration is frequently accompanied by a rapid gain in body weight and development of obesity *(Hausberger, 1958)*.

c) Dietary-induced obesity

To produce obesity by dietary manipulation it is necessary to induce the animal to overfeed. This can be achieved in several ways, e.g., by increasing the energy density of the diet. A high fat diet *(Lemonnier,1972; Schemmel & Mickelson, 1973)* may be associated with rapid weight gain and fat deposition. It is important however to maintain the correct protein/energy ratio in the diet, as low protein diets can cause weight loss *(McCracken & Gray, 1976; Rothwell et al., 1982c)*. Obesity can also be induced by feeding a highly palatable diet, e.g. sucrose *(Schemmel et al., 1982; Kanarek & Hirsch, 1977)* or a 'cafeteria' diet *(Sclafani & Springer, 1976; Stock & Rothwell, 1979)*. With all of these diets the rat increases its energy intake and on long term feeding deposits extra body fat. The degree of obesity depends on the duration of the diet and the age and nutritional state of the animals when the diet is introduced *(Schemmel & Mickelson, 1973; Sclafani & Gorman, 1977; Rothwell & Stock, 1979b, 1980b)*. The genetic background can also influence the effectiveness of the diet *(Rothwell & Stock, 1980b; Rothwell et al., 1982e)*. *Miller (1979)* found large variations in fat content, in different strains of rats maintained on a high fat diet. However, he noticed that lipid deposition was more related to the efficiency of energy utilization than to the level of energy intake. *Rothwell et al. (1982e)* examined four strains of rat (Sprague-Dawley, Lister Hooded, Alderley Park and a hybrid Wild/Sprague-Dawley strain), fed with a 'cafeteria' diet and reported that Sprague-Dawley and Alderley Park strains gained more fat than the other two, and concluded that the weight gain was due to strain differences in energetic efficiency and energy expenditure. Generally though, the energetic efficiency of young rats fed a 'cafeteria' diet is reduced *(Rothwell & Stock, 1979a)*, and little excess fat deposition occurs despite a large increase in energy intake. This ability to compensate for overfeeding appears to be reduced in older rats, in which 'cafeteria' feeding is associated with appreciable body fat deposition *(Sclafani & Gorman, 1977; Rothwell & Stock, 1982a)*. When rats are returned to a normal chow diet there often follows a period of hypophagia, and in some cases the metabolic rate remains increased *(Rothwell & Stock, 1979a, 1979b)* which results in a return to normal body weight.

1.2.2 *Genetic models of obesity*

Animals having obesities resulting from a single, recessive gene lesion, have been the most extensively studied. Genetic theory suggests that a single-gene defect leads to the production of a defective protein, which may in turn lead to a multiplicity of metabolic abnormalities *(Festing, 1979)*. Theoretically the primary biochemical lesion should be detectable, however, the metabolic abnormalities arising as a consequence of the lesion are so numerous, that so far it has proved impossible to detect. The range of animal models of obesity has been extensively reviewed *(Bray & York, 1971a, 1979)*. A number of metabolic disturbances are shown consistently in all models of obesity, although the severity of these changes and their age of onset may vary considerably. One of the models most extensively used in the study of obesity has been the Zucker 'fatty' (fa/fa) rat and a brief review of the major characteristics of this mutant are discussed below.

1.3 *The Zucker 'fatty' (fa/fa) rat*

The spontaneous fa/fa mutation was first described by *Zucker & Zucker (1961)* and appeared in a cross between Sherman and Merck stock M rats *(Zucker & Zucker, 1963)*. The obesity in these rats is due to a single gene mutation and the trait is inherited in a mendelian recessive manner *(Zucker & Zucker, 1961; Yen et al., 1977)*. The symbol fa (recessive) is given to the fat allele and Fa (dominant) for the normal allele. The obese (fa/fa) male rats are occasionally fertile, the females are uniformly sterile, and the phenotypically normal heterozygotes (Fa/fa) are usually used for breeding, one in four of the resulting offspring being obese.

1.3.1 *Development of obesity in the fa/fa rat*

One of the earliest demonstrable defects in the fa/fa rat is a decreased thermogenesis, as detected by a reduction in oxygen consumption *(Planche et al., 1983)*, which was noticed as early as 5 days of age. As young as 7 days of age, a reduced core temperature can be detected in the fa/fa rat *(Planche et al., 1983)* and this, it has been suggested, may indicate a reduced capacity for thermoregulatory thermogenesis. Also detectable at 5 - 7 days of age in the pre-obese fa/fa rat, is an adipose cell hypertrophy and over-

development of adipose tissue *(Boulange et al., 1979b)* possibly associated with their diminished metabolic rate *(Godbole et al., 1978; Kaplan, 1977)*. Adipose tissue thymidine kinase and DNA polymerase, enzymes that are associated with proliferative activity, showed marked increases in the pre-obese phase *(Boulange et al., 1979a)* as does lipoprotein lipase (LPL), an enzyme associated with storage of circulating triglyceride. This elevation in LPL activity occurs during the first week of life, before hyperphagia and hyper-insulinemia *(Boulange et al., 1979b)* and persists well into adulthood, peaking at 15 weeks of age, at which time the cell size is maximal *(Gruen et al., 1978)*.

Hyperphagia is absent in the pre-obese suckling fa/fa rats, since measurements of ^3H_2O intake from maternal milk show similar values for all the pups *(Bell & Stern, 1977; Boulange et al., 1979b)* However, there is evidence to suggest that pre-obese fa/fa pups may be characterised by an early intake of solid food in the third week of life *(Boulange et al., 1979b)*. At the second postnatal week, when the body weight of pre-obese fa/fa and lean littermates were still similar, it seems that even in the absence of hyperphagia or hyper-insulinemia, incoming substrate is preferentially shunted to body fat *(Bell & Stern, 1977)*. Increased LPL activity in the fa/fa rat is not a secondary consequence of hyperphagia, since life long food restricted rats still showed increased LPL activity, in epididymal fat pad adipose cells, when compared to food restricted and ad lib fed lean rats *(Cleary et al., 1980)*.

The time of onset of hyperinsulinemia appears to be around 17 days of age *(Bazin & Lavau, 1982; Zucker & Antoniades, 1972)*, just prior to weaning. This is the time when pre-obese fa/fa pups begin to show hyperphagia if allowed access to solid chow diet *(Stern & Johnson, 1977; Bazin & Lavau, 1982)*. After weaning, under free feeding conditions, the hyperphagia accelerates the developing hyperinsulinemia. When pre-obese fa/fa and lean rats were allowed access to a high carbohydrate chow diet, adipose tissue lipogenesis was increased to higher levels in the pre-obese fa/fa group than in the lean group *(York et al., 1981)*. Also when the secretory response of the pancreatic β cells was investigated in response to a glucose load *(York et al., 1981; Rohner-Jeanrenaud et al., 1983)*, pre-obese fa/fa rats showed an enhanced and prolonged insulin secretory response.

This hypersecretion of insulin was returned to normal by acute pre-treatment with atropine *(Rohner-Jeanrenaud et al., 1983)*, indicating increased parasympathetic activity in the pre-obese fa/fa rat. The above experiments suggest that the increase in insulin secretory capacity is already present before weaning. However, recent in vitro experiments with isolated perfused pancreas preparations from suckling fa/fa rats, failed to show a hypersecretion of insulin in response to glucose *(Chan et al., 1983)*. Despite this, enlarged pancreatic islets were present in the suckling fa/fa rat. Around the time of weaning (21 days), the rate of hepatic and adipose tissue lipogenesis is increased significantly, when compared to the lean rat *(Godbole & York, 1978; Godbole et al., 1978; Jeanrenaud, 1978; Smith & Kaplan, 1980)*. These increases have been attributed mainly to their hyperinsulinemia and hyperphagia *(Godbole et al., 1978; Zucker & Antoniades, 1972; Martin, 1974; Martin & Lamprey, 1975)*.

Hyperphagia, a characteristic trait of the weaned fa/fa rat *(Boulange et al., 1979b;Godbole et al., 1981)*, is associated with a rapid deposition of body fat in all adipose tissue depots, particularly in subcutaneous sites. Around this time (3 to 4 weeks of age) the fa/fa rat can be visibly detected as obese *(Planche et al., 1983)*. The increase in fat deposition is accommodated by an increase in the adipocyte size and also by an increase in the number of adipocytes *(Bray, 1977; Johnson et al., 1971)*. Weaned fa/fa rats develop excess adiposity even when 'pair-fed' to lean rats *(Bray et al., 1973; Deb et al., 1976; Cleary et al., 1980)*, confirming that hyperphagia is not the primary cause of overdevelopment of adipose tissue in the fa/fa rat.

At 16 days of age, lean and fa/fa rats have similar body protein content, but thereafter, the rate of protein deposition in the body and skeletal muscle mass is decreased in the fa/fa rat *(Reeds et al., 1982)*. After weaning the phenotypic differences in protein deposition are less marked. However, if the fa/fa rat is 'pair-fed' to its lean littermate, the deposition of body protein is markedly reduced when compared with that of the lean rat *(Radcliffe & Webster, 1976)*. This suggests that lipid is laid down at the expense of protein, when food intake is restricted in the fa/fa rat.

1.3.2 *Endocrine status in the obese (fa/fa) rat*

a) Insulin

The association of obesity and hyperinsulinemia in the fa/fa rat has been well documented *(Stern & Hirsch, 1971; Bray, 1977; Bray & York, 1979)*, and appears in all forms of experimentally induced animal obesity (see reviews by *Bray & York, 1971a, 1979;* and *Herberg & Coleman, 1977,* for references). Insulin increases lipoprotein lipase activity in vitro *(Salaman & Robinson, 1966)* and in vivo in the rat *(Garfinkel et al., 1976)*. Therefore, hyperinsulinemia may be important in further increasing LPL activities in the developing fa/fa rat and thus contributing to maintaining the obese state *(Chan & Stern, 1982)*.

Parasympathetic activity may be increased in the fa/fa rat *(Rohner-Jeanrenaud et al., 1983)*, since electrical stimulation of the vagus nerve, preceding a glucose load, potentiated the in vivo glucose-induced insulin release to a greater extent in the fa/fa rat than in the lean rat. Also, vagotomy of Zucker rats resulted in an immediate decrease in glucose-induced insulin secretion in fa/fa rats, but not in lean rats *(Rohner-Jeanrenaud et al., 1983)*. However, hyperinsulinemia is not a primary factor in causing the onset of obesity, since the pre-obese suckling rat has been shown to have an increased accumulation of triglyceride in the first and second weeks of life, alongside an increased LPL activity, at which time hyperinsulinemia was not apparent *(Boulange et al., 1979b; Gruen et al., 1978)*.

The fa/fa rat is also known to show pancreatic islet hyperplasia *(Larsson et al., 1977)* and hypertrophy *(Shino et al., 1973)*, and more recent work has shown that 21 day old foetuses bearing the fa/fa gene have higher plasma insulin levels than homozygous (Fa/Fa) lean foetuses *(Turkenkopf et al., 1982)*. These workers suggest that this hyperinsulinemia is modulated by food restriction whilst suckling, and reappears around the time of weaning. Neither restriction of food intake prior to weaning *(Johnson et al., 1973)*, nor restriction of carbohydrate intake after weaning *(Stern et al., 1975)*, prevents the development of hyperinsulinemia in the fa/fa rat.

Peripheral insulin resistance gradually develops in the fa/fa rat, as the serum insulin levels increase with age *(Zucker & Antoniades, 1972; Stern et al., 1972)*. The tissues most markedly affected are

muscle and adipose tissue *(Stern et al., 1975; Zucker & Antoniades, 1972; Crettaz et al., 1978)*. At the tissue level, insulin resistance is expressed by a decrease in both sensitivity and maximal response of metabolic pathways, to insulin stimulation. Insulin resistance is a secondary adaptation of the tissues to the continual stimulation by high endogenous concentrations of the hormone *(York & Bray, 1973a,b; Stern et al., 1975)*.

b) Thyroid function

The possibility of dysfunction in the pituitary-thyroid system was suggested by the lower metabolic rate of the fa/fa rats compared to lean controls *(Bray, 1969a)*. This is supported by the observations that oxygen consumption *(Kaplan, 1977; Planche et al., 1983)* and body temperature *(Godbole et al., 1978; Planche et al., 1983)* are both depressed at a very early age. Obese (fa/fa) rats also show abnormalities in such indices of thyroid function as uptake and release of ^{131}I by the thyroid *(Bray & York, 1971b)*. Serum protein bound iodine (PBI) and thyroxine (T_4) concentrations are also reduced in fa/fa rats *(Bray & York, 1971b; Martin et al., 1978)*. The normal concentration of thyroid stimulating hormone (TSH) in serum and pituitary, suggest that the defects in thyroid function are not attributable to a lack of TSH stimulation *(Bray & York, 1971b)*. Thyroid releasing hormone (TRH) injection produced a normal increase in circulating TSH in fa/fa rats. However, serum TSH only rose slightly in fa/fa rats treated with propylthiouracil *(York et al., 1972)*, suggesting that the fa/fa rat may have an impairment in the formation and/or release of TSH from the hypothalamus, in addition to an impaired thyroid response to circulating TSH.

The serum thyroxine (T_4) concentration in fa/fa rats has been found to be lower than in lean rats *(Autissier et al., 1980)*, but the levels of triiodothyronine (T_3), the active hormone, were similar in lean and fa/fa rats. However, as reverse T_3 concentration was significantly higher in fa/fa rats, a defective peripheral deiodination of T_4 to T_3 was suggested *(Autissier et al., 1980)*.

c) Adrenal corticosteroids

Adrenalectomy of fa/fa rats prevented further excess weight gain, reduced serum insulin levels, increased their rate of

longitudinal growth and abolished the characteristic hyperphagia of fa/fa rats *(Yukimura et al., 1978; Yukimura & Bray, 1978; York & Godbole, 1979)*. When adrenalectomised fa/fa rats were treated with corticosterone the hyperphagia and obesity were restored, but corticosterone had little effect on food intake of adrenalectomised lean rats. It is unlikely that the apparent normalisation of weight gain, after adrenalectomy, was simply a result of the consequent reduction in food intake, since studies have shown that restriction of food intake in fa/fa rats does not prevent the obesity *(Bray et al., 1973; Radcliffe & Webster, 1976)*. *Martin et al. (1978)* reported a change in the diurnal pattern of serum corticosterone, such that the levels of serum corticosterone were elevated over lean values throughout most of the day. However, *Yukimura et al. (1978)* and *Shargill et al.* (1983) were unable to show any differences in corticosterone levels between lean and fa/fa rats at any time of the day. *Yukimura et al. (1978)* also reported that plasma ACTH was normal in fa/fa rats, and that it responded normally to adrenalectomy and corticosterone replacement. Hypophysectomy also prevented excessive weight gain in fa/fa rats *(Powley & Morton, 1976)*, and therefore it has been suggested that a defect in the pituitary control of adrenal function may contribute to the obesity of the fa/fa rat.

1.3.3 Energy balance in the obese (fa/fa) rat

The regulation of energy balance is central to the development of obesity, since the accumulation of fat implies that energy intake exceeds expenditure. Although an increase in food intake is not necessary for the development of obesity in the fa/fa rat *(Zucker, 1975; Bray et al., 1973)*, hyperphagia which develops around the time of weaning, is a major factor in contributing to the massive obesity in the adult fa/fa rat *(Bray & York, 1972; Dilettuso & Wangsness, 1977)*. At normal laboratory temperatures, the food intake in the fa/fa rat is around 40% higher than that of the lean rat *(Haberey et al., 1980; Deb et al., 1976)*, from 4 until 19 weeks of age *(Dilettuso & Wangsness, 1977)*. However, when food intake was expressed per unit body weight (to compensate for differences in body weight) fa/fa rats were only hyperphagic from 3 to 7 weeks of age *(Dilettuso & Wangsness, 1977)*.

Both the increase in body fat per gram of food eaten, and the

energy deposited per metabolisable energy intake, are higher in the fa/fa rat than in the lean rat, indicating an increased efficiency of food (energy) utilisation *(Bray et al., 1973; Deb et al., 1976; Zucker, 1975; Marchington et al., 1983)*. The energy requirement for maintenance is lower in fa/fa rats than in lean rats *(Pullar & Webster, 1974; Zucker, 1975; Haberey et al., 1980; Mowrey & Hershberger, 1982)*, so contributing to the increased energetic efficiency of the fa/fa rat. However, similar maintenance requirements have also been recorded *(Deb et al., 1976; Marchington et al., 1983)*. The adult fa/fa rat has a lower level of voluntary activity *(Mowrey & Hershberger, 1982)*, but since the decrease in activity does not occur until after weaning, subsequent to the initial increase in body fat and the onset of hyperphagia *(Stern & Johnson, 1977)*, it is apparent that this loss of activity is not a major factor in the increased energetic efficiency. Indeed experiments using forced exercise have shown that it has little effect on the full development of obesity in the fa/fa rat *(Walberg et al., 1982; Deb & Martin, 1975)*.

The regulation of food intake in the fa/fa rat has been extensively studied. The fa/fa rat eats larger and less frequent meals than its lean littermates *(Becker & Grinker, 1977)*. Also, the diurnal feeding pattern is disturbed in the fa/fa rat, although the largest portion of the food intake is still taken during the dark period *(Wangsness et al., 1978; Martin et al., 1978; Haberey et al., 1980)*.

Food intake and growth in rats is markedly influenced by the protein content of the diet *(Young et al., 1980)*. Lean weanling and mature rats eat more in response to a moderate dilution of diet with non-digestable fillers *(Peterson & Baumgardt, 1971)*. When given the opportunity, lean rats will self-select from a variety of diets differing in protein content, in order to keep the ratio of protein intake to total energy intake, within favourable limits *(Musten et al., 1974)*. When lean rats were fed a low protein diet *(Young et al., 1980)*, they over-ate (when food consumption was expressed in relation to body weight or metabolic body size). They also showed a decreased efficiency of energy utilisation, and increased oxygen consumption and serum T_3 concentrations. These results are consistent with an adaptive thermogenesis, in which excess non-protein energy consumed, could be dissipated through excess heat production. However, when fa/fa rats

are fed a diluted diet *(Bray & York, 1972; Bray, 1977)* they showed a more limited response than the lean control rats. When fa/fa rats were fed a low protein diet *(Young et al., 1980)*, only a small increase in food consumption was noticed. In contrast to the lean rat, the fa/fa rat which was fed a low protein diet, showed only a slight decrease in the efficiency of utilisation of metabolisable energy, and no increase in oxygen consumption or serum T_3 concentration was observed. *Pullar & Webster (1974)* reported that on ad lib. feeding, the heat losses were similar for lean and fa/fa rats, in spite of greater energy intake in the fa/fa rat; also, when fa/fa rats were pair-fed to the intake of the lean rat, heat losses were lower in the fa/fa group. *Young et al. (1980)* proposed that the fa/fa rat did not show the same thermogenic increase in response to a low protein diet as did the lean rat, because they are already overeating for protein and storing the excess as fat. However, more recent evidence has shown that fa/fa rats do not select to take more protein when offered a choice, but increase the fat component of their diet *(Castonguay et al., 1982)*.

Although the fa/fa rat is more efficient at utilising metabolisable energy for growth, when the entire carcass including fat was considered, the efficiency of protein utilisation was lower in the fa/fa rat *(Deb et al., 1976; Pullar & Webster, 1977; Zucker, 1975; Radcliffe & Webster, 1976)*. Thus the fa/fa rat is characterised by an abnormal partitioning of ingested energy between true growth (protein deposition) and fat storage.

The resting metabolic rate (RMR) has been shown to be either similar in lean and fa/fa rats *(Rothwell et al., 1981a)*, or reduced in fa/fa rats *(Planche et al., 1983)*. Obese fa/fa rats showed a reduced thermogenic response to feeding *(Rothwell et al., 1981a; Marchington et al., 1983)*. Other experiments have shown that feeding responses of fa/fa rats to various stimuli are defective *(Ikeda et al., 1980; Rothwell et al., 1981a, 1983b)*. Intraventricular administration of 2-deoxy-D-glucose (2DG), induced hyperphagia and cocomitant hyperglycemia, in lean rats *(Ikeda et al., 1980)*. Blood glucose was elevated in fa/fa rats, but food intake was unaltered after 2DG administration, suggesting that the glucosensitive site for food intake regulation, was impaired in fa/fa rats. However, intraventricular administration of noradrenaline, stimulated food intake in both lean

and fa/fa rats *(Ikeda et al., 1980)*, indicating that the fa/fa rat is sensitive to noradrenaline but not to 2DG. When 2DG was given peripherally to lean and fa/fa rats, it produced a 26% decrease in metabolic rate in lean rats but only an 8% decrease in the fa/fa rat. The low response of fa/fa rats to 2DG could be due to insensitivity to central or peripheral glucopenia *(Rothwell et al., 1981a)*. Feeding responses to 2DG are also impaired in rats with VMH lesions *(King et al., 1978)*, suggesting that the 2DG sensitive site may be in the ventromedial hypothalamus, or neurally connected to the VMH area. Supporting this suggestion, *Shiraishi & Mager (1980a,b)* observed that injection of 2DG into the hypothalamus caused hypothermia.

When lean and fa/fa rats were atropinised before intragastric feeding of a 40kJ meal, fa/fa rats showed a thermogenic response equivalent to that of the lean animal *(Rothwell et al., 1981a)*. As fa/fa rats have a high level of vagal activity, resulting in hyper-insulinemia *(Jeanrenaud, 1978; Rohner-Jeanrenaud et al., 1983)*, it is possible that atropine may block any parasympathetic inhibition of thermogenesis. VMH lesions also result in hyperinsulinemia in the rat, and the excess weight gain of these animals can be inhibited by vagotomy *(Powley & Opsahl, 1974)*.

When lean and fa/fa rats were injected peripherally with insulin, fa/fa rats increased food intake to a greater extent than did lean rats *(Ikeda et al., 1980)*. The consequent induced hypoglycemia returned to normal levels in the fa/fa group 120 minutes after insulin injection, whereas the lean group still remained hypoglycemic after this time. Therefore, the regulation of food intake appears to be more sensitive to insulin in the fa/fa rat than in the lean rat *(Ikeda et al., 1980)*.

The fa/fa rat is known to have high circulating and pituitary levels of β-endorphin *(Margules et al., 1978; Recant et al., 1983b)*. Treatment with nalaxone (an endorphin antagonist) reduces the obesity and food intake in the fa/fa rat, without affecting the behaviour of the lean littermate controls *(Margules et al., 1978)*. β-endorphins act on the pancreas to stimulate the release of insulin *(Ipp et al., 1978)*. It is possible therefore, that high circulating levels of β-endorphins may contribute to the regulation of food intake in the fa/fa rat.

1.4 *Thermogenesis and energy balance*

The equation representing energy balance is given in Figure 1.1. Metabolisable energy represents the energy obtained from food, after allowing for losses of energy in faeces and urine. The metabolisable energy is utilised for maintenance and work (growth, production and external physical work), and these transformation processes will involve inevitable losses in the form of heat. Maintaining homeothermy in cold environments involves utilisation of energy for the production of heat, by the processes of shivering and non-shivering thermogenesis (NST). Heat is also produced with the assimilation of dietary energy, diet-induced thermogenesis (DIT). The energy costs of nutrient absorption, transport, and storage, result in obligatory heat losses which form part of DIT. In addition to this, however, there is an adaptive component of DIT which helps to maintain energy balance, by dissipating the metabolisable energy consumed in excess of requirements *(Rothwell & Stock, 1981c)*.

The genetically obese (fa/fa) rat has been described as energetically efficient i.e. it has an increased efficiency of energy utilisation when compared to lean rats. Since hyperphagia is not a requirement for the development of obesity in the fa/fa rat, the explanation for this increase in efficiency must be sought in the components of energy expenditure. The resting metabolic rate of fa/fa rats is not significantly different from that of lean rats, when metabolic mass is expressed as body protein *(Kaplan, 1981)*; and a decrease in physical activity is not seen until the obese state is well established *(Stern & Johnson, 1977)*. Therefore, considerable interest has focussed on the possibility that these animals suffer from defective thermogenesis.

1.4.1 *Non-shivering thermogenesis (NST)*

NST is the heat produced when the temperature drops below the thermoneutral zone (around 28°C for the adult rat and man). The resulting increase in metabolic rate is to be distinguished from that part of the cold induced increase in metabolic rate that is due to muscle activity (shivering thermogenesis). Production of heat by shivering is usually an acute response to cold, and is not an effective mechanism for long term heat production, as it interferes with locomotion and coordination of the animal. Also, shivering thermogenesis is not an efficient mechanism of heat production, since increased

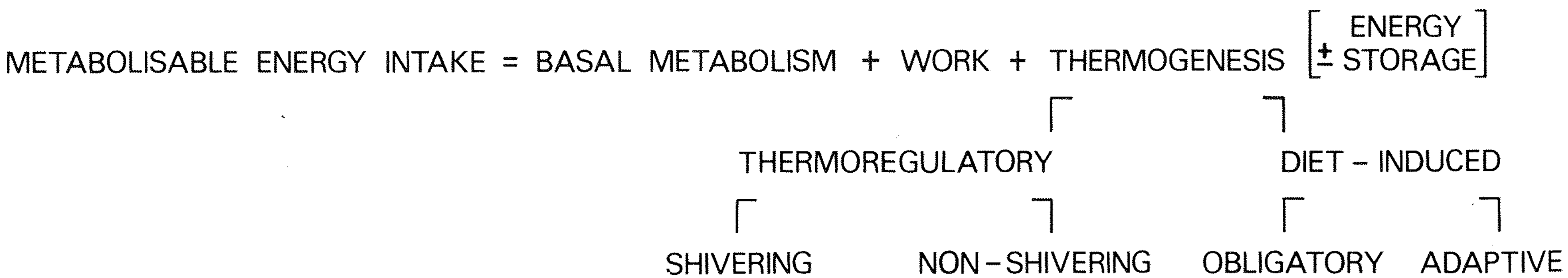


Fig I.I The utilisation of metabolisable energy represented in terms of energy balance.

muscular activity and peripheral blood flow tend to increase heat loss, and so chronic cold exposure results in switching from shivering thermogenesis to the more effective NST.

NST is an adaptive process which occurs in newborn animals of certain species (e.g. rat, rabbit, guinea pig and human), in hibernating mammals (e.g. bat, hamster and ground squirrel) and in certain species after acclimation to cold temperatures (e.g. rat, mouse and rabbit). It does not occur to as great an extent in these same species when they are adult, when they are not hibernating or when they are not acclimatised to cold temperatures. NST is a facultative process; it does not occur continuously, but can be switched on and off in accordance with the requirements of the animal. The regulation of NST is mediated by the sympathetic nervous system (SNS), principally by noradrenaline released from sympathetic nerve endings. Evidence for this includes:

i) that the increase in NST during the first few weeks of cold exposure is paralleled by an increase in the capacity to respond to noradrenaline with an increase in metabolic rate *(Girardier & Seydoux, 1978)*,

ii) the ability of noradrenaline, but not adrenaline, to increase oxygen uptake and maintain body temperature in cold exposed rats treated with hexamethonium *(Hsieh et al., 1957; Leduc, 1961)*,

iii) that the increase in NST seen in cold acclimatised rats can be inhibited by β adrenergic blockade with propranolol *(Rothwell & Stock 1980a)*.

1.4.2 Brown adipose tissue (BAT)

BAT has now been established as the main site of NST in the rat *(Foster & Frydman, 1978, 1979)*.

BAT is an organ distinct from white adipose tissue. The function of white adipose tissue is the storage of energy in the form of triglycerides. In contrast, the function of BAT is the production of heat *(Smith & Horwitz, 1969; Lindberg, 1970; Himms-Hagen, 1976)*. To support this function BAT also stores some triglyceride, but only for its own use as a substrate for heat production. Whereas the function of white adipose tissue is controlled primarily by the level of circulating insulin, the function of BAT is principally controlled

by its sympathetic innervation *(Smith & Horwitz, 1969; Cottle, 1970)*.

a) Morphology

BAT occurs at multiple sites within an individual animal *(Smith & Horwitz, 1969; Afzelius, 1970)*, and can constitute between 1 and 5% of body weight. In small rodents, however, BAT constitutes in the region of 1 - 2% of body weight, the largest deposit of the tissue being found in the interscapular area *(Smith & Horwitz, 1969; Afzelius, 1970)*. Other sites include the subscapular, cervical, axillary and perirenal regions *(Afzelius, 1970)*. Each discrete mass contains up to 80% adipocytes, and the remainder is composed of a rich vasculature, with a finely divided capillary bed, and connective tissue *(Afzelius, 1970; Barnard, et al., 1980)*. BAT is particularly developed in neonates, hibernators and some cold acclimatised non-hibernators. An autopsy study in the human *(Heaton, 1972)*, showed that in the first decade of life, there is a consistently wide distribution of BAT, but that during the next 6 decades BAT disappears from most areas, persisting however, around the kidneys, suprarenals, aorta, neck and mediastinum. BAT is readily distinguished from white fat by both appearance and topology. The colour of BAT ranges from pale buff to dark reddish brown *(Afzelius, 1970)*, depending on seasonal (in the case of hibernators), nutritional and environmental conditions. The colour derives largely from the blood haemoglobin and to some extent, from a high level of heme porphyrins (largely cytochromes), *(Hook & Barron, 1941; Joel & Ball, 1962)*, and flavin compounds *(Smalley & Dryer, 1967)* in the mitochondria. Infiltration with white fat turns the colour towards beige. When examined under the electron microscope, brown adipocytes are characterised by numerous small lipid inclusions in the cytoplasm, and an extremely high content of highly invaginated mitochondria *(Afzelius, 1970)*. White adipose tissue however has adipocytes which have a single large lipid vacuole, and few, sparsely distributed mitochondria, which have a less dense internal structure (few cristae).

b) Mitochondria

BAT is characterised by an abundance of mitochondria, which provide the brown adipocyte with the most distinct and reliable morphological criterion for identification *(Afzelius, 1970)*. The

cristae typically traverse the whole width of the mitochondrion and are usually tightly packed *(Afzelius, 1970; Flatmark & Pedersen, 1975)*. This pattern is a characteristic feature of mitochondria with a high respiratory capacity. BAT mitochondria are rich in respiratory chain enzymes *(Flatmark & Pedersen, 1975)* and low in ATP synthase.

c) Blood supply

The main function of BAT in NST is to heat the blood passing through it. This process is facilitated by its rich capillary network, a feature which distinguishes it from the white adipose tissue. The vascularity of the BAT is four to six times greater than that of the white adipose tissue in the rat *(Hausberger & Widelitz, 1963)*, and the vascular system of BAT is the main extracellular compartment surrounding the brown adipose cells *(Fain et al., 1967; Smith & Horwitz, 1969)*. The high blood flow satisfies the high requirement for oxygen and oxidisable substrates, by the tissue, during thermogenic periods.

d) Innervation

BAT appears to be innervated predominantly by fibres of the sympathetic nervous system (SNS) *(Smith & Horwitz, 1969; Cottle, 1970)*. The interscapular BAT mass comprises two bilateral lobes each of which receives a discrete nerve supply *(Cottle, 1970)*. Nerves entering each lobe run in the interlobular septa. Small branches of these nerves are found along the major interlobular arteries, and in addition, a large number of fine fibres can be noticed, which appear to run independently of the blood vessels and to terminate on the adipose cells. The sympathetic origin of BAT innervation is supported by evidence to show that BAT contains a high concentration of noradrenaline *(Barnard et al., 1980)* and that BAT can be stimulated by administration of noradrenaline. Furthermore, when BAT is in the thermogenic state, noradrenaline turnover is increased in BAT *(Young et al., 1982)*, and this effect can be blocked by β-adrenergic blockade *(Rothwell & Stock, 1979a)*.

1.4.3 Development of BAT in the rat

BAT of young mammals has a very high respiratory capacity *(Barnard & Skala, 1970)*. The ontogeny of interscapular BAT has been studied in some detail in the rat *(Barnard & Skala, 1970; Barnard et al.,*

1980; Sundin & Cannon, 1980). The weight of BAT increases steadily during the perinatal period, until around 5 weeks of age *(Barnard & Skala, 1970).* The ratio of the weight of BAT to body weight is highest around birth and declines slowly during postnatal development. The percentage of total protein in the tissue increased from around 10% (of wet weight) at birth, to about 20% by thirty days after birth *(Barnard & Skala, 1970).* The major cause of the increase in BAT weight after birth is lipid accumulation. Respiratory activity, as measured by the quantity of some electron transport enzymes, shows a rapid increase from 1 day before birth until 5 days post-partum, and then a slower increase until 17 days after birth. Thereafter, it declined until at least the 30th postnatal day *(Barnard & Skala, 1970).* The noradrenaline content of the tissue, and the mitochondrial development, followed a similar pattern, maximising around 17 days after birth. It is at approximately 17 days of age that the increase in oxygen consumption of the rat, in response to noradrenaline injection, is greatest *(Barnard & Skala, 1970).* Therefore, the in vitro respiratory capacity of BAT seems to reflect the level of in vivo tissue respiration. There is an active cell proliferation during the first two weeks of postnatal life *(Smalley, 1970)*, after which time the tissue grows through hyperplasia of the adipocytes. After the fifth postnatal week there is a general slow involution of the tissue; the mitochondria become less packed, and appear to contain fewer cristae; the respiratory enzyme activities decline, and the catecholamine metabolism of the tissue is reduced. This tissue involution continues for the remainder of the life of the rat. However, it may be reversed by cold acclimatisation, whereby the total respiratory capacity of the tissue can be still further increased by growth of the tissue mass *(Barnard & Skala, 1970; Sundin & Cannon, 1980).*

1.4.4 Evidence for involvement of BAT in NST

BAT was initially dismissed as a major site of NST *(Hemingway, 1963)*, because it was small and because calculations of in situ oxygen consumption, based upon the rate of in vitro oxygen uptake, only accounted for a small part of the oxygen uptake of cold adapted rats *(Smith & Roberts, 1964; Himms-Hagen, 1967).* However, the involvement of BAT in thermogenesis in the rat was shown in experiments

reported by *Foster & Frydman (1978)*, using radio-labelled microspheres for measurement of blood flow and fractional distribution of blood flow. They showed that BAT could account for 60% of the increase in oxygen consumption seen in the cold adapted rat *(Foster & Frydman, 1979)*. The remaining proportion, it was suggested, could be accounted for firstly, by the increased work of the heart and respiratory muscles, that play a supporting role in providing the increased oxygen supply to BAT, and secondly, by the induced action of the increase in body temperature on the metabolic rate of the tissues in general.

During infusion of catecholamines to BAT, the blood flow to the tissue increases dramatically. *Foster & Frydman (1978)* reported that BAT (all depots) can receive up to one third of the total cardiac output. This represents one of the highest blood flows to any tissue in the body. In spite of the high blood flow, BAT can use all the oxygen supplied, and in the thermogenic state venous blood is usually completely oxygen depleted.

BAT, particularly the interscapular depot, is the warmest region in the body when NST is occurring *(Brück, 1970; Smith & Horwitz, 1969)*. Stimulation of the SNS increases the temperature, blood flow and oxygen consumption of the tissue *(Brück, 1970; Foster & Frydman, 1978, 1979)*. Moreover, the size of BAT is correlated with the requirement for NST. Thus BAT is particularly abundant in the newborn, in hibernating, and in cold acclimated animals; and BAT hypertrophies in rats during the period of adaptation to a cold environment.

BAT has been reported *(Foster & Frydman, 1979)* to be the source of much of the heat produced during cold acclimation. These authors reported that decreasing the environmental temperature from 21°C to -19°C, increased the oxygen consumption of a 370g cold adapted rat by 13.8 ml O_2/min. As 60% of the extra oxygen consumed is used by BAT (estimated total weight 5.25g), this represents a dissipative power of 500 W/kg BAT *(Rothwell & Stock, 1983b)*, which is enough to heat isolated BAT at a rate between 5°C and 10°C per minute. The blood flow (10.8 litres/kg/min in the cold adapted rat) is large enough to carry away the heat produced *(Foster & Frydman, 1978, 1979)* with an arterial-venous temperature differential as low as 0.7^{o}C, assuming that the thermal equilibration time is small compared with blood transit time. It is apparent, therefore, that BAT can be

responsible for much of the heat produced during NST in cold acclimatised rats.

1.4.5 *Mechanism of heat production in BAT*

In most tissues respiration is controlled by the cellular demand for ATP, and this respiratory control minimises the dissipation of chemical energy as heat. The chemiosmotic theory explains the control of respiration as being a consequence of the electrochemical work required to translocate protons out of the matrix, against a proton electrochemical gradient *(Mitchell & Moyle, 1969)*. As this gradient increases, the energy required to translocate further protons, more closely balances that available from the redox span of the electron transport chain and so the rate of respiration is decreased. Normally the rate of respiration is controlled by the rate of proton re-entry into the mitochondrial matrix via the proton translocating ATP-synthetase. In BAT, however, the ATP synthetase activity is exceptionally low *(Bulychev et al., 1972)*, suggesting that the control of respiration must have some unusual features. Also, the rate of proton re-entry via the ATP synthetase is inadequate to account for the observed respiratory rates *(Bulychev et al., 1972)*. Indeed BAT mitochondria have a unique uncoupling mechanism in the form of a high conductance ion uniport *(Nicholls, 1976a, 1979)* through which H^+ (or the experimentally equivalent OH^-) can cross the inner membrane without obligatory ATP synthesis (see Fig. 1.2), thus preventing induction of respiratory control, by continually dissipating the proton electrochemical gradient *(Nicholls, 1976a)*.

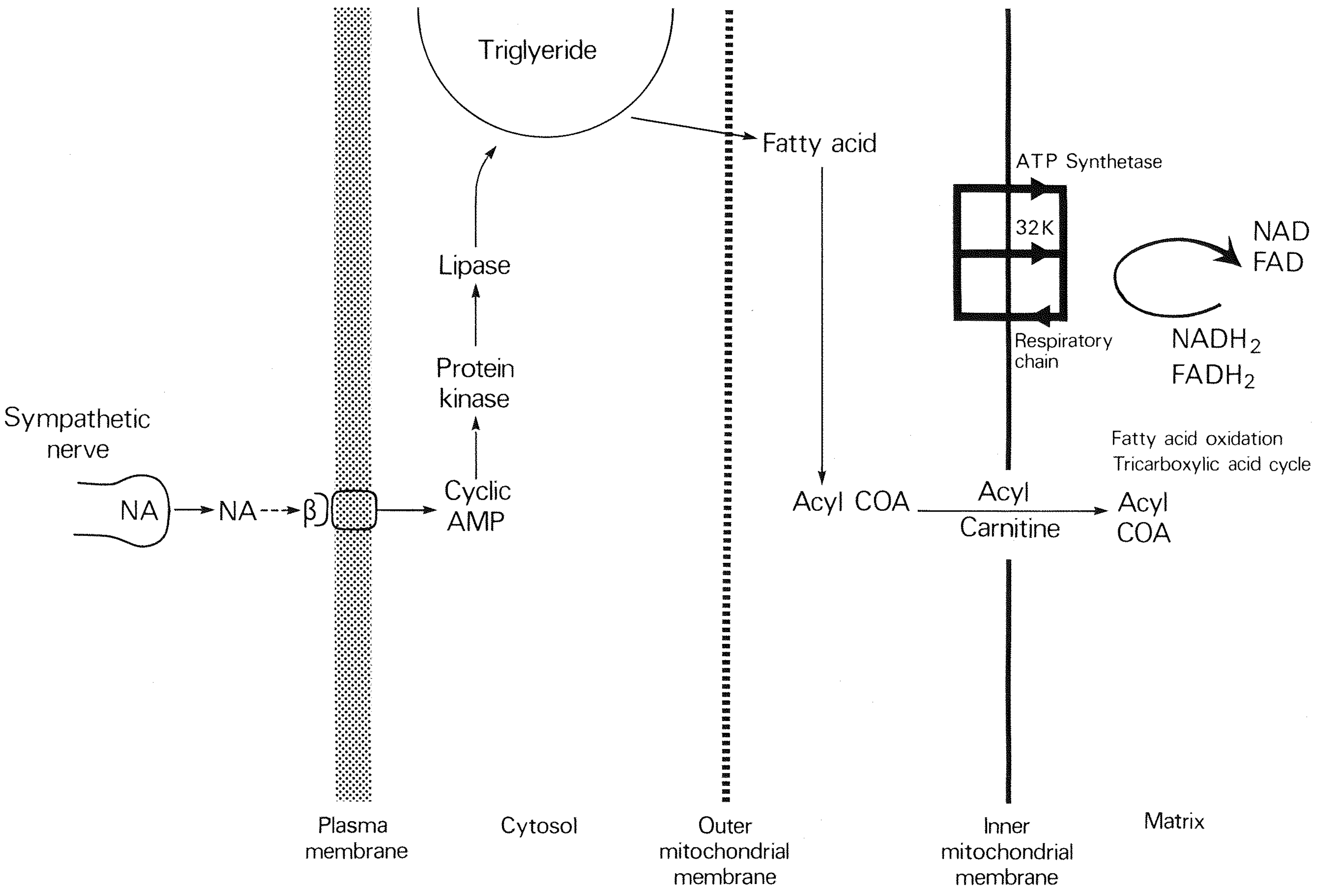
The energy dissipating ion uniport has been shown, in isolated mitochondria, to be inhibited by purine nucleotides, in particular GDP and ADP *(Hohurst & Rafael, 1968; Pedersen, 1970; Nicholls, 1976b)*. Mitochondria isolated from BAT are largely uncoupled *(Nicholls & Lindberg, 1973)* and coupling can be restored by adding purine nucleotides *(Pedersen, 1970)*.

The matrix of isolated BAT mitochondria contains millimolar concentrations of adenine nucleotides *(Pedersen & Grav, 1972)*. Since the addition of only micromolar concentrations of exogeneous nucleotides are required to couple isolated BAT mitochondria, it follows that the matrix nucleotides do not have access to the regulatory site *(Nicholls, 1976b, 1979)*. Purine nucleotides in vitro bind avidly to

FIG. 1.2 *MECHANISMS INVOLVED IN THE THERMOGENIC RESPONSE IN THE BROWN FAT CELL*

Arrows in bold print indicate movement of protons across the inner mitochondrial membrane; being pumped out by the respiratory chain, and re-entering via the 32K protein channel and the ATP synthetase.

NA = noradrenaline



Triglyeride
Fatty acid
ATP Synthetase
32K
NAD
FAD
Lipase
Respiratory chain
NADH2
FADH2
Protein kinase
Sympathetic nerve
Fatty acid oxidation
Tricarboxylic acid cycle
NA
NA
β
Cyclic AMP
Acyl COA
Acyl
Carnitine
Acyl COA
Plasma membrane
Cytosol
Outer mitochondrial membrane
Inner mitochondrial membrane
Matrix

a specific high affinity site on the outer surface of the inner mitochondrial membrane. The nucleotides do not bind covalently and are not modified chemically during their interaction with the regulatory site *(Nicholls, 1976b)*. The nucleotides, on binding to the site, decrease the proton conductance and restore respiratory control. This mechanism is independent of the adenine nucleotide translocator *(Nicholls, 1976a,b)*.

Further identification of the regulatory site was achieved by the covalent binding of 8-Azido-adenosine 5' triphosphate (γ-^{32}P labelled) to the site *(Heaton et al., 1978)*. Then, by separating the inner membrane proteins and locating the labelled components, a 32K molecular weight protein was identified as the regulatory site of proton conductance *(Heaton et al., 1978)*.

The 32K protein has been purified *(Ricquier et al., 1979a; Lin & Klingenberg, 1982)* and hydrodynamic evidence suggests that it may function in the membrane as a dimer, of molecular weight 64K *(Lin et al., 1980)*.

The nucleotide found to have the highest affinity for the regulatory site was GDP. The levels of this 32K protein in the inner membrane correlate with the degree of thermogenic adaptation of the animal *(Heaton et al., 1978; Desautels et al., 1978; Ashwell et al., 1983)*. The 32K protein has been found to be located exclusively in BAT mitochondria *(Cannon et al., 1982; Lean et al., 1983)* and to be universally present in thermogenically active BAT of different species. Hence the binding of labelled purine nucleotides, particularly [^{3}H]-GDP, has become a widely used parameter to quantitate the thermogenic state of the tissue.

Another factor that has been proposed to contribute to the high metabolic rate of BAT, is the hydrolysis of ATP by the membrane bound [Na^+K^+]-ATPase *(Horwitz, 1973)*. Its activity correlates with increased stimulation of BAT, i.e. the activity is increased in cold adapted and 'cafeteria' fed rats and on noradrenaline stimulation; depressed in fasted animals, and stimulated by thyroid hormones *(Stock & Rothwell, 1981; Ricquier et al., 1981b, 1982d)*. However, the energy cost of sodium pumping in BAT has been directly measured and found to represent only a small fraction of the total metabolism of the brown fat cell *(Chinet et al., 1977)*, therefore, it does not contribute significantly to overall thermogenesis in BAT.

1.4.6 *The substrate for BAT thermogenesis*

Fatty acids are the predominant fuel for thermogenesis in BAT, and oxidation of fatty acids takes place in the mitochondria. The substrate for fatty acid oxidation is fatty acyl-CoA and the fatty acyl-CoA synthetase is located on the outer mitochondrial membrane *(Pedersen et al., 1975)*. The inner mitochondrial membrane is not permeable to fatty acyl-CoA and its translocation depends on a supply of carnitine and the activity of carnitine acyl-CoA transferases. Fatty acyl-CoA goes through the inner mitochondrial membrane via the carnitine shuttle, and acyl-CoA is the initial substrate for oxidation. The oxidation capacity of the system in BAT is extremely high *(Nicholls et al., 1972)* and proceeds via the β-oxidation pathway, to yield acetyl CoA, NADH and $FADH_2$. The tricarboxylic acid cycle is responsible for the further oxidation of acetyl CoA to carbon dioxide. The level of tricarboxylic acid cycle intermediates is maintained by a particularly high level of pyruvate carboxylase in brown fat cells *(Cannon & Johansson, 1980)*. It is during the re-oxidation of NADH and reduced flavoproteins, by the respiratory chain, that protons are pumped out across the inner mitochondrial membrane. Lack of control of this activity (uncoupling), results in the very high rates of substrate oxidation which are responsible for the high rates of heat production in BAT.

1.4.7 *Evidence for sympathetic regulation of BAT*

The tissue content of noradrenaline, which reflects the extent of sympathetic innervation, is approximately 1.5 μg per gram of tissue in rat interscapular BAT *(Barnard et al., 1980)*. This is higher than the concentration found in the heart *(Young et al., 1982)*. Measurement of uptake and release of noradrenaline in BAT has revealed a rapid turnover which is accelerated in cold adapted rats *(Young et al., 1982)*.

The functional significance of BAT sympathetic innervation has been demonstrated by electrical stimulation of nerves supplying BAT. Stimulation elicited a biphasic temperature response *(Erskine, 1975; Flaim et al., 1976)* a depolarisation of the tissue, accompanied by a small transient fall in temperature (probably due to α mediated vasoconstriction *(Flaim et al., 1977)*), followed by a rise in temperature, which could be inhibited by β-adrenergic blockade.

Surgical denervation reduces lipolysis *(Slavin & Bernick, 1974)*. In some studies the effects of surgical denervation have only partially

impaired the response of BAT to cold exposure *(Steiner et al., 1970)*, but complete surgical denervation of BAT has not been achieved *(Barde et al., 1975)*. However, in chemically sympathectomised rats the trophic response to cold acclimation in BAT is inhibited *(Mory et al., 1982)*. Brown fat cells have a high density of dihydroalprenalol binding sites, and the relative potency of adrenergic agonists in displacing dihydroalprenalol indicates a β_1-receptor subtype *(Bukoweiki et al., 1980; Svobada et al., 1979)*. However, neither β_1 nor β_2 agonists completely prevented the rapid increase in BAT mitochondrial GDP binding when noradrenaline was administered to cold acclimatised rats, whereas in combination total blockade could be achieved *(Rothwell et al., 1982b)*. This indicated that a mixed β_1/β_2 receptor population mediates the mitochondrial response to noradrenaline in vivo. The possible involvement of α_1 and α_2 receptor systems in the control of BAT thermogenesis, has also been reported *(Rothwell et al., 1982b)*.

Synaptic release of catecholamine seems to be more important than adrenal medullary release in BAT thermogenesis, since adrenalectomy impairs thermoregulation less than immunosympathectomy *(Himms-Hagen, 1975)*. Also, the concentration of exogenous noradrenaline and adrenaline required for maximal stimulation of metabolic rate in BAT, was far in excess of their circulating levels. This indicated that BAT thermogenesis is mainly dependent on synaptic release of noradrenaline *(Mejsnar & Jirak, 1981)*. The effective concentration of noradrenaline in the local environment of the adipocyte that induces half maximal respiratory response, has been estimated at 10^{-8}M *(Petterson & Valin, 1976; Depocas et al., 1979)*. Half maximal lipolysis requires a higher dose *(Lindberg et al., 1976)*. Adrenaline is also calorigenic, but is considered to be of secondary importance in the defence against cold *(Leduc, 1961)*.

1.4.8 Acute regulation of thermogenesis in BAT

The noradrenaline stimulation of BAT thermogenesis in intact cells is thought to occur via the following series of events *(Nedergaard & Lindberg, 1982)*. Sympathetic nerve fibres directly innervate the brown adipocytes while separate innervation passes to the adjacent blood vessels. The nervous stimulation is mediated by noradrenaline which is released to interact with the brown adipocytes, predominantly through β-adrenoreceptors on the brown adipocyte cell surface (see Fig. 1.2).

The binding of noradrenaline to the brown adipocytes is followed by a change in membrane potential and ion conductance of the plasma membrane *(Seydoux & Girardier, 1977)*. Noradrenaline also activates adenylate cyclase in the plasma membrane, which leads to an increase in concentration of cyclic AMP *(Petterson & Valin, 1976)*. Cyclic AMP initiates a cascade, activating protein kinase which activates the inactive form of hormone sensitive triglyceride lipase (HSTL). This HSTL catalyses the hydrolysis of triglycerides, ultimately to fatty acids and glycerol in the brown adipocyte. The multilocular formation of the triglyceride stores in brown adipocytes provides a large surface area of substrates for lipase action, facilitating the rapid provision of fatty acids for uptake and oxidation in the mitochondria.

On noradrenaline stimulation, respiration of BAT can increase 40 fold within one minute *(Prusiner et al., 1968a,b,c)*, and there is a shift towards a more oxidised state of both cytochrome b and NADH *(Prusiner 1968c; Williamson, 1970)*. This shift is indicative of a decrease in membrane potential across the inner mitochondrial membrane. A similar rate of reversal to basal conditions, is seen upon addition of the β-adrenergic antagonist propranalol *(Petterson & Valin, 1976)*.

In the non-thermogenic state the proton conductance pathway is presumably closed, since if the conductance rate remained high, and substrate supply was limited, membrane potential would collapse and ATP production would stop.

When rats are briefly exposed to cold, or injected with noradrenaline *(Desautels et al., 1978; Desautels & Himms-Hagen, 1979)*, isolated BAT mitochondria show a large increase in purine nucleotide binding. This occurs without any change in mitochondrial membrane composition, but is associated with an ultrastructural change and an 'unmasking' of purine nucleotide binding sites present in the mitochondrial membrane. The 'unmasking' response is rapid, and is readily reversible *(Desautels & Himms-Hagen, 1980)*. Therefore, the extent of binding of purine nucleotides is not directly related to the concentration of the 32K protein, but is a measure of the extent of exposure of binding sites in the mitochondrial membrane.

The 'unmasking' response in the brown fat cell is thought to be mediated by noradrenaline and via an intracellular messenger, which causes an increase in the proton conductance of the inner mitochondrial membrane and so uncouples the mitochondria from normal

respiratory control *(Desautels & Himms-Hagen,1979; Nicholls, 1976a,b, 1979)*. The messenger causing the uncoupling of BAT mitochondria is not known. However, *Nicholls' group (Heaton & Nicholls,1976; Locke & Nicholls, 1981; Locke et al., 1982a,b)* and others *(Bukoweicki et al., 1981)* consider that the most likely controlling agent should be a normal component of the reaction sequence from the β-receptor on the plasma membrane, to the mitochondrion. This would provide a simple mechanism, since the levels would correlate with the induction and termination of lypolysis *(Locke et al., 1982a)*. a suggested candidate *(Locke et al., 1982a,b; Bukoweicki et al., 1981)* has been the free fatty acids, since they possess the capacity for increasing the proton conductance *(Locke & Nicholls, 1981; Locke et al., 1982a,b)*.

A dual role of fatty acids as both substrates and uncouplers in BAT has previously been proposed *(Williamson, 1970; Bulychev et al., 1972; Bukowiecki et al., 1981)*, but since fatty acids will uncouple any mitochondria when added at sufficient concentration *(Pressman & Lardy, 1956)*, the proposal was regarded as over simplistic. However, recent work *(Locke et al., 1982a)* has shown that in vitro, free fatty acids can satisfy the criteria for an acute regulator of proton conductance. They are tissue specific, i.e. they increase proton conductance in isolated BAT mitochondria, 30 times as much as in liver mitochondria, which have bound the same amount of fatty acid *(Heaton & Nicholls, 1976)*. The proton conductance increases after fatty acid infusion, and when the infusion is terminated the conductance change reverses automatically, restoring membrane potential and normal respiratory control. This indicates that no damage to the integrity of the mitochondria has occurred.

The concentration of fatty acid required to increase proton conductance is very low, of the order of 0.2 μM. Although the physiological concentration of the unbound free fatty acid in the brown adipocyte cytosol is unknown *(Locke et al., 1982a)*, it is likely to be within a similar range. Fatty acids also mimic the respiratory effects of the normal response to noradrenaline. Mitochondria isolated from cold adapted rats were far more sensitive to fatty acid uncoupling than mitochondria from warm adapted rats *(Locke et al., 1982b)* and respiration in mitochondria isolated from cold adapted rats increased proportionally with the rate of fatty acid infusion. This was not the case for isolated mitochondria from warm adapted rats.

The criterion that intra-cellular fatty acids should increase and decrease in synchrony with the thermogenic response has not yet been satisfied.

1.4.9 The adaptive response

On continued sympathetic stimulation BAT wet weight increases as a result of hyperplastic growth *(Bukoweicki et al., 1978; Himms-Hagen et al., 1981)*. This was indicated by the increase in total tissue DNA, protein and cytochrome oxidase activity *(Bukoweicki et al., 1978; Himms-Hagen et al., 1981; Desautels et al., 1978)*. The growth of BAT results in an enhanced total capacity for thermogenesis.

Noradrenaline appears to be the mediator for the acute unmasking of purine nucleotide binding sites in BAT mitochondria *(Desautels & Himms-Hagen, 1979)* and is thought to be also responsible for the hyperplasia and hypertrophy of BAT *(Barnard et al., 1980)*. During these periods catecholamine metabolism is elevated and there is a raised sympathetic tone (measured by the rate of noradrenaline turnover), *(Barnard et al., 1980)*. The hypothesis that noradrenaline, in addition to its action as a stimulus for heat production might also exert a trophic influence on BAT has been examined by chronic administration of noradrenaline, to rats kept warm, in an attempt to mimic cold activation of the sympathetic nerves. The capacity for thermogenesis increases in these rats and BAT hypertrophies as a result of cell proliferation and growth *(Heick et al., 1973; Barnard et al., 1980)*.

The acclimatisation of rats to cold leads to a general stimulation of sympathetic activity *(Leduc, 1961, Tedesco et al., 1977)*. However, the adaptive changes in BAT induced by long term acclimatisation to cold cannot be attributed entirely to noradrenaline. On cold acclimatisation, a selective change in synthesis of mitochondrial proteins occurs *(Desautels et al., 1978)*, such that the mitochondria have an increased relative proportion of the 32K polypeptide *(Desautels et al., 1978;Ricquier & Kader, 1976;Ricquier et al., 1979a,b; Ashwell et al., 1983)*. These mitochondria require a higher concentration of purine nucleotides to achieve a fully coupled state in vitro *(Desautels & Himms-Hagen, 1981)*. During the growth of BAT induced by infusion of noradrenaline or overfeeding *(Himms-Hagen et al., 1980; Brooks et al., 1980; Barnard et al., 1980)*, there is no such change in polypeptide composition, and the increase in BAT mitochondrial GDP binding is attributed to an 'unmasking' of binding sites already present in the mitochondrial membrane, and to the increase in BAT mass

(Desautels et al., 1978; Desautels & Himms-Hagen, 1980, Brooks et al., 1980, 1982). The nature of the mediator for the cold induced change in mitochondrial polypeptide composition is unknown.

The method previously used for the detection of the 32K protein, was polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecylsulphate (SDS). Cold adapted rats showed an increase in the amount of protein in the 32K band separated from BAT mitochondria *Ricquier & Kader, 1976; Desautels et al., 1978; Ricquier et al., 1979b).* However, the 32K band on an SDS PAGE gel is a heterogeneous mixture of different proteins, so that a change in the amount of the specific 32K protein may be partially masked by the presence of other components, and therefore go undetected. A more reliable quantitative method of determining the amount of uncoupling protein in BAT mitochondria is radioimmunoassay *(Cannon et al., 1982; Ashwell et al., 1983; Lean et al., 1983)* and results so far show a correlation between temperature of chronic acclimatisation of animals and the amount of 32K protein *(Ashwell et al., 1983; Lean et al., 1983).* Experiments with mice have shown that between 33°C (thermoneutral temperature for mice) and -2°C, there was a progressive increase in the amount of the 32K protein in BAT mitochondria. At -2°C the mitochondrial concentration of the protein was 9 times higher than at 33°C *(Ashwell et al., 1983).*

Evidence for a functional association between the amount of 32K protein present in BAT mitochondrial membranes and thermogenesis is shown by BAT mitochondrial GDP binding. The extent to which BAT mitochondria bind purine nucleotides such as GDP is accepted as an assessment of the activity of the protein conductance pathway *(Desautels et al., 1978; Sundin & Cannon, 1980).* The binding capacity of purine nucleotides varies in synchrony with the proton conductance, and thus the thermogenic activity of the tissue *(Rafael & Heldt, 1976).* BAT mitochondrial GDP binding is lowest at thermoneutral temperatures *(Triandafillou & Himms-Hagen, 1983; Ashwell et al., 1983)* and increases progressively as the temperature decreases. Also, isolated brown fat cells prepared from cold and warm adapted animals *(Locke et al., 1982b)* showed differing responses to noradrenaline. The respiratory response to noradrenaline is considerably depressed in the brown adipocytes from warm adapted animals. Isolated mitochondria from cold adapted animals were highly sensitive to fatty acid uncoupling, and this

sensitivity is lost in the mitochondria from the warm adapted animals. Although a lower yield of mitochondria is obtained from BAT of warm adapted animals *(Locke et al., 1982b)*, the properties of the inner mitochondrial membrane, i.e. phospholipid composition *(Ricquier et al., 1978)* protein composition *(Heaton et al., 1978)*, and enzyme activities *(Triandafillou & Himms-Hagen, 1983)* are conserved, with the notable exception of the 32K protein.

1.4.10 Diet-induced thermogenesis (DIT)

DIT refers to the increase in metabolic rate following ingestion of food. This term (DIT) includes both an obligatory and an adaptive component. Obligatory thermogenesis is produced by the ingestion of food (i.e. energy cost of digestion, absorption and assimilation of nutrients and synthesis of proteins, etc.) and adaptive thermogenesis is a mechanism serving to dissipate energy consumed in excess of requirements. These two mechanisms are often difficult to separate, since the energy costs of obligatory processes vary in some individuals and may serve in adaptive responses to alter energy balance.

The existence of DIT and its importance in the regulation of energy balance has been recognised for some time *(Stirling & Stock, 1968; Miller & Payne, 1962; Tulp et al., 1977)*. Only recently, however, has BAT been implicated as being the major tissue responsible for the increase in heat production (and thus the reduction of energetic efficiency) associated with overfeeding *(Rothwell & Stock, 1979a; Himms-Hagen, 1983b)* and also with nutritionally unbalanced diets, such as a low protein diet *(Rothwell et al., 1982c)*.

Rothwell & Stock (1979b) showed that rats overate when presented with a 'cafeteria' diet, consisting of a variety of palatable food items. Energy balance studies showed that in spite of an increase in energy intake of 80%, the weight gain of cafeteria-fed rats was only 27% greater than that of the control group *(Rothwell & Stock, 1979a)*. The energy cost of weight gain (g. gain/MJ eaten) and energetic efficiency were significantly lower in 'cafeteria' rats than in controls. *Rothwell & Stock (1979b)* also reported that 'cafeteria' fed animals increased their total energy expenditure by 100%. Such an increase could not be accounted for by an increase in physical activity or by extra energy required for the excess fat deposition. Indeed, obligatory DIT has been estimated to account for only 10% of the increased heat production

seen in 'cafeteria' fed rats *(Rothwell & Stock, 1982b)*. The magnitude of the adaptive component can be considerable, and even in older rats, fed a 'cafeteria' diet, the degree of obesity was attenuated by the increase in heat production *(Rothwell & Stock, 1982a)*.

Adaptive DIT and NST involve similar mechanisms and this is evidenced by the following observations:

i) Cold exposure results in a marked increase in sympathetic activity which is thought to be largely responsible for the elevated metabolic rates of cold adapted animals *(Himms-Hagen, 1976; Jansky, 1973)*. Hyperphagic 'cafeteria' fed rats also show raised metabolic rates *(Rothwell & Stock, 1979a)*, and the increase in oxygen consumption can be abolished by β-adrenergic blockade with propranalol *(Rothwell & Stock, 1979a)*.

ii) 'Cafeteria' fed rats adapt more quickly to cold exposure *(Rothwell & Stock, 1980a)* and the onset of DIT is more rapid in previously cold exposed rats *(Rothwell & Stock, 1981b)*. This suggests that the two thermogenic stimuli produce equivalent metabolic adaptations *(Rothwell & Stock, 1980a)*.

iii) Blood flow studies and measurements of oxygen extraction in 'cafeteria' fed rats, show that the enhanced thermogenic capacity can be entirely accounted for by the increase in oxygen consumption of BAT *(Rothwell & Stock, 1981b)*, indicating that BAT is also the major effector tissue for adaptive DIT.

iv) The increases in BAT mass, protein content, mitochondrial proliferation, respiratory enzyme activities and GDP binding, seen in the cold acclimated rat *(Desautels & Himms-Hagen, 1979; Sundin & Cannon, 1980)* are also apparent in the BAT of 'cafeteria' fed rats *(Brooks et al., 1980, 1982; Himms-Hagen et al., 1981; Tulp et al., 1982)*.

v) Scatchard plots of purine nucleotide binding to BAT mitochondria reveal an increase in the maximum number of binding sites in 'cafeteria' fed rats *(Bryant et al., 1983)* without any change in affinity. This was also the case for BAT mitochondria isolated from cold adapted rats *(Bryant et al., 1983; Sundin & Cannon, 1980)*.

vi) Unlike cold adapted animals, 'cafeteria' fed rats do not show an increase in the concentration of the 32K protein

in BAT mitochondria *(Himms-Hagen et al., 1981)*. However, the method used for quantifying this protein (i.e. SDS PAGE) has been shown to be imprecise, when compared to a recently employed immunoassay technique *(Ashwell et al., 1983)*. This technique detected fine temperature-dependent changes in the concentration of the 32K protein, which were not detected by SDS PAGE, and also showed that the concentration of the 32K protein was underestimated by SDS PAGE. It is possible then, that the smaller increase in BAT mitochondrial GDP binding capacity seen in 'cafeteria' fed rats, may have resulted in a small increase in the concentration of the 32K protein, which was not detected by SDS PAGE.

1.4.11 Central control of thermogenesis

The hypothalamus is known to play an important role in thermoregulation *(Jansky, 1973)*. Both shivering and NST are under the control of the hypothalamic temperature regulating centre. Shivering is mediated by the somatic motor efferents and NST is mediated by the SNS *(Banet et al., 1978)*. The preoptic and supraoptic regions of the hypothalamus contain neurones which respond to local variations in temperature. The hypothalamus also receives inputs from other temperature sensors, situated in the brain, spinal cord and peripherally. Cooling of the preoptic area of the hypothalamus in the rat, increased oxygen consumption (without inducing shivering) and this was blocked by propranalol, indicating a neural link with BAT *(Banet et al., 1978)*. In animals which had been acclimatised to a thermoneutral temperature, there was little increase in oxygen consumption following cooling of the preoptic area. This is consistent with a reduced thermogenic capacity in these animals, as evidenced by an involution of BAT and a decreased BAT mitochondrial GDP binding *(Triandafillou & Himms-Hagen, 1983)*.

Two areas of the hypothalamus that are specifically involved with food intake are the ventromedial (VMH) and the lateral (LH) hypothalamus. Destruction of the LH results in aphagia, weight loss and increased metabolism *(Anand & Brobeck, 1951; Von de Porten & Davis, 1979; Yoshida et al., 1983)*. Conversely, lesions of the VMH induce hyperphagia (in adult but not weanling rats) and obesity.

However, rats with VMH lesions can become obese without overeating *(Bernardis & Goldman, 1976; Han, 1967, 1968)*, and the obesity is not entirely dependent on altered pancreatic endocrine function *(Sclafani, 1981)*. This suggests that the VMH lesioned rat may fail to regulate efficiency of energy retention (i.e. reduce energy expenditure). BAT in VMH lesioned rats is atrophied and has been reported to be insensitive to noradrenaline stimulation *(Seydoux et al., 1981)*. VMH lesioned rats show a reduced BAT mitochondrial GDP binding and a reduced concentration of 32 K protein in the inner mitochondrial membrane *(Seydoux et al., 1981, 1982)*.

The involvement of the VMH in control of BAT thermogenesis is supported by the observation that electrical stimulation of the VMH causes an increase in temperature of the interscapular BAT depot *(Perkins et al., 1981)*. This is similar to the effect of sympathetic nerve stimulation and can be inhibited by β-adrenergic blockade *(Perkins et al., 1981; Rothwell & Stock, 1982c)*. *Shimazu (1981)* showed that electrical stimulation of the VMH enhanced lipogenesis in BAT, whereas white adipose tissue lipogenesis was unchanged and hepatic lipogenesis was decreased.

After cold acclimatisation VMH lesioned rats show a normal increase in BAT mitochondrial GDP binding *(Seydoux et al., 1982; Hogan et al., 1982)* and the concentration of the 32K protein (uncoupling protein) in the inner mitochondrial membrane is increased. This increase was similar to that seen in control cold acclimatised rats. It is possible, however, that diet-induced, sympathetically mediated activation of BAT function may require an intact VMH region, since DIT was not observed in hyperphagic VMH lesioned rats *(Hogan et al., 1982)*.

1.4.12 Endocrine control of BAT thermogenesis

The primary control of processes involving thermogenesis in BAT is mediated by noradrenaline released from sympathetic nerve endings within the tissue. Other hormones can alter the thermogenic capacity of BAT by modifying its sensitivity to noradrenaline, or by promoting its growth or regression, and a brief summary of some of the effects of three of the hormones are given below.

a) Thyroid hormones

Thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), are known to increase basal metabolic rate (*Ismail-Beigi et al.*, *1970*). T_4 is thought to express its metabolic effects after deiodination to the more potent form, T_3 (*Hardeveld et al., 1979a,b*). T_3 is produced from T_4 by a process of 5'-monodeiodination in peripheral tissues (*Schimmel & Utiger, 1977*). Hyperthyroidism increases metabolic rate, whereas hypothyroidism causes a depression of energy expenditure (*Girardier, 1977; Leblanc & Villemaire, 1970*). Thyroid hormones have been implicated because an increase in the level of serum T_3 is seen in animals exhibiting both NST and DIT (*Scammel et al., 1981; Hardeveld et al., 1979a,b; Rothwell & Stock, 1979a; Rothwell et al., 1982b; Tulp et al., 1982*). Thyroid hormones enhance the thermogenic response to noradrenaline (*Leblanc & Villemaire, 1970*). Thus the thermogenic effect of catecholamines are increased in hyperthyroidism (*Gibson, 1981; Axelrod, 1975*) and decreased in hypothyroidism (*Fregly et al., 1979*).

Thyroidectomised rats, acutely exposed to cold, do not show the normal increase in BAT mitochondrial purine nucleotide binding (*Triandafillou et al., 1982*) and lipid mobilisation (*Mory et al., 1981*). The noradrenaline content of BAT in adult thyroidectomised rats is normal (*Kennedy et al., 1977*), and it seems likely that the failure of the thyroidectomised rat to respond to cold exposure is due to a reduced sensitivity to noradrenaline, in BAT. This may be due to a reduced affinity of its β-adrenergic receptors for noradrenaline (*Gibson, 1981; Giraridier, 1981*). Thyroidectomised rats survive in the cold if provided with a low maintenance dose of thyroid hormone (*Sellers et al., 1974*), when a normal thermogenic response of BAT is seen (*Triandafillou et al., 1982*). Thus thyroid hormones are required for the normal BAT thermogenic response to cold. However, this effect is considered to be permissive rather than direct. In experimentally-induced hyperthyroidism in rats, the hypertrophy of BAT is largely due to an increase in lipid content (*Heick et aL, 1973; Leblanc & Villemaire, 1970*). There were no changes in either BAT mitochondrial content or in total cytochrome oxidase activity in comparison to euthyroid control rats (*Heick et al., 1973*). *Rothwell & Stock (1983a)* were unable to show any increase in BAT mitochondrial GDP binding in hyperthyroid rats. However, as

thyroid hormones are responsible for maintaining basal metabolic rate, it is possible that thyroid hormones may reduce the requirements of regulatory heat production in the cold, by their effects on increasing basal metabolism. Reduced activity of the SNS has been shown in hyperthyroidism *(Axelrod, 1975; Tedesco et al., 1977; Gibson, 1981)*. This explanation can to some extent be supported by experiments with hyperthyroid rats acclimatised to differing environmental temperatures *(Sundin, 1981)*. BAT mitochondrial GDP binding was reduced in hyperthyroid rats maintained at both thermoneutral (28 - 30°C) and mildly cold (22°C) housing temperatures. Only in rats housed at 5°C was BAT thermogenesis enhanced in hyperthyroid rats, presumably since the hypermetabolism caused by T_3 was insufficient at this low temperature, for the rat to maintain its normal body temperature.

Changes in the level of food intake and the nutrient composition of the diet can profoundly affect thyroid hormone concentration. Rats fed a diet which is low in protein show an increased thermogenesis and plasma T_3 levels *(Tyzbir et al., 1981; Rothwell et al., 1982c)*. Fasting results in a reduced resting oxygen consumption and a parallel decrease in serum T_3 levels *(Donati et al., 1963; Rothwell et al., 1982f)*, whereas feeding is associated with an increase in both *(Rothwell et al., 1982f)*. The reduction in plasma T_3 levels observed during fasting, results mainly from the reduced peripheral conversion of T_4 into T_3 by the 5'-deiodinase *(Harris et al., 1978; Kaplan, 1979a)*. Fasting is also associated with decreased SNS activity *(Young & Landsberg, 1977a)* and hyperphagic rats exhibit an increased sympathetic activity *(Landsberg & Young, 1978)*.

Thyroid hormones have been shown to influence β-adrenergic receptor number *(Williams et al., 1977; Gibson, 1981)*, and propranalol (a β antagonist) reduces the in vitro conversion of T_4 to T_3 *(Van Noorden et al., 1979)*. These effects suggest that the SNS may be involved in the control of peripheral thyroid hormone metabolism. This can be supported by the observation that chronic noradrenaline treatment caused an increase in plasma T_3 levels, but not T_4 which would be more consistent with an increase in peripheral conversion of T_4 to T_3, rather than a direct effect on thyroidal secretion *(Rothwell et al., 1982f)*. Overfeeding with a 'cafeteria' diet increases both metabolic rate and serum T_3 levels, and these increases can be abolished with

propranalol *(Rothwell & Stock, 1979a)*. The diet induced changes in serum T_3 may play an important permissive function in regulating the sensitivity to sympathetic stimulation.

Hyperthyroid rats had a potentiated metabolic response to 'cafeteria' feeding *(Rothwell et al., 1983a)*. They also showed an increase in energy expenditure and an increase in BAT mass and mitochondrial content. However, BAT mitochondrial GDP binding in hyperthyroid rats was unaltered, either when fed a control diet or a 'cafeteria' diet *(Rothwell et al., 1983a)*.

Recently it has been reported that the SNS may increase the rate of T_4 conversion to T_3 by BAT type II 5'-deiodinase (5'DII) *(Silva & Larsen, 1983)*. BAT is known to have a high activity of this enzyme *(Leonard et al., 1983)*. Both noradrenaline injection and cold exposure specifically increased BAT 5'D II activity. *Silva & Larsen (1983)* suggested that an increase in activity of this enzyme could contribute to the higher serum T_3 to T_4 ratios observed in cold adapted and hyperphagic rats.

The results indicate that a synergistic relationship exists between thyroid hormones and the SNS in the rat, which could amplify the thermogenic response.

b) Adrenal corticosteroids and ACTH

The importance of the adrenal cortex in maintaining body temperature and regulating fuel reserves and metabolism has been known for many years *(Selye, 1946)*. Early work with BAT showed that adrenalectomy led to a progressive loss of tissue lipid and that replacement with cortisone caused an increase in the fat content and weight of the BAT *(Lachance & Page, 1953; Mazzucchelli et al., 1961)*; these increases were also seen when intact lean rats were treated with cortisone *(Lachance & Page, 1953)*. The reduction in lipid content of BAT that followed hypophysectomy was similar to that reported after removal of the adrenals *(Fawcett & Jones, 1949; Mazzucchelli et al., 1961)*. Both ACTH and cortisone replacement to hypophysectomised animals prevented lipid depletion, and the BAT retained its normal histological appearance. However, the response to ACTH was absent in adrenalectomised animals, indicating that the effect was mediated by release of glucocorticoids *(Smith & Horwitz, 1969)*.

ACTH has also been shown to increase blood flow to BAT *(Kuroshima et al., 1968)* and to increase basal metabolic rate (by 20%

during chronic treatment). The calorigenic effect of noradrenaline was increased by 50% with ACTH *(Laury & Portet, 1977)*, whereas glucocorticoids had no such effect *(Smith & Horwitz, 1969)*, indicating that ACTH and glucocorticoids may exert independent direct effects on BAT. Brown adipocytes have been shown to possess specific glucocorticoid receptors, indicating that BAT may be a target organ for these hormones *(Feldman, 1978)*.

1.5 Aim of the project

Genetically obese (fa/fa) rats have been extensively used to help determine factors that regulate efficiency of energy utilisation *(York 1979)*, and the obesity of the fa/fa rat is associated with an increase in energetic efficiency. This primarily results from an impaired energy expenditure rather than excessive food intake.

The capacity of BAT to dissipate large amounts of energy via NST and DIT, obviously suggests that any impairment in this system would lead to an increase in energetic efficiency and pre-dispose to obesity.

Adrenalectomy prevents the development of obesity in fa/fa rats *(Yukimura & Bray, 1978)*, it abolishes the hyperphagia and decreases serum insulin to values observed in lean rats *(York & Godbole, 1979)*. However, fa/fa rats still deposit excess fat stores when their food intake is paired to that of lean control rats *(Bray et al., 1973)*. This suggests that adrenalectomy may increase thermogenesis as well as decrease food intake in order to prevent obesity.

The aim of the present work was to investigate:

a) whether the fa/fa rat had a defective BAT thermogenesis

b) whether this suggested defect was sensitive to corticosterone

and c) whether this suggested defect was related to the primary gene lesion.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Sources of chemicals used are detailed below. Those not mentioned were of reagent grade from BDH Chemicals, Poole, Dorset, U.K. or Sigma Chemicals, Poole, Dorset, U.K.

Aldosterone	Ciba, Horsham, Sussex, U.K.
Atractyloside	Sigma Chemicals, Poole, Dorset, U.K.
Bovine serum albumen (fraction V)	Sigma Chemicals, Poole, Dorset, U.K.
Charcoal (Norit GSX)	Hopkins and Williams Ltd., from BDH, Poole, Dorset.
Corticosterone	Sigma chemicals, Poole, Dorset, U.K.
[^{3}H]Corticosterone	Radiochemical Centre, Amersham, U.K.
Dextran T-70	Pharmacia, Fine Chemicals Ltd., Milton Keynes, U.K.
Dimethyl bis phenyl oxazoyl benzene (POPOP)	G. & G. Chemicals Ltd., Berks., U.K.
Diphenyloxazole (PPO) (scintillation grade)	G. & G. Chemicals Ltd., Berks., U.K.
Fentanyl-fluanisone	Hypnorm. Crown Chemicals Co., Kent, U.K.
Folin-Ciocalteu	BDH Chemicals Ltd., Poole, Dorset, U.K.
Gelatin (swine-skin G-2500)	Sigma Chemicals, Poole, Dorset, U.K.
Glucose oxidase (sp.act.ca.200U/mg)	Boehringer Corporation Ltd., Lewes, East Sussex, U.K.
Guanosine diphosphate	Sigma Chemicals, Poole, Dorset, U.K.
[^{3}H]-Guanosine diphosphate	Radiochemical Centre, Amersham, U.K.
Insulin radioimmunoassay kit	Wellcome Reagents Ltd., Dartford, U.K.
NCS tissue solubilizer	Radiochemical Centre, Amersham, U.K.
Non-esterified fatty acid kit	Boehringer Corporation Ltd., Lewes, East Sussex, U.K.
Noradrenaline	Sigma Chemicals, Poole, Dorset, U.K.
Peroxidase (sp.act.ca.100 U/mg)	Boehringer Corporation Ltd., Lewes, East Sussex, U.K.
Propranalol	Sigma Chemicals, Poole, Dorset, U.K.
Rat insulin standard	Novo Laboratories, Denmark.

[^{14}C]-Sucrose	Radiochemical Centre, Amersham, U.K.
Streptozotocin	Sigma Chemicals, Poole, Dorset, U.K.
Synperonic NXP	Cargo Fleet Chemicals Co., Middlesborough, Cleveland, U.K.
Thyroid powder	Sigma Chemicals, Poole, Dorset, U.K.
Triiodothyronine radioimmunoassay kit	RIA (UK) Ltd., Newcastle, U.K.
Valium	Roche Products, Herts, U.K.

Scintillation cocktails used were Beckman Ready-Solv. (High Wycombe, U.K.) and Tritoscint (0.4% (w/v) PPO, 0.05% (w/v) POPOP and 33.3% (v/v) Synperonic NXP, in xylene).

2.2 *Animals*

Zucker rats *(Zucker and Zucker, 1961)* were used in all experiments. Animals were bred from heterozygote parents (Fa/fa) of the Southampton University colony. Such matings produced obese (fa/fa) rats in the usual proportion of 1 in 4. Unless otherwise stated, animals subsequently referred to as lean are taken to be Fa/?. For the gene dosage experiment, homozygous lean rats were obtained from matings of known homozygous (Fa/Fa) parents. Heterozygous (Fa/fa) lean, and obese (fa/fa) rats were also produced from crossings of heterozygous (Fa/fa) lean females and young obese (fa/fa) males.

All litters were weaned on day 21 onto rat P.R.D. diet (Christopher Hill Ltd., Poole, Dorset, U.K.) (See Table 2.1), unless otherwise stated.

2.2.1 *Sacrifice of animals*

Where a blood sample was required the animals were killed by decapitation, blood collected, centrifuged, the serum sample drawn off, frozen and stored at -20°C until required. Otherwise the animals were stunned and killed by cervical dislocation.

2.2.2 *Animal maintenance*

All animals were housed at 23°C, under a 14 h. light and 10 h. dark lighting schedule, with lights on from 0600 h. to 2000 h. They had free access to laboratory chow and drinking water, unless otherwise stated.

After adrenalectomy, the normal drinking water was substituted with a 0.9% (w/v) solution of sodium chloride.

2.3 *Sucrose supplemented diet*

Overfeeding was induced in lean and obese (fa/fa) rats by allowing free access to a 35% (w/v) sucrose solution, in addition to the laboratory chow and drinking water normally offered. Animals which had been adrenalectomised, were given the above diet plus 0.9% (w/v) saline solution.

2.4 *Pair feeding*

In order to restrict the food intake of the lean and obese (fa/fa) 'intact' rat to that of the lean and obese (fa/fa) adrenalectomised rat, a pair feeding technique was used.

TABLE 2.1

COMPOSITION OF STANDARD PRD FOOD PELLETS FROM CHRISTOPHER HILL LTD

	% of Diet by Weight
Fat	3.0
Protein	20.0
Carbohydrate	54.0
Fibre	5.0
Minerals) Vitamins)	1.8
Metabolisable energy	9.5 MJ/Kg

Each rat was housed individually, and each lean and obese (fa/fa) 'intact' rat was given the weight of food consumed on the previous day by an adrenalectomised lean and obese (fa/fa) rat respectively.

Food intake was measured twice daily for each adrenalectomised rat, for the period 9.00 - 17.00 h. and 17.00 - 09.00 h. The pair-fed rats were then fed twice daily, in an attempt to prevent major changes in the diurnal pattern of feeding that can occur in food-restricted animals.

2.5 *Identification of pre-obese rats*

Suckling, pre-obese (fa/fa) rats were identified by their lower rectal temperature on day 8 - 14, using an electric thermometer (Light Laboratories, Brighton, England), *(Godbole et al., 1978)*. Litters were removed from their mothers just before the rectal temperatures were taken. Animals which had consistently lower rectal temperatures for three consecutive days were designated as 'pre-obese'. On sacrifice, the inguinal fat pad was dissected out and weighed to confirm such identification. In all cases, where an animal had been labelled as pre-obese, the fat pad was at least two-fold heavier than that of a lean littermate.

2.6 *Regulation of environmental housing temperature*

Rats were housed individually in wire mesh cages in either a warm room (30-32°C) or cold room regulated to either 4°C or 14°C, for the times stated in the results section.

Control rats were kept at normal room temperature (23°C).

2.7 *Adrenalectomy*

Adrenalectomy or sham operations were performed through bilateral flank incisions after anaesthesia with valium (0.25 mg/100g body weight, intraperitoneally (i.p.)) and fentanylfluanisone (1 mg/100g body weight, intramuscularly) *(Green 1975)*. After such treatment righting reflex was lost in less than 5 minutes, and the rats were completely immobilised for 30-60 minutes. After identification, the adrenals were carefully removed with minimum damage to the capsule. The wounds were sewn up and animals were allowed to recover. Full

recovery took between 2 and 12 h., and during this time animals were kept at a warm (26°C) temperature, in a quiet room.

After adrenalectomy, the animals were maintained at normal laboratory temperatures, and given 0.9% (w/v) saline instead of drinking water. Control animals were sham operated and after recovery from anaesthesia, given standard laboratory chow and drinking water.

2.8 *Hormone, streptozotocin and propranolol treatments*

2.8.1 *Aldosterone*

Lean and obese (fa/fa) adrenalectomised and 'intact' rats were injected sub-cutaneously (s.c.) with aldosterone (10 μg/100g body weight) in a 0.9% (w/v) saline vehicle. Control rats received a similar volume (0.1 ml/rat) of vehicle only.

2.8.2 *Corticosterone*

Lean and obese adrenalectomised and 'intact' rats were injected s.c. with corticosterone in an ethanol, dimethylformamide, 0.9% (w/v) saline (2:1:7 v/v/v) vehicle. Doses ranged from 0.01 to 1 mg/100g body weight.

Control animals were injected with vehicle only.

2.8.3 *Addition of thyroid powder to the diet*

Hyperthyroidism was induced in lean and obese (fa/fa) rats by the addition of 0.03% thyroid powder to standard laboratory chow *(Levin et al., 1982)*. The pelleted chow was ground to a dry powder, (Christy and Morris grinder, Chelmsford, England), and the thyroid powder added (0.3g thyroid powder/kg chow) and stirred in a Hobart mixer for one hour, to ensure an even distribution of the thyroid granules. Water was carefully added to the mixture until it began to hold together. The mixture was then repelleted using a Lister pelleter (R. A. Lister and Co. Ltd., Dursley, England), and dried on trays in an oven, at 35°C, overnight. The dried diet was stored in a cool, dry place until required. A control diet was prepared using the same method.

Animals were housed individually, and either given a control or thyroid supplemented diet for 6 days.

2.8.4 *Induction of diabetes with streptozotocin*

Experimental diabetes was induced in the rat with the antibiotic streptozotocin *(Rakieten et al., 1963)*. A freshly prepared solution of streptozotocin in 0.05 M sodium citrate buffer, pH 4.3, was injected as a single dose (8.0mg/100g body weight), i.p., to lean and obese (fa/fa) rats.

Control rats received a similar volume (0.1 ml) of buffer. The development of diabetes was confirmed by measuring blood glucose, and fall in rate of body weight gain *(Rakieten et al., 1963)*. Animals were sacrificed 7 days after injection.

2.8.5 *Noradrenaline*

A freshly prepared solution of noradrenaline in a 0.9% (w/v) saline solution, was injected s.c. (50 μg/100g body weight) to lean and obese (fa/fa) rats. Control rats were injected with vehicle only.

2.8.6 *Propranolol*

Lean and obese (fa/fa) rats were injected s.c. with propranolol (2 mg/100g body weight) prepared in a 0.9% (w/v) saline solution. Control animals received vehicle only.

2.9 *Preparation of interscapular brown adipose tissue (BAT) mitochondria*

Isolation buffer

0.25 M Sucrose
0.2 mM EDTA (disodium salt)
1 mM HEPES pH 7.2 at $0^{o}C$

BAT mitochondria were prepared by a modification of the method described by *Cannon & Lindberg (1979)*.

Rats were sacrificed, and the interscapular brown fat pad quickly removed and placed in an ice cold buffer. The tissue was cleared of any muscle, blood, connective tissue and overlying white adipose tissue, rinsed with buffer, blotted and weighed. The tissue was coarsely chopped with scissors, suspended at 5% (w/v) in buffer and homogenised in a glass homogenising tube by four or five passes with a close fitting teflon pestle. The homogenate was centrifuged

at 700g for 10 minutes at 4°C, and the fat 'cake' formed on the top of the supernatant removed and discarded. The supernatant was carefully poured off into an ice-cold, clean centrifuge tube, the remaining pellet was rehomogenised in a similar volume of buffer and the spin repeated. The two supernatants were combined and centrifuged at 8500g for 14 minutes. The resulting mitochondrial pellet was washed, by resuspending in buffer including 2% (w/v) fatty acid free, bovine serum albumen (BSA) (to remove endogenous free fatty acids) and centrifuging again at 8500g for 14 minutes. The pellet was washed once more with buffer (not including BSA) and the final pellet resuspended in 0.25 M sucrose, at a concentration between 1 and 2 mg/ml, and stored on ice for no longer than 1 h. before use.

2.10 *Measurement of specific binding of [^{3}H]-guanosine diphosphate (GDP) to BAT mitochondria*

The binding of [^{3}H]-GDP to BAT mitochondria was measured essentially as described by *Nicholls (1976b)* with certain modifications.

Incubation buffer

100 mM Sucrose
20 mM Tris   pH 7.1 at room temperature (23°C)
10 mM Choline chloride
1 mM EDTA (disodium salt)
5 μM Rotenone
100 μM Potassium atractyloside
0.2 μCi [U^{14}C]-Sucrose

Assay procedure

Plastic microfuge tubes (1.5 ml capacity) were used for the assay. Freshly prepared brown adipose tissue mitochondria (200-400 μg in 200 μl 0.25 M sucrose) were added to 285 μl of the above incubation medium plus or minus 50 nmoles GDP. The tubes were preincubated at 23°C for one minute. The reaction was initiated by adding 5 nmoles of GDP containing 0.625 μCi [8^{3}H]-GDP to make a final volume of 500 μl. The tubes were vortexed for 5 seconds and then incubated in a gently shaking water bath, at 23°C, for 8 minutes. The reaction was terminated by separating the mitochondria by centrifugation in a Beckman microfuge operated at full speed for two minutes. The supernatant was removed

with a fine tipped glass pipette attached to a vacuum line. The base end of the microfuge tube containing the mitochondrial pellet was cut off and placed into a scintillation vial. N.C.S. tissue solubiliser (0.5 ml) was added to each vial, and incubated for approximately one hour at 45°C, with frequent vortexing to dissolve the mitochondrial pellet. The tissue solubiliser was then neutralised with glacial acetic acid (18 µl), and Beckman Ready-Solv (5 ml) scintillation fluid added. The $^{3}H/^{14}C$ ratio was determined for each sample by dual label counting in a Phillips scintillation counter for 5 minutes per vial. Specific binding was calculated as the [^{3}H]-GDP binding displaced by 100 µM GDP. [^{14}C]-sucrose was included in the assay to measure intercellular water trapped in the mitochondrial pellet.

2.11 Protein determination using the Lowry assay

Protein concentrations were assayed by the method of *Lowry et al., (1951)*. Reduction of phosphomolybdate, present in Folin-Ciocalteu reagent, by a preformed protein-copper complex and, directly, by tyrosine and tryptophan residues, results in the formation of a blue colour, which is measured by absorbance at 750 nm.

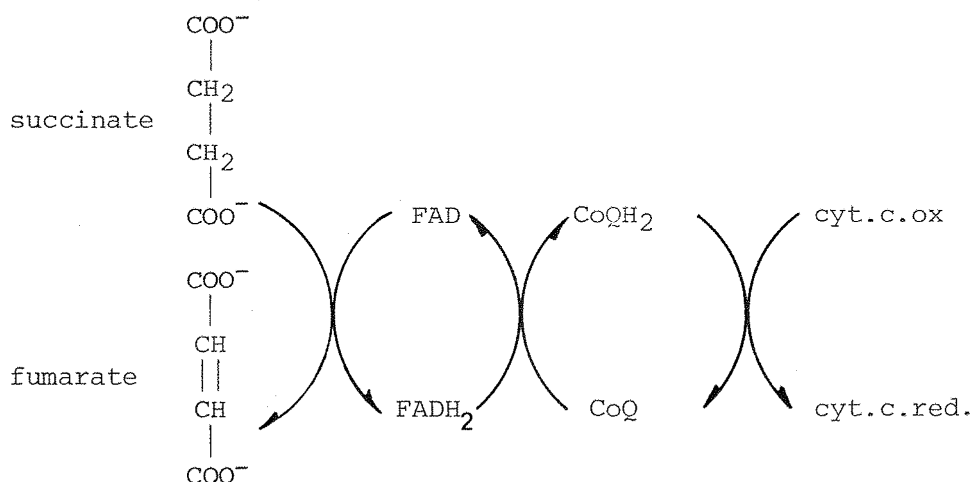
Reagents

1%	Copper sulphate solution	) Alkaline reagent
2%	Sodium potassium tartrate	)
10%	Sodium deoxycholate	)
2%	Sodium carbonate in 0.1M sodium hydroxide	)

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

Between 20 and 60 µg (in a small volume) of protein sample was taken and 1.1 mls of the alkaline reagent added. This was vortexed and left to stand for 10 minutes at room temperature. 100 µl of Folin-Ciocalteu reagent (diluted 1:1 (v/v) with water just before use) was then added to the tube, mixed and incubated in a water bath at 37°C for 15 minutes, after which time the optical density was read at 750 nm against a blank. Standard curves were prepared each time using BSA (0 - 80 µg protein) as standard protein.

2.12 *Assay of succinate-cytochrome c oxidoreductase (succ.cyt.c.O-R)*



Assay

The activity of succ. cyt. c.O-R was assayed essentially as described by *Tisdale (1967)*. The assay was performed in a final volume of 1 ml of 10 mM potassium phosphate buffer, pH 7.4, containing 1 μmole sodium azide; 200 nmoles Na_2EDTA; 5 mg BSA; 1mg ferricytochrome c; 10 μmoles sodium succinate and 10 - 20 μg protein. Solutions were warmed to 37°C, and the reaction was started by addition of the succinate. The rate of the reaction at 37°C was followed using a Unicam SP 1800 recording spectrophotometer measuring the rate of increase in optical density at 550 nm.

The extinction coefficient used for cytochrome c (reduced-oxidised) was $18.5 \times 10^3 M^{-1} cm^{-1}$ (at 550 nm) and the activities are expressed as μmoles cytochrome c reduced/minute, related either to the amount of mitochondrial protein or to the whole tissue depot.

2.13 *Removal of fatty acids from bovine serum albumen (BSA) by charcoal treatment*

Charcoal treatment removes at least 99% of albumen bound fatty acids under optimal conditions *(Chen, 1967)*.

Activated charcoal (35g) was washed with distilled water (500 mls), by soaking overnight. The residue which floated on the surface of the water, was removed using a vacuum line, and any loss in volume was made up with distilled water. BSA (fraction V) (70g) was added to the charcoal suspension, and stirred continuously until dissolved.

The pH was lowered to 3.0 by the addition of 0.2M HCl (approx. 100 ml. over 1 h). The solution was placed on an ice bath and stirred for a further hour, and then the charcoal removed by centrifugation at 30,000g for 1 h at $4^{o}C$. The solution was immediately decanted and filtered, using a millipore filter, to remove any remaining charcoal. The pH of the filtered solution was brought to 7.0 by the careful addition of 0.2M NaOH (approx. 100 mls over 1 h). The resulting solution was frozen in 10 ml aliquots at $-20^{o}C$ until required.

The protein concentration was determined by the Lowry method (Section 2.11), and free fatty acid content determined with the Boehringer assay kit (below). Samples containing <0.01 meq/g BSA were regarded as fatty acid free.

2.14 Serum assays

2.14.1 Determination of serum free (non esterified) fatty acid concentration

Serum free fatty acids were determined using an assay kit (Boehringer) with certain modifications.

Principle of assay

Free fatty acids are converted to chloroform-soluble copper salts and the copper in the organic layer is subsequently measured colorimetrically. The concentration of free fatty acids is proportional to the absorbance of the copper-containing chloroform *(Duncombe, 1964)*.

Standard solution

A standard solution of fatty acid (0.5 meq. fatty acid/litre) was prepared, using palmitic acid A.R.

Extraction of copper salts

The extraction was carried out in 10 ml. ground glass stoppered centrifuge tubes. To each of the tubes containing either 0.1 ml of palmitic acid (standard), 0.1 ml redistilled water (blank), or 0.1 ml serum (sample), 2.5 mls of chloroform was added. To this mixture, 0.1 ml of a solution containing 0.45 M triethanolamine buffer (pH 7.8) and 0.27 M cupric nitrate was added. The tubes were shaken vigorously

(using a mechanical shaker) for 5 minutes, and then centrifuged in a bench centrifuge for 5 minutes. The blue-green aqueous layer was carefully drawn off (this layer also contained the protein), by means of a fine tipped glass pipette attached to a vacuum line. One ml of the remaining chloroform layer was removed into test tubes containing 1 ml of 9 mM diethyldithiocarbamate solution, vortexed, and after standing for 10 minutes, the absorbance (A) read at 436 nm against the blank. All samples and the standard were prepared in duplicate.

Calculation

The concentration (C) of free fatty acids in serum

$$C = 0.5 \times \frac{\text{A sample}}{\text{A standard}} \quad \text{(meq/1000 ml)}$$

2.14.2 Measurement of serum triglyceride concentration

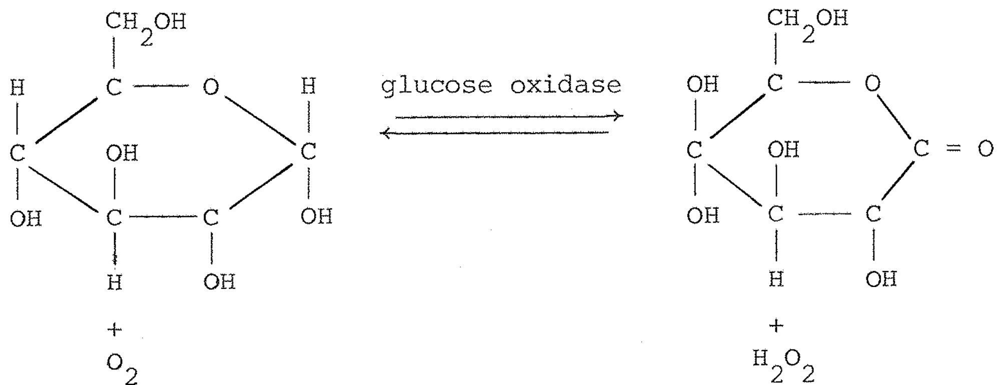
Serum triglycerides were measured after extraction in iso-propanol by a fully automated method on the Autoanalyser II system (Technicon Technical Publication AA II - 23 1971).

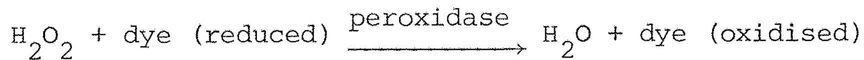
100 µl of serum was extracted in 1.9 mls of 90% (v/v) iso-propanol by vortexing for 20 seconds. 2g of zeolite mixture was added, the tubes were capped and carefully mixed by vortexing for 30 seconds. The samples were allowed to stand for 30 minutes with intermittent mixing. The zeolite was sedimented by centrifugation at 2000g for 5 minutes, and the supernatant (triglyceride extract) decanted into sample cups.

An aliquot of the isopropanol extract was then sampled on the autoanalyser into alcohol and 0.8 M potassium hydroxide mixture. Saponification of triglycerides to glycerol takes place on stream in a 50°C heating bath. This was followed by the oxidation (by perchlorate) and condensation (with acetylacetone) reactions which also occur on stream in a second heating bath at 50°C. The stream exits the second heating bath and is sent through the fluorimeter where in a 2 mm internal diameter flow cell, the fluorophore is activated and fluorescence measured. Triolein was used as a standard.

2.14.3 *Measurement of serum glucose concentration*

Principle





Reagents

The glucose oxidase reagent was prepared by mixing 9.6 mg peroxidase with 600 mg glucose oxidase and 1.2g of A.B.T.S. in 1.2ℓ of 0.1 M sodium phosphate buffer, pH 7.0. This was stored at 4°C until required.

Procedure

50 µl serum samples were mixed with 450 µl 6% (w/v) perchloric acid. The tubes were vortexed and the precipitated protein removed by centrifugation for 10 minutes, in a bench centrifuge.

A 100 µl aliquot of the supernatant was added to 2.5 ml of the glucose oxidase reagent. The samples were then incubated at 37°C for 15 minutes. The absorbtion was determined on an SP 1800 spectrophotometer at 620 nm.

A standard curve, using 0 - 100 µg glucose, was determined in all assays.

2.14.4 *Measurement of serum insulin concentration using radioimmunoassay*

Insulin in rat serum was measured using the 'Wellcome' kit by a modification of the *Hales and Randle (1963)* method.

The principle of the assay is the reaction of a limited fixed

quantity of anti-insulin serum, with a mixture of the sample of insulin to be assayed, together with a constant amount of $[^{125}I]$ insulin. The binding of labelled insulin to the antibody is progressively inhibited by increasing amounts of unlabelled insulin, due to competition for specific binding sites on the antibody.

The insulin-antibody complex is soluble, and this method separates free insulin from antibody bound insulin using a double antibody procedure, employing a pre-precipitated antibody (anti-guinea pig globulin).

Insulin binding reagent

The freeze dried contents of the mixture of guinea pig anti-insulin serum and rabbit anti-guinea pig globulin serum in 40 mM sodium phosphate, pH 7.4, containing 20 mM EDTA, 0.1% sodium azide and 0.5 % BSA were reconstituted with 8 mls deionised water and mixed by gentle inversion.

Buffers

(A) 50 mM phosphate buffer pH 7.4 containing 0.5% BSA, and 0.025% thiomersal,

(B) 50 mM phosphate buffer pH 7.4 containing 0.5% BSA, 0.025% thiomersal and 0.9% sodium chloride.

Standard insulin solution

Rat serum standard used in the assay was serially diluted in buffer (B) to give a range from 6.25 μU/ml to 100 μU/ml.

The assay was performed with triplicates of each sample in 2 ml glass tubes.

The tubes were set up as follows:

i) 'Wash' blanks to which 100 μl buffer (B) was added.

ii) 'Zeros' to which 100 μl buffer (B) was added.

iii) 'Standards' 100 μl of each dilution of rat insulin standard added.

iv) 'Unknown' 100 μl unknown serum sample added (diluted if necessary).

To (i) 100 μl of buffer (A) was added (as control of the decanting procedure). Tubes in catgegories (ii), (iii) and (iv) received 100 μl

of reconstituted binding reagent. The contents of all tubes were vortexed and left at $4^{o}C$ for 6 h. 100 μl of the working solution of iodinated (^{125}I) insulin (0.25 μCi/ml) was then added to all tubes, plus three other tubes for 'total' counts. All tubes were mixed and left at $4^{o}C$ for 18 h. 1 ml of buffer (B) was added to all tubes except 'totals' at the end of the incubation, and tubes centrifuged at 2000g for 20 minutes and supernatants decanted. Each tube was then counted for 1 minute, in a Beckman γ-counter with an efficiency for ^{125}I of 80%.

Radioactivity bound was expressed as a % of the total radioactivity added, after background count was subtracted:

$$\frac{\text{Bound-Blank}}{\text{Total-Blank}} \times 100$$

The means of each set of triplicates were determined, and plotted as mean % bound versus concentration of standard insulin, or log concentration of standard insulins. The curve was used to determine the insulin concentration in the unknown samples.

2.14.5 *Measurement of serum triiodothyronine (T_3) using radioimmunoassay*

Serum T_3 concentration was measured by specific radioimmunoassay using a Gamma-B T_3 kit. A pre-precipitated antibody complex (500 μl) was added to serum samples (50 μl), vortexed gently and incubated at $37^{o}C$ for 30 minutes. This was followed by the addition of [^{125}I]-T_3 tracer (200 μl) vortexed gently, and incubated for 30 minutes at $37^{o}C$. The samples were centrifuged ($4^{o}C$) at 760g for 20 minutes, the supernatants were then decanted and the tubes containing the pellets allowed to drain on a pad of absorbant tissue, and blotted gently to remove any remaining drops of liquid.

Tubes were then counted in a Beckman Biogamma counter for 1 minute each.

Non-specific binding was calculated by including tubes which contained 0.4% BSA-borate buffer (550 μl) instead of serum and antibody complex. A standard curve was prepared using a set of 10, T_3 standards (provided in the kit), ranging from 0 to 12.8 nmoles/l. Radioactivity bound was expressed as a % of the total radioactivity added (background count was subtracted from all figures)

$$\frac{\text{Bound-Blank}}{\text{Total-Blank}} \times 100$$

The means of each set of duplicates were determined and plotted as % bound versus concentration of standard T_3. The curve was used to determine the T_3 concentration in the unknown samples.

2.14.6 *Measurement of total serum corticosterone concentration using radioimmunoassay*

The animals used for measurement of serum corticosterone were sacrificed by decapitation within 5 seconds of holding to avoid stress induced release of adrenal corticosteroids.

Total serum corticosterone concentration was measured using a specific radioimmunoassay. A supply of antiserum to corticosterone was obtained from Dr. A. Thomas of Southampton University. The basis of the assay was adapted from the method described by *Fahmy et al. (1975)*, for the assay of cortisol in human plasma.

Reagents

i) Assay buffer and dextran/charcoal mixture

The assay buffer was 0.01 M sodium phosphate, containing 0.15 M sodium chloride (pH 7.4), with gelatin (0.1% w/v). The dextran/charcoal suspension was prepared by dissolving 25 mg of dextran in 100 ml assay buffer. 250 mg charcoal (Norit GSX) was then added to the buffer and stirred contiuously to form a fine suspension. This suspension was stirred, over ice, for at least one hour before use.

ii) Standard corticosterone solutions

A corticosterone standard solution of 100 ng/ml in ethanol was stored at $4^{o}C$ until required. A solution of *[1,2,6,7-^{3}H]*-corticosterone (10 μCi/ml, 91 Ci/mmole) in ethanol was prepared, and stored at $4^{o}C$. For each set of assays, a portion of this solution was dried under a stream of nitrogen and re-dissolved in assay buffer to a final activity of approximately 2×10^5 dpm/ml.

Assay procedure

Standards were prepared by diluting the 100 ng/ml solution of corticosterone in ethanol, to give a series of tubes (in duplicate), containing 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1.0 ng of

corticosterone, each in 100 µl ethanol. The same volume of ethanol was added to two further tubes to form a zero standard, thus measuring the total amount of [^{3}H]-corticosterone bound. Two tubes were prepared without antibody to assess non-specific binding.

Serum samples were extracted (in duplicate) in 3 times their volume of ethanol, vortexed for 30 seconds, and the precipitated protein was sedimented by centrifugation for 15 minutes at 500g at 4°C. Half of the ethanolic supernatant was transferred into an assay tube. The standard solutions and the ethanolic extracts were evaporated to dryness, under nitrogen at 40°C. The optimum antiserum dilution was prepared, and 100 µl was added to each tube with 100 µl of buffer, except for the total and non-specific tubes which each received 200 µl of buffer only. After vortexing briefly the tubes were allowed to stand in a waterbath for 30 minutes at 30°C. 100 µl of [^{3}H]-corticosterone solution was then added to all tubes and after vortexing briefly again, the tubes were returned to the water bath and incubated for a further hour at 30°C. At the end of the incubation the tubes were placed on ice for 15 minutes, and 0.5 mls of dextran/charcoal was added to all tubes except totals. The tubes were vortexed briefly and allowed to stand on ice for a further 15 minutes before centrifugation at 500g for 10 minutes at 4°C. The supernatant was decanted into a scintillation vial, 7 mls of tritoscint was added and the radioactivity [^{3}H] counted in a Phillips liquid scintillation counter.

The standard curves were constructed by plotting the counts bound, expressed as a % of the total counts added, against the amount of cold corticosterone in the standard tubes. The curve was used to determine the total corticosterone concentration in the unknown samples.

2.15 Preparation of BAT for electron microscopy

Interscapular brown adipose tissue was removed from the animal, and immediately immersed in 3% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, at 4°C. The tissue was dissected free of any connective tissue and overlying white adipose tissue under fixative. The tissue was diced into tiny pieces and fixation continued for 5 h. The fixative was decanted, and the tissue washed overnight (several

changes), in 0.1 M phosphate buffer pH 7.4 at 4°C. The tissue was then post fixed in 1% OsO_4 for 90 minutes. After two brief washings in distilled water, the tissue was dehydrated in graded ethanol, immersed in propylene oxide and finally embedded in Araldite. Ultrathin sections were cut with glass knives on a Cembridge-Huxley microtome and mounted on copper grids. The sections were counterstained with uranyl acetate and lead citrate and examined under a Hitachi HU-12 electron microscope.

2.16 *Determination of radioactivity*

Radioactivity in samples was measured by liquid scintillation counting using Phillips liquid scintillation analysers, models PW4510 or PW4540. Counting efficiency was 63% for ^{14}C and 28% for ^{3}H. Variations in quenching were corrected by external standardisation using preprogrammed quench curves, and the counts expressed as disintegrations per minute (dpm). The scintillants used were either Beckman Ready-Solv or tritoscint (dimethyl POPOP and Triton x-100 in xylene).

2.17 *Statistics*

Results have been presented as the mean ± standard error of the mean (S.E.M.). Statistical significance limits have been determined using the student's 't' test for unpaired data, or by one-way analysis of variance (gene dosage experiments).

CHAPTER 3

RESULTS AND DISCUSSION

SECTION 3.1 BAT Function in the Obese (fa/fa) Rat

The obesity of the fa/fa rat results primarily from a defect in energy expenditure, which is apparent at an early age, before any increase in food intake or serum insulin can be detected. Since BAT has been shown to be the principal site of thermogenesis, in response to both cold and dietary stimuli, the initial experiments described in this section were designed to investigate whether BAT function was defective in the fa/fa rat.

The thermogenic function of BAT may be biochemically estimated by measurement of the specific binding of $[^{3}H]$-GDP (henceforth referred to as GDP binding) to the 32K mol. wt. mitochondrial protein (32K protein) which is responsible for the uncoupled proton translocation. Initially, it was important to investigate the characteristics of BAT mitochondrial GDP binding, and to establish that the optimal conditions for binding were similar in BAT mitochondria from lean and fa/fa rats.

If a defect in BAT thermogenesis was responsible for the reduction in energy expenditure of fa/fa rats, it would be expected that this defect would be observed in young, suckling pre-obese fa/fa rats, at the time when excess fat deposition can first be detected. Hence, BAT function was studied at different stages of the development of the obesity in the fa/fa rat.

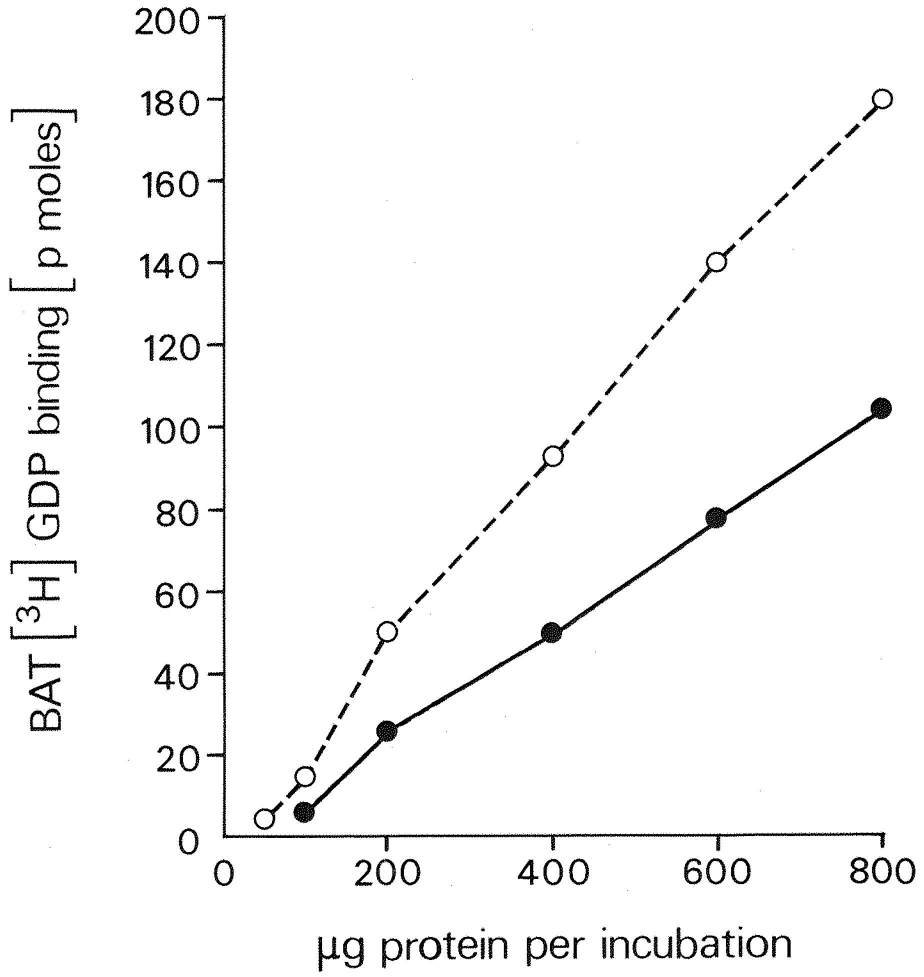
RESULTS

3.1.1 Effect of protein concentration on BAT mitochondrial GDP binding in lean and fa/fa rats

Fig. 3.1.1 demonstrates that BAT mitochondrial protein concentrations ranging from 200 μg to 800 μg perincubation (final volume 1 ml), gave a linear increase in GDP binding for both lean and fa/fa rats (Fig. 3.1.1); indicating that the amount of GDP used was not limiting in either case. A protein concentration between 200 μg and 500 μg per incubation was routinely used. The difference in the amount of GDP bound to lean and fa/fa mitochondria will be discussed later in this section.

FIG. 3.1.1   DEPENDENCE OF BAT MITOCHONDRIAL GDP BINDING ON MITOCHONDRIAL PROTEIN CONCENTRATION FOR LEAN AND OBESE (fa/fa) RATS

BAT from 4 lean (O) and 4 fa/fa (●) rats were pooled and mitochondria prepared as described in Section 2.9. BAT mitochondrial GDP binding assays were performed using the protein concentrations indicated, in a total volume of 1 ml. All other details of the assay were as stated in Section 2.10.



BAT [^{3}H] GDP binding [p moles]
200
180
160
140
120
100
80
60
40
20
0
0
200
400
600
800
μg protein per incubation

3.1.2 *Effect of pH on BAT mitochondrial GDP binding in lean and fa/fa rats*

Fig. 3.1.2 shows that BAT mitochondrial GDP binding was stable between pH 6.8 and 7.2 in both lean and fa/fa groups. Thus the difference in GDP binding cannot be attributed to an altered pH optimum, and the nature of the binding is similar in both groups. The pH routinely used was 7.1 which was near physiological pH. Small changes either side of this value are unlikely to lead to errors in measured GDP binding.

3.1.3 *Time course of association of GDP with BAT mitochondria from lean and fa/fa rats*

Fig. 3.1.3 shows a typical example of the association pattern of GDP binding to lean and fa/fa BAT mitochondria, with time. There was an initial rapid association of GDP, so that maximal binding was largely attained after the first sample was taken (at 15 seconds). This was followed by a very slow increase, which was completed after 8 minutes in lean and fa/fa BAT mitochondria.

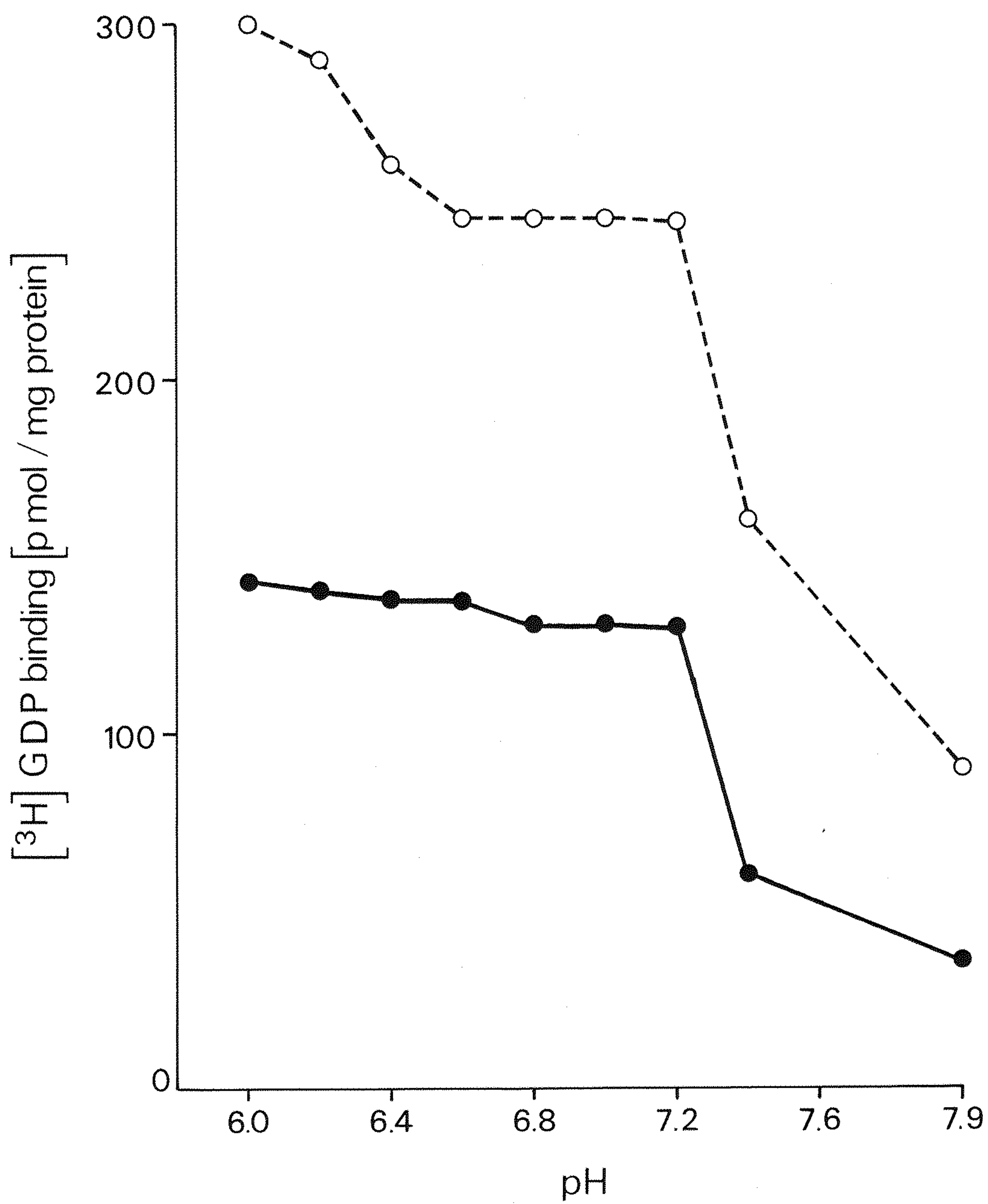
A 9 minute incubation time was routinely used for the assay of both lean and fa/fa BAT mitochondrial preparations.

3.1.4 *Effect of the temperature of incubation on GDP binding in BAT mitochondria from lean and fa/fa rats*

For the effect of temperature on BAT mitochondrial GDP binding, HEPES (20 mM) buffer was substituted in the assay incubation medium (Section 2.10) for TRIS (20 mM). HEPES retains a more stable pH over a wider temperature range. BAT mitochondrial GDP binding values were found to be unaltered by this substitution (Lean, 262 ± 6, and 258 ± 8; pmol GDP bound/mg protein and fa/fa, 138 ± 5 and 141 ± 4 pmoles GDP bound/mg protein, using TRIS and HEPES buffers, respectively). Fig. 3.1.4 shows that optimum BAT mitochondrial GDP binding occurs between 16°C and 30°C for both lean and fa/fa rats, and so room temperature (23°C) was routinely used as the incubation temperature for all assays.

FIG. 3.1.2 *EFFECT OF pH ON GDP BINDING TO BAT MITOCHONDRIA OF LEAN AND OBESE (fa/fa) RATS*

BAT from 4 lean (O) and 4 fa/fa (●) rats was pooled and mitochondria prepared as described in Section 2.9. BAT mitochondrial GDP binding assays were performed as described in Section 2.10, at the pH values indicated.



300
200
100
0
[3H] GDP binding [p mol / mg protein]
6.0
6.4
6.8
7.2
7.6
7.9
pH

Fig. 3.1.3 *ASSOCIATION OF GDP TO LEAN AND OBESE (fa/fa) BAT MITOCHONDRIA AS A FUNCTION OF TIME*

BAT from 4 lean (O) and 4 fa/fa (●) rats was pooled for each group, and mitochondria prepared as described in Section 2.9. After a pre-incubation with mitochondria for one minute (as described in Section 2.10), the GDP binding assay was initiated by adding the [^{3}H]-GDP to the incubation medium, and the reaction terminated by centrifugation after 15 seconds, 2,4,6,8 and 20 minutes. Other details of the assay are as described in Section 2.10.

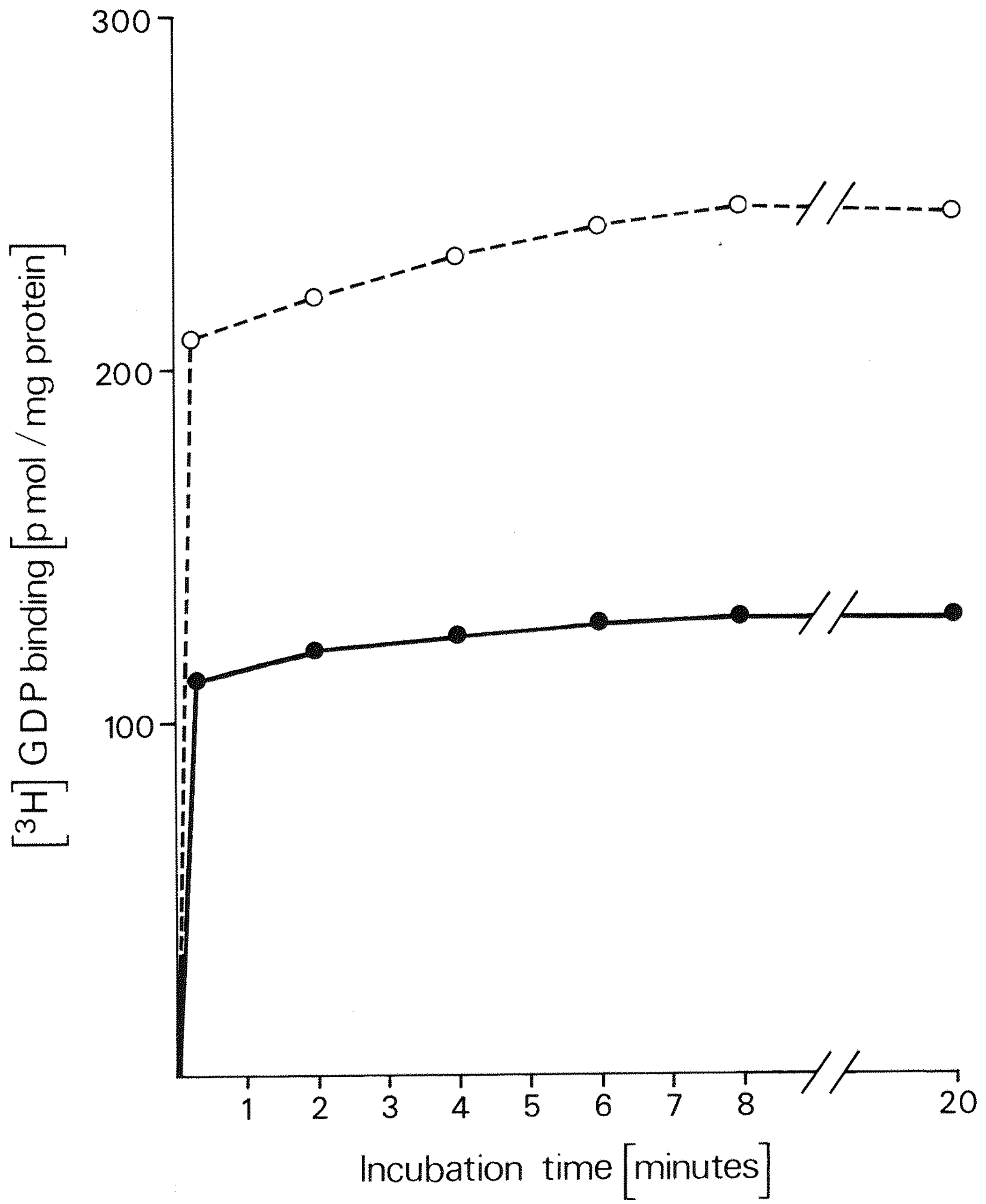
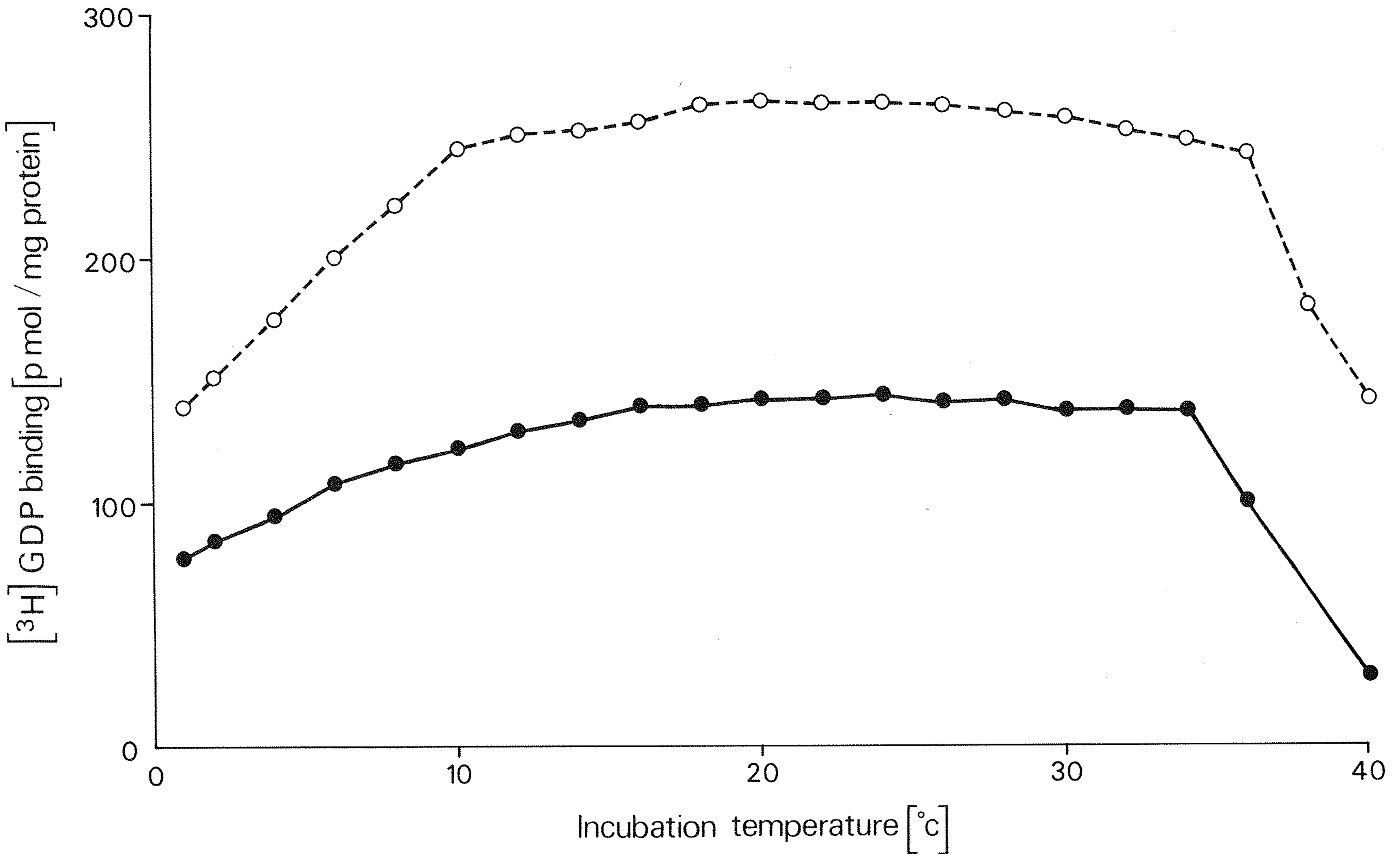

300
200
100
[3H] GDP binding [p mol / mg protein]
1
2
3
4
5
6
7
8
20
Incubation time [minutes]

FIG. 3.1.4 ASSOCIATION OF GDP TO BAT MITOCHONDRIA OF LEAN AND OBESE (fa/fa) RATS, AS A FUNCTION OF TEMPERATURE

BAT mitochondria were prepared from 6 lean (O) and 6 fa/fa (●) rats in each case, as described in Section 2.9. BAT mitochondrial GDP binding assays were performed in a heating block, across which there was a linear temperature gradient, from $1^{O}C$ to $40^{O}C$. The incubation buffer contained 1 mM HEPES (which replaced TRIS), as this maintained a constant pH for the temperature range used for this experiment. Other details of the assay were as stated in Section 2.10.


[3H] GDP binding [p mol / mg protein]
300
200
100
0
0
10
20
30
40
Incubation temperature [°C]

3.1.5 *Treatment of BAT mitochondria from lean and fa/fa rats with 2% (w/v) BSA (fatty acid free)*

Free fatty acids are thought to cause uncoupling in isolated BAT mitochondria *(Locke & Nicholls, 1981)*. As the BAT of fa/fa rats contains more triglyceride than that of lean rats (to be discussed later), it was possible that differences in GDP binding could result from differences in free fatty acid concentration. Endogenous free fatty acids were therefore routinely removed by the addition of a 2% solution of BSA,fatty acid free) during the isolation of the mitochondria *(Sundin & Cannon, 1980)*.

When BAT mitochondria were prepared including a 2% BSA (fatty acid free) wash, GDP binding in lean and fa/fa BAT mitochondria was increased by 60 and 50% respectively (Fig. 3.1.5). Increasing the concentration of BSA to 4% did not increase the binding further (253 ± 13 and 146 ± 10 pmol GDP bound/mg mitochondrial protein, for lean and fa/fa rats respectively, using a 4% BSA wash in the mitochondrial preparation). A 2% BSA wash was routinely used in the preparation of BAT mitochondria. This treatment also reduced the variation in BAT mitochondrial GDP binding (Fig. 3.1.5), thus stabilising the assay.

3.1.6 *Characteristics of BAT in lean and fa/fa rats*

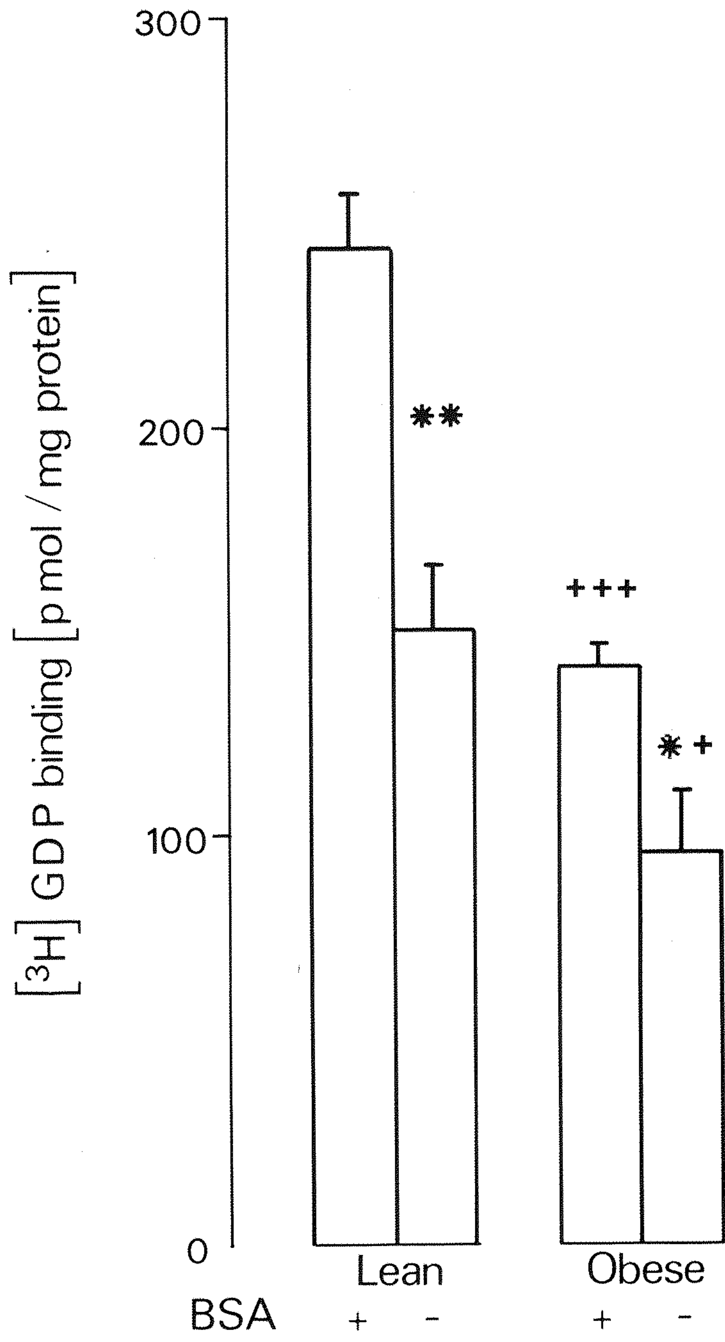
Preliminary measurements with 5-6 week old animals (Fig. 3.1.6) show the fa/fa group to be hyperphagic when compared to the lean group. The body weight of fa/fa rats was not significantly higher than that of lean rats at this age, however they were markedly obese, as shown by the two to three-fold increase in inguinal fat pad weight. Fig. 3.1.6 also shows that fa/fa rats have a decreased rectal temperature compared to the lean rat, at normal housing temperatures (23°C).

The weight of the BAT depot was increased in the fa/fa rat (Table 3.1.1). Since the total protein content was slightly (but not significantly) decreased when compared to the lean animal, this suggests that the increased weight of BAT was not due to a true hypertrophy of the tissue, but to an accumulation of lipid. This assumption is supported by the visible appearance of BAT in the fa/fa rat, which is pale in comparison to the lean rat, and by the large fat component of the homogenate seen during the mitochondrial preparation (Section 2.9).

FIG. 3.1.5 EFFECT OF INCLUSION OF A 2% BSA (FATTY ACID FREE) WASH IN THE PREPARATION OF BAT MITOCHONDRIA FROM LEAN AND OBESE (fa/fa) RATS

BAT from 5 lean and 5 fa/fa rats was pooled for each group, and mitochondria prepared as described in Section 2.9, except that the BSA wash was omitted for 50% (BSA -) of the mitochondrial preparation in each case. BAT mitochondrial GDP binding assays were then performed as described in Section 2.10.

Values represent means ± S.E.M. for 3 experiments in each case.
* $p < 0.05$, ** $p < 0.01$, compared with equivalent untreated group;
† $p < 0.05$, ††† $p < 0.001$, compared with equivalent lean group.



300
200
100
0
[3H] GDP binding [p mol / mg protein]
**
+++
* +
Lean
Obese
BSA
+
-
+
-

FIG. 3.1.6 *FOOD INTAKE, BODY WEIGHTS, INGUINAL FAT PAD WEIGHTS AND RECTAL TEMPERATURES OF LEAN AND OBESE (fa/fa) RATS*

4-5 week old lean (open bars) and fa/fa (cross-hatched bars) rats were housed individually at 23°C for 7 days. Food intake and rectal temperatures were monitored daily during this period. Rats were 5-6 weeks of age on sacrifice. After sacrifice, inguinal fat pads were dissected out and weighed.

Values represent means ± S.E.M. for 10 animals in each group; * $p < 0.05$, *** $p < 0.001$, compared with lean group.

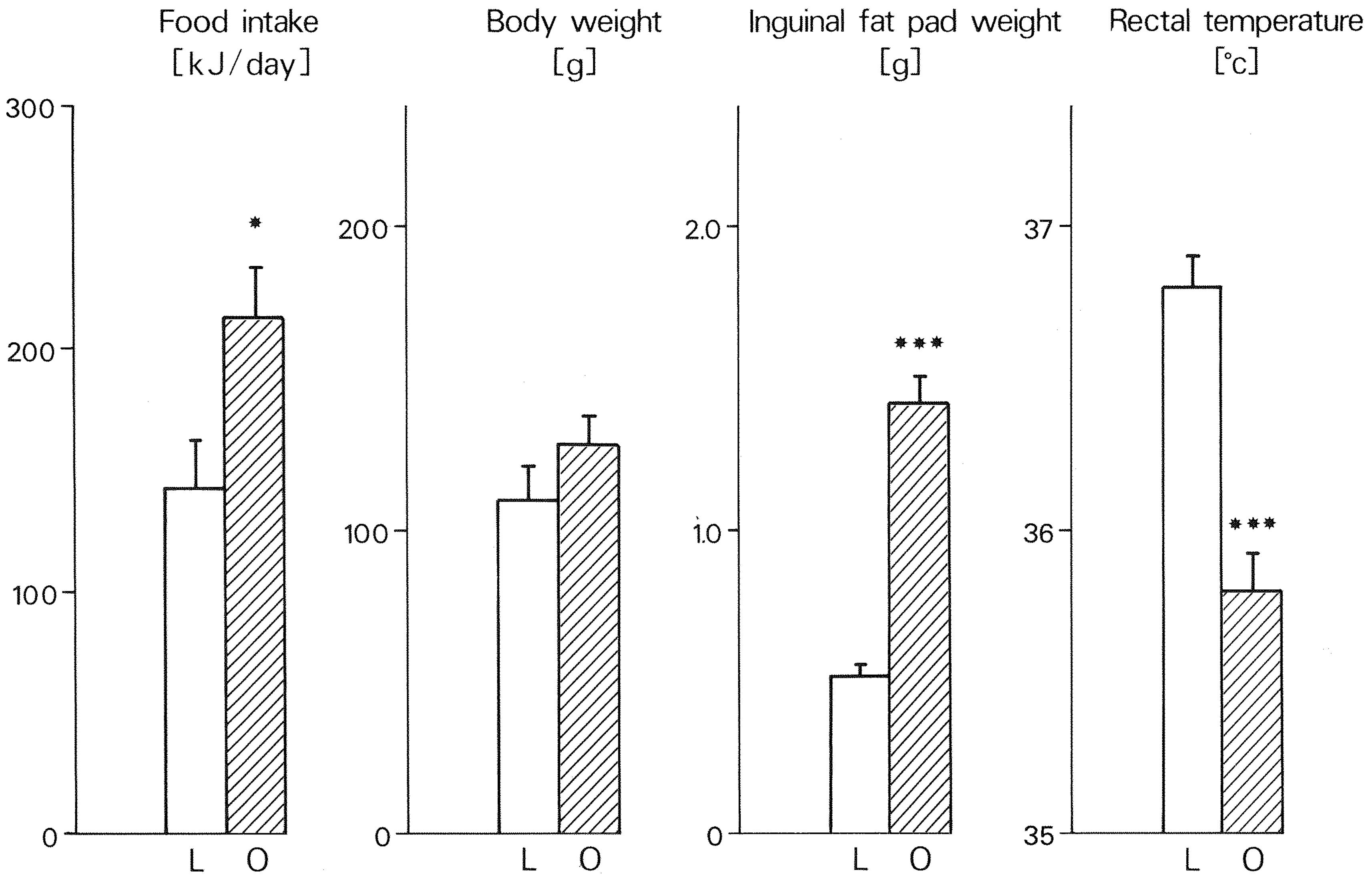

Food intake
[kJ/day]
300
200
100
0
L
O
*
Body weight
[g]
200
100
0
L
O
Inguinal fat pad weight
[g]
2.0
1.0
0
L
O
***
Rectal temperature
[°c]
37
36
35
L
O
***

TABLE 3.1.1

CHARACTERISTICS OF BAT IN 5-6 WEEK OLD LEAN AND OBESE (fa/fa) RATS

Brown Adipose Tissue	Lean	Obese (fa/fa)
Wet weight (g)	0.27 ± 0.02	0.40 ± 0.02***
Protein (mg)	28.2 ± 2.1	24.2 ± 2.4
[^{3}H] GDP binding (pmol/mg protein)	252.0 ± 14.2	127.0 ± 10.6***
Total [^{3}H] GDP binding (pmol/tissue)	1844 ± 50	1205 ± 80***
Succ.cyt.c.O-R activity (μmol/mg mitochondrial protein/min)	0.23 ± 0.01	0.22 ± 0.01
Total succ.cyt.c.O-R activity (μmol/min/depot)	1.57 ± 0.23	1.28 ± 0.24
Mitochondrial recovery (%)	18.1 ± 2.2	12.0 ± 1.2*

BAT mitochondria were prepared from lean and fa/fa rats as described in Section 2.9. BAT protein content, succ.cyt.c.O-R activity and BAT mitochondrial GDP binding determined as described in Section 2.11, 2.12 and 2.10 respectively. Recovery of mitochondria was measured as % recovery of succ.cyt.c.O-R activity from the whole tissue homogenate. Total tissue binding was calculated based on 100% recovery of mitochondria.

Values represent means ± S.E.M. of 10 animals in each group.

* $p < 0.05$; *** $p < 0.001$, compared to lean group.

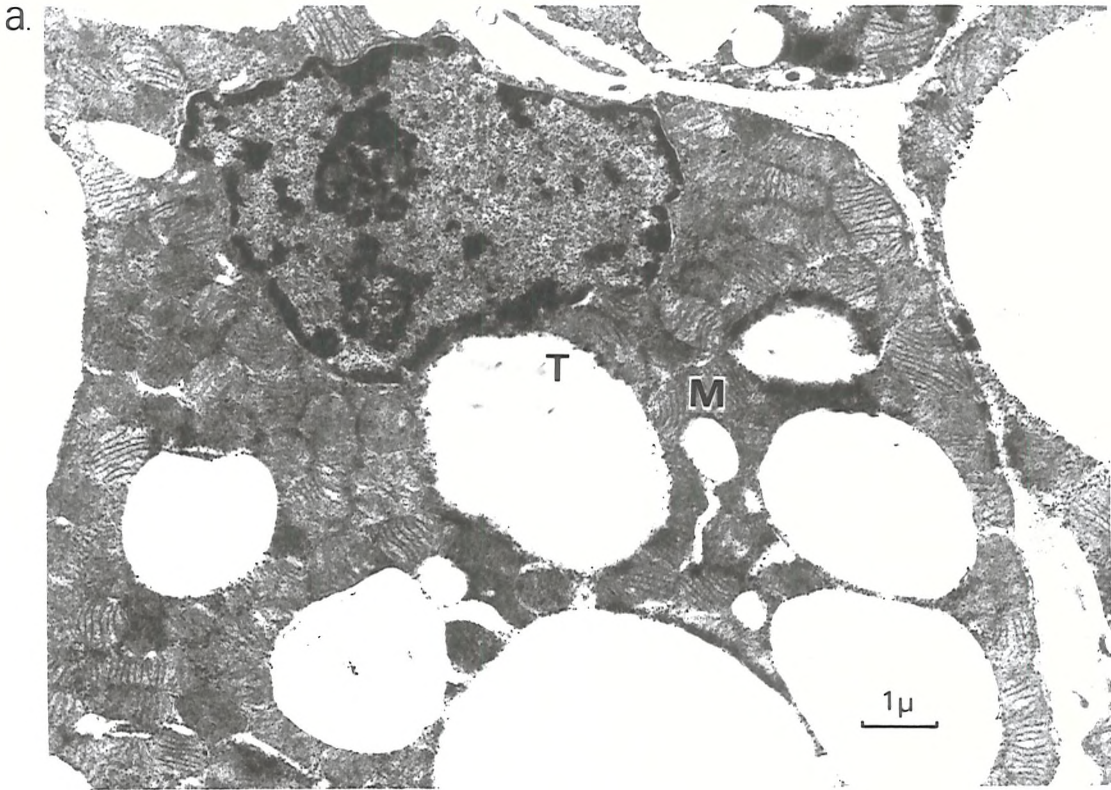
The total activity of succ.cyt.c.O-R activity, a mitochondrial marker, in BAT of the fa/fa rat was not significantly different when compared to the value for the lean rat, indicating a near normal mitochondrial population in this experiment. BAT mitochondrial GDP binding was reduced by 50% in fa/fa rats (Table 3.1.1). This decrease was not due to a difference in purity between lean and fa/fa mitochondrial preparations, since the specific activity of succ.cyt.c.O-R in the mitochondrial fraction was similar in both groups. The total GDP binding, expressed for the BAT depot (interscapular pad), was decreased in the fa/fa rat when compared to the lean rat. This was mainly due to a reduced GDP binding per mg of mitochondrial protein, and to a lesser extent a decreased mitochondrial population. The yield of mitochondria from the BAT mitochondrial preparation, was routinely less in the fa/fa rat when compared to the lean.

Histological study of BAT from lean and fa/fa rats confirm some of the above biochemical observations (Plate 3.1a and 3.1b). BAT of 5 week-old fa/fa rats contained numerous large lipid droplets and enlarged mitochondria, which had fewer cristae, when compared to the lean rat. This suggests that BAT in the fa/fa rat is thermogenically less active than that in the lean rat.

3.1.7 Scatchard analysis of BAT mitochondrial GDP binding in lean and fa/fa rats

Scatchard plots of the specific binding of GDP to BAT mitochondria from lean and fa/fa rats (Fig. 3.1.7) indicate the presence of high and low affinity binding sites. In the absence of computer facilities to analyse the data, the apparent dissociation constant (Kd) and maximum binding capacity (B_{max}) of the high and low affinity sites were estimated graphically. The apparent dissociation constants (0.26 μM and 0.26 μM for the high affinity binding sites, and 3.3 μM and 2.9 μM for the low affinity binding sites, for lean and fa/fa rats respectively) suggests that a change in affinity is not responsible for the reduced BAT mitochondrial GDP binding observed in fa/fa rats. However, the B_{max} was decreased from 405 pmol/mg in lean rats to 255 pmol/mg in fa/fa rats, for the low affinity binding site and from 110 pmol/mg in lean to 85 pmol/mg in the fa/fa rat, for the high affinity site. These results indicate that there is a large

a.



b.

Fig 3.1 Electron micrographs of BAT of 5 week old lean (a) and obese (b) rats

BAT was removed from the rats and processed as described in section 2.15. Magnification x 15,000

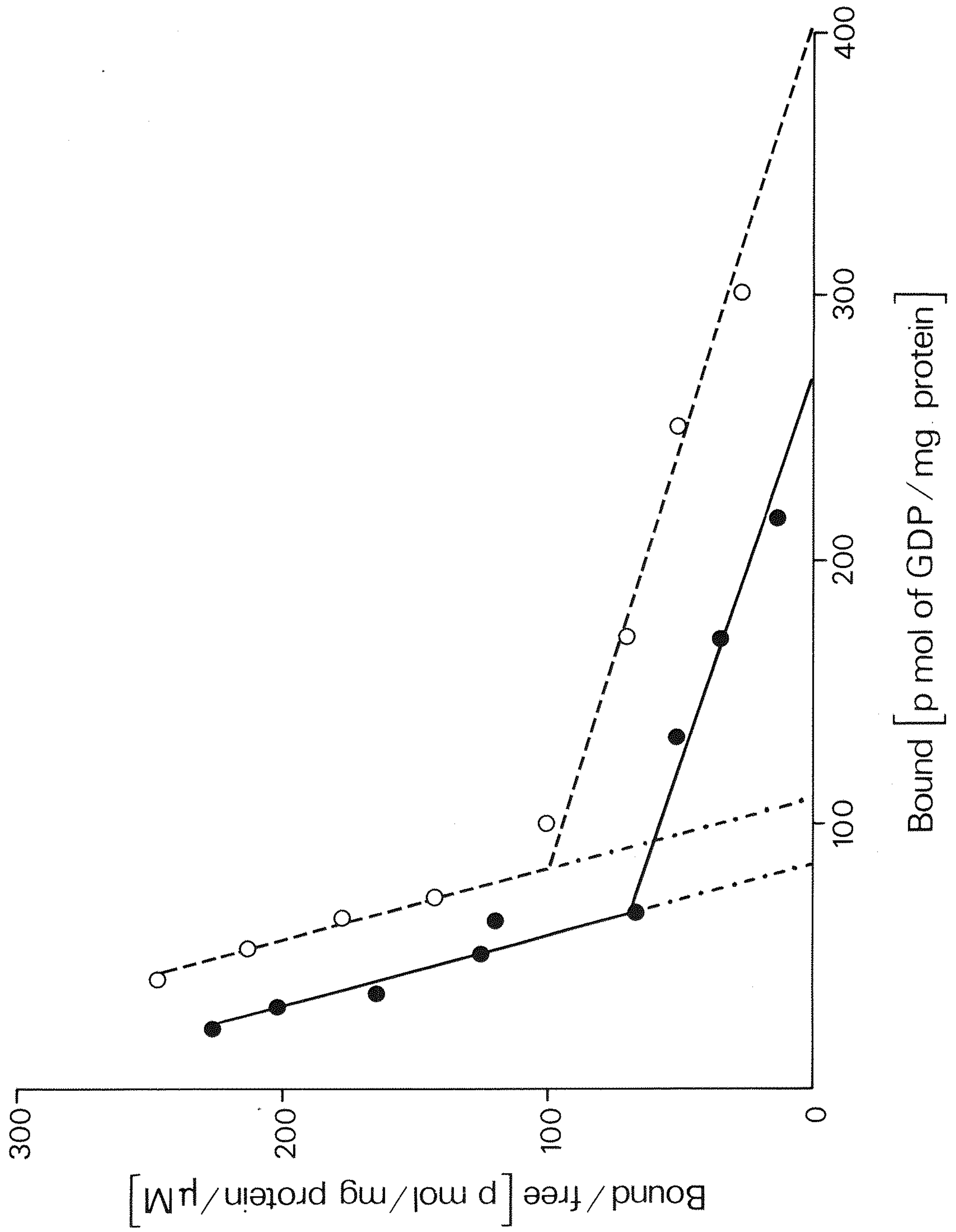
M = Mitochondrion

T = Triglyceride droplet

FIG. 3.1.7 *SCATCHARD PLOTS OF [^{3}H]-GDP BINDING TO BAT MITOCHONDRIA OF LEAN AND OBESE (fa/fa) RATS*

4 lean (O) and 4 fa/fa (●) rats, (5-6 weeks of age) were sacrificed, BAT pooled for each group, and mitochondria prepared as described in Section 2.9. Scatchard analysis was performed using GDP concentrations ranging from 20 µM to 0.05 µM, other details of assay were as stated in Section 2.10.

—•—• extrapolation for B_{max} of high affinity binding sites.



Bound / free [p mol / mg protein / µM]
0
100
200
300
Bound [p mol of GDP / mg. protein]
100
200
300
400

fall in the number of GDP binding sites in the BAT of fa/fa rats. This may be due either to a decrease in the amount of 32K protein in the mitochondria or to a masking of the binding sites.

3.1.8 Effect of age on BAT thermogenesis in lean and fa/fa rats

The present report confirms the previous finding that pre-obese fa/fa rats can be identified before weaning by a reduced rectal temperature (*Godbole et al., 1978*) (Fig. 3.1.8), and by an increased inguinal fat pad weight *(Boulange et al., 1979b; Bell & Stern, 1977)*. At 10 days of age the body weights of lean and pre-obese fa/fa rats were similar, as were the weights of BAT. The BAT protein contents of lean and pre-obese fa/fa rats were similar, as were the total tissue succ.cyt.c.O-R activities (Table 3.1.2), suggesting a normal mitochondrial content in the pre-obese fa/fa rat. The highest measured values of BAT mitochondrial GDP binding in lean and fa/fa rats were at 10 days of age, after which, levels fell to reach minima at 20 and 28 days respectively. Subsequently, there was little age dependent variation (Fig. 3.1.8). BAT mitochondrial GDP binding in the 10 day old pre-obese fa/fa rats was 20% lower than values in lean rats of the same age. This difference increased with age to 45% just before weaning, and 55% after weaning. After weaning, food intake was significantly higher in the fa/fa rat. Also, at this time, a significant increase in BAT wet weight became apparent, which in the fa/fa rat further increased with age. This difference presumably reflected an increase in BAT lipid content since BAT protein content and succ.cyt.c.O-R activity were not sifnificantly different in lean and fa/fa rats at any age (Table 3.1.2). Despite similar body weights until 6 weeks of age, a rapid deposition of excess lipid was apparent in the inguinal fat pad of the developing fa/fa rat.

3.1.9 Discussion

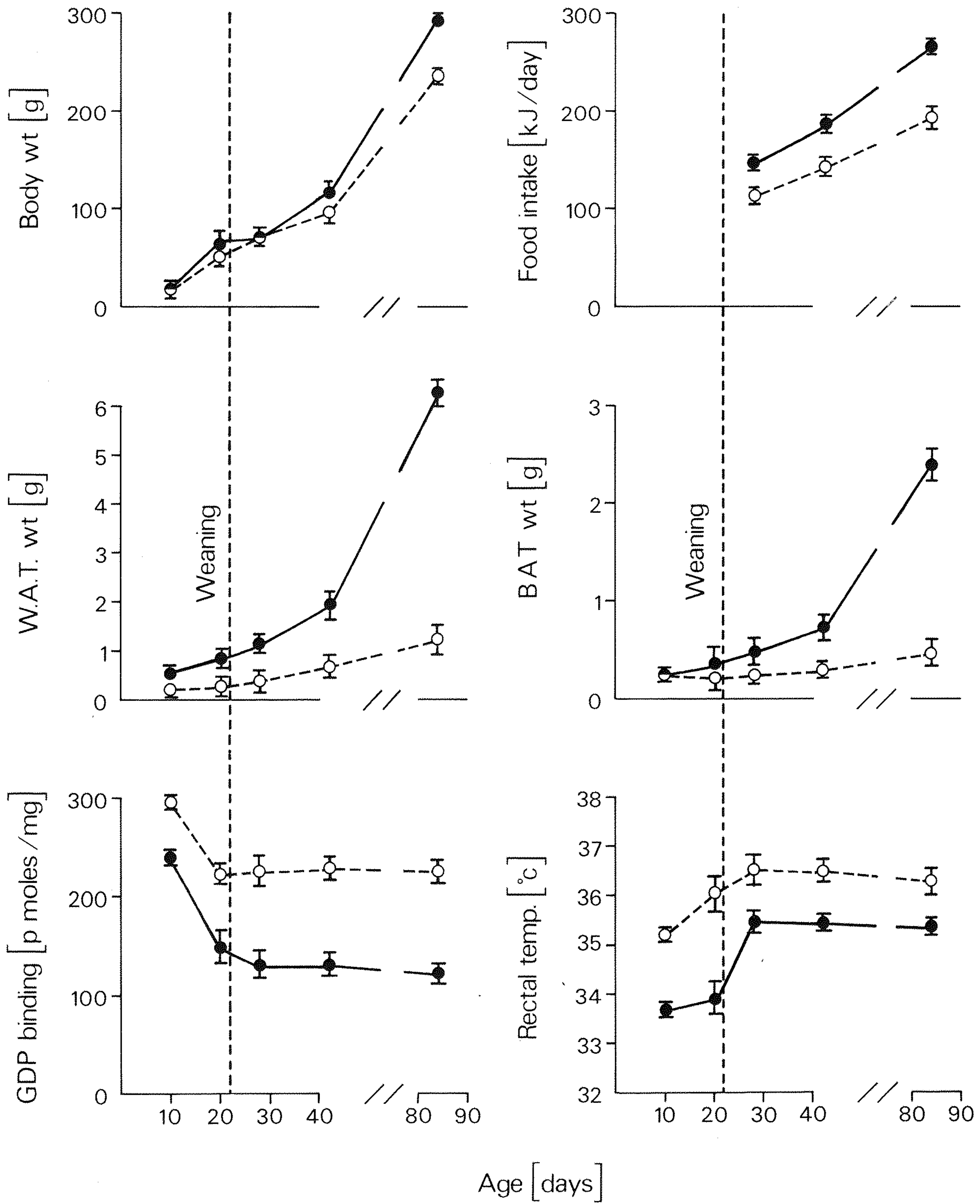
a) BAT in the 5-6 week old fa/fa rat and the characteristics of the GDP binding sites

The animals used in the preliminary part of the investigation into the activity of BAT in fa/fa rats, were 5 to 6 weeks of age and body weight was similar in lean and fa/fa groups. However, the body fat content appeared to be elevated in fa/fa rats, since inguinal fat pad weight was greatly increased. Increased body lipid in fa/fa rats

FIG. 3.1.8 AGE-DEPENDENT CHANGES IN BODY WEIGHT, FOOD INTAKE, INGUINAL WHITE ADIPOSE TISSUE WEIGHT, BAT WEIGHT, RECTAL TEMPERATURE AND [^{3}H]-GDP BINDING TO BAT MITOCHONDRIA OF LEAN AND OBESE (fa/fa) RATS

Lean (O) and fa/fa (●) rats were housed individually and food intake and rectal temperatures monitored daily for 3 consecutive days; the values on the figure represent the mean values of the 3 measurements for each animal. Animals were sacrificed at the ages indicated and body weight, inguinal white adipose tissue (WAT), and BAT weight determined. BAT mitochondria were prepared and GDP binding assays performed as described in Section 2.9 and 2.10 respectively.

Values represent means ± S.E.M. for 6 animals at each point.



Body wt [g]
300
200
100
0
Food intake [kJ/day]
300
200
100
0
W.A.T. wt [g]
6
5
4
3
2
1
0
Weaning
B A T wt [g]
3
2
1
0
Weaning
GDP binding [p moles/mg]
300
200
100
0
Rectal temp. [°c]
38
37
36
35
34
33
32
10
20
30
40
80
90
Age [days]

TABLE 3.1.2

EFFECT OF AGE ON BAT PROTEIN CONTENT AND SUCC.CYT.C.O-R ACTIVITY IN LEAN AND OBESE (fa/fa) RATS

Age (d)	Lean		Obese (fa/fa)	
	Protein (mg)	Succ.Cyt.C.O-R Activity (μmol/min/depot)	Protein (mg)	Succ.Cyt.C.O-R Activity (μmol/min/depot)
10	30.2 ± 2.0	1.20 ± 0.26	30.0 ± 2.3	1.26 ± 0.20
20	31.4 ± 2.2	2.32 ± 0.22	28.2 ± 1.8	1.89 ± 0.26
28	30.0 ± 1.9	2.52 ± 0.24	25.2 ± 2.6	1.81 ± 0.38
42	32.0 ± 2.4	2.24 ± 0.26	26.0 ± 2.7	1.74 ± 0.25
84	46.4 ± 2.8	4.18 ± 0.41	48.0 ± 4.6	3.60 ± 0.32

Lean and fa/fa rats were sacrificed at the ages indicated. BAT protein content and succ.cyt.c.O-R activities determined as described in Section 2.11 and 2.12 respectively.
Values represent means ± S.E.M. for 5 animals in each group.

at 5 weeks of age has previously been shown *(Zucker & Antoniades, 1972)*, and more recent experiments have confirmed an increased body energy density in fa/fa rats at this age *(Marchington et al., 1983)*.

BAT in the young (5-6 week old) fa/fa rat appeared to be relatively normal, as judged from the protein content. The total amount of mitochondria in BAT was also normal, as indicated by the total succ.cyt.c.O-R activity, and the above findings suggest that the defect in thermogenesis cannot be ascribed to a lack of this tissue or its mitochondria. The mitochondria of BAT in the fa/fa rat, however, do not appear to function normally. The mitochondrial GDP binding was reduced by 50% in the fa/fa rat maintained at normal laboratory temperatures, suggesting a reduced activity of the proton conductance pathway, with which the binding is normally correlated, and thus a reduced capacity for thermogenesis.

The morphological appearance of BAT of fa/fa rats was consistent with their lack of thermogenesis and the greatly elevated rate of lipogenesis that has been reported in this tissue of 30 day old rats *(Lavau et al., 1982)*. The BAT mitochondria of fa/fa rats were enlarged and contained fewer cristae than those of lean rats. Similar biochemical and structural changes have been reported in obese (ob/ob) and diabetic (db/db) mice *(Himms-Hagen & Desautels, 1978; Hogan & Himms-Hagen, 1980; Goodbody & Trayhurn, 1981)*.

Scatchard analysis showed a reduced number of BAT mitochondrial GDP binding sites to be responsible for the reduced GDP binding in the fa/fa rat. This could result from either a decrease in the amount of 32K protein in the mitochondria, or a masking of these binding sites. As the fa/fa rat has been shown to respond to noradrenaline administration with a rapid increase in BAT mitochondrial GDP binding, to levels close to those observed in lean rats (Section 3.4), it is likely that the GDP binding sites are masked to a large extent in BAT mitochondria of the fa/fa rat. BAT mitochondrial GDP binding has been reported to be sensitive to pH *(Nicholls, 1974, 1976b)* and the present results show an increase in GDP binding with reduced pH in both lean and fa/fa rats. This presumably resulted from an increase in affinity for GDP binding, since others have shown a reduced apparent dissociation constant (Kd) with a decrease in pH in isolated BAT mitochondria from hamster *(Nicholls, 1976b)* and lean rats *(Bryant et al., 1983)*. In theory,

increasing the cytosolic pH could activate the proton conductance pathway in situ by reducing the affinity for purine nucleotides. It is unlikely, however, that an increased cytosolic pH plays a messenger role in stimulating BAT thermogenesis, since incubating hamster BAT with 10 mM NH_4Cl, inhibits noradrenaline stimulated respiration *(Cannon & Nedergaard, 1979)*. Also, the respiratory response of isolated hamster brown adipocytes to noradrenaline, is enhanced if a Krebs-phosphate medium is bubbled with CO_2 even though the extra-cellular pH falls from 7.3 to 6.9 *(Pettersson,1977)*, indicating a substantial acidification can occur with no adverse effects on thermogenesis.

b) *BAT and development in the fa/fa rat*

In contrast to the adult fa/fa rat, the abnormalities present at the onset of, or prior to obesity, are poorly documented, due to lack of genetic markers. Previous developmental studies on the fa/fa rat, have indicated that excess fat deposition is of very early onset. By 7 days of age pre-obese fa/fa rats may be identified by the hypertrophy of adipocytes in the inguinal fat pad depot *(Bell & Stern, 1977; Boulange et al., 1979b)*. Inguinal fat pad weight is also increased by this age, and may be used to determine the pre-obese fa/fa genotype before any increase in body weight or serum insulin can be observed *(Boulange et al., 1979b;Lavau & Bazin, 1982)*. Hyperphagia, which is one of the characteristic traits of the weaned fa/fa rat is absent in the suckling 1 week old fa/fa pup *(Boulange et al., 1979b;Godbole et al., 1981)* and only appears in the third week of life *(Bell & Stern, 1977; Stern & Johnson, 1977)*. These findings therefore exclude the possibility that the onset of obesity in the fa/fa rat could result from excess energy intake.

BAT is thought to be the most important organ for production of heat during NST and DIT *(Foster & Frydman, 1978; Rothwell & Stock, 1979a)*. At birth, the rat pup experiences a cold shock, which can be monitored by a high level of BAT mitochondrial GDP binding *(Sundin & Cannon, 1980)*. This peaks in the first few days after birth, and slowly declines to a stable level around 4 weeks of age. The present results agree with this data, BAT mitochondrial GDP binding being highest at 10 days of age in both lean and fa/fa rats (the earliest age of measurement).

This suggests that the high level of BAT thermogenesis observed may be interpreted as a reflection of the need of the animal for extra heat. As the thermoneutral temperature decreases with age, in the rat (*Conklin & Heggeness, 1971)*, the requirement for adaptive thermoregulatory thermogenesis is reduced, this is consistent with an elevation of body temperature with increasing age.

The present results demonstrate that the reduced capacity for thermogenesis in the fa/fa rat appears as early as 10 days of age. Therefore, the level of BAT mitochondrial GDP binding is a sensitive determinant of genotype in the Zucker rat, and demonstrates that defective BAT thermogenesis may be closely related to the gene defect. Recently, *Bazin et al. (1983)* have shown a reduced level of BAT mitochondrial GDP binding in fa/fa rats, as early as 5 days of age. Thus the initial energy inbalance of suckling fa/fa rats may result from a defective BAT thermogenesis. The consequent decrease in energy expenditure, in the presence of normal food intake, would result in the development of obesity. It has been suggested that the decrease in oxygen consumption observed in the 7 day old pre-obese fa/fa rat, compared to the lean rat, is due to a decreased thermoregulatory thermogenesis *(Planche et al., 1983)*. Conclusive evidence for this assumption has not yet been shown. The reduced oxygen consumption of the 7 day old fa/fa rat *(Planche et al., 1983)* was observed during housing at 33°C, a temperature which is below the thermoneutral temperature for rats of this age. Some requirement for NST would still exist in these rats. Thus it is possible that the reduction in BAT mitochondrial GDP binding of fa/fa rats, could reflect either a defective NST or DIT. This question is investigated further in Section 3.3, when evidence is presented to suggest that the difference in BAT function reflects a loss of DIT. Support for an impaired response of fa/fa rats to diet, is provided by the observations of smaller increases in oxygen consumption in response to feeding *(Rothwell et al., 1982a; Marchington et al., 1983)* in the fa/fa rat compared to the lean rat. The response to diet has not been examined in suckling rats, but *Rothwell & Stock (1983a)* have recently shown that adult fa/fa rats have a diminished metabolic response to carbohydrate and, particularly to fat meals. As the suckling diet is high in fat, a low DIT in these animals, may be responsible for the reduced BAT mitochondrial GDP binding observed in the present results.

SECTION 3.2 The Effect of Adrenalectomy on BAT of the Obese (fa/fa) Rat

A defective BAT thermogenesis in the fa/fa rat has been established. This defect is present at an early age, before hyper-insulinemia and hyperphagia, and may be one of the earliest detectable changes associated with the mutant gene. The increase in energetic efficiency in the fa/fa rat may be related to the impairment in the thermogenic function of BAT mitochondria.

It is known that adrenalectomy prevents the obesity of the Zucker fa/fa rat *(Yukimura & Bray, 1978; Yukimura et al., 1978)*. It decreases serum insulin and hepatic lipogenesis to values close to those in lean rats *(York & Godbole, 1979)*, and abolishes the hyperphagia. Since 'intact' fa/fa rats still deposit excess fat stores when their food intake is restricted to that of lean control rats *(Bray et al., 1973)*, adrenalectomy must alter the energy balance, and may reduce the energetic efficiency by increasing BAT thermogenesis. The following section reports the effect of adrenalectomy on BAT mitochondrial GDP binding in the fa/fa rat. The results suggest that the thermogenic capacity of BAT in the fa/fa rat is suppressed by adrenal steroids.

RESULTS

3.2.1 Effect of adrenalectomy on BAT in lean and fa/fa rats

The present results (Table 3.2.1) confirm previous findings that adrenalectomy abolishes the hyperphagia of fa/fa rats *(Yukimura et al., 1978; York & Godbole, 1979)*. Food intake of the lean rat was reduced by 20% following adrenalectomy (Table 3.2.1), although this was not statistically significant. The fa/fa rat showed a 40% decrease in food intake after adrenalectomy to reach values observed in lean rats.

Adrenalectomy had no significant effects on either the weight of BAT, or the BAT protein content, the mitochondrial content (as indicated by succ.cyt.c.O-R activity), or the specific binding of GDP to BAT mitochondria in the lean rat. However, 7 days after adrenalectomy, BAT weight in the fa/fa rat had fallen slightly towards lean values, presumably reflecting a loss of tissue lipid, since BAT protein content was significantly increased. The succ.cyt.c.O-R activity was

TABLE 3.2.1

EFFECT OF ADRENALECTOMY ON BODY WEIGHT, FOOD INTAKE AND BAT, OF LEAN AND OBESE (fa/fa) RATS

	Control		Adrenalectomised	
	Lean	Obese (fa/fa)	Lean	Obese (fa/fa)
Body wt (g)	118.0 ± 7.0	140.0 ± 9.0	112.6 ± 10.2	120.6 ± 7.2
Food intake (kJ/day)	142.0 ±12.2	198.6 ±18.8 *	112.6 ± 6.2	120.0 ±20.2 †
Brown adipose tissue:				
Wet wt (g)	0.28± 0.02	0.48± 0.03***	0.28± 0.02	0.42± 0.02***
Protein (mg)	22.4 ± 2.0	17.6 ± 1.5	19.3 ± 1.5	24.2 ± 1.0*,††
Succ.cyt.c.O-R activity (μmol/min/depot)	1.58 ± 0.30	0.97± 0.20	1.24± 0.16	1.18± 0.20
[^{3}H]GDP binding (pmol/mg protein)	281.6 ±15.0	150.0 ± 8.6***	282.0 ±32.0	238.2 ±20.2 †††

Lean and fa/fa rats (5-6 weeks of age) were adrenalectomised or sham-operated and maintained as described in Section 2.7, for 7 days. BAT protein content, succ.cyt.c.O-R activity and mitochondrial GDP binding assays were performed as described in Section 2.11, 2.12 and 2.10 respectively.
Values represent means ± S.E.M. for 6 animals in each group. * $p < 0.05$, *** $p < 0.001$, compared with lean rats in the same treatment group; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent control group.

also slightly increased in the fa/fa rat but the difference did not reach statistical significance in this experiment. BAT mitochondrial GDP binding in the fa/fa rat was increased by almost 50%, reaching values observed in the lean rat.

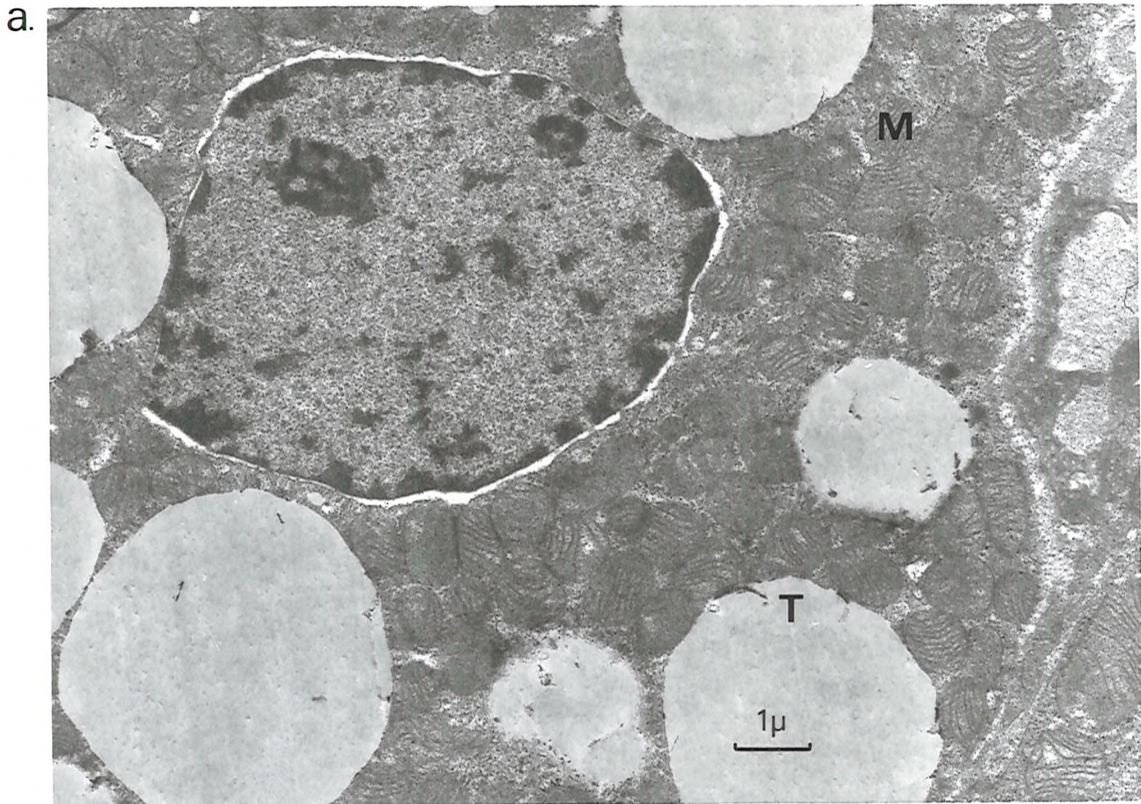
BAT of fa/fa rats contained numerous large lipid droplets (Section 3.1, Plate 3.1 b) and enlarged mitochondria with few cristae, when compared with the lean rat. However, 7 days after adrenalectomy, the appearance of the BAT of fa/fa rats (Plate 3.2 a and b) resembled the lean 'intact' and lean adrenalectomised rat. The lipid content was reduced, triglyceride droplets were smaller, and the mitochondrial content was increased per unit area of tissue when compared to the fa/fa 'intact' rat. The mitochondria also appeared to have a more dense internal structure (more cristae), similar to the lean adrenalectomised and lean intact rat.

3.2.2 Scatchard analysis of BAT mitochondrial GDP binding in adrenalectomised lean and fa/fa rats

The increase in BAT mitochondrial GDP binding seen in the adrenalectomised fa/fa rat may be due to either an increase in the number of binding sites, or a change in the affinity for the ligand. Scatchard analysis was performed to investigate these possibilities.

Scatchard analysis of BAT mitochondrial GDP binding has been previously reported (Section 3.1, Fig. 3.1.7), and revealed the presence of high and low affinity binding sites in lean and fa/fa rats. The dissociation constants (Kd) of both the high and low affinity sites were similar in lean and fa/fa rats; the difference in binding was due to an increased number of both types of site present in the mitochondria of the lean rat. After adrenalectomy Scatchard plots again indicate the presence of high and low affinity binding sites (Fig. 3.2.1). In the absence of computer facilities to analyse the data, the apparent dissociation constant (Kd) and maximum binding capacity (B_{max}) of the high and low affinity binding sites were estimated graphically (as in Section 3.1). The Kd values (0.36 μM for the high affinity binding sites and 3.1 μM for the low affinity binding sites, for both lean and fa/fa rats) indicate that there is no major change in affinity after adrenalectomy in lean or fa/fa rats. However, the maximum binding capacity (B_{max}) of BAT mitochondria of adrenalectomised fa/fa

a.



b.

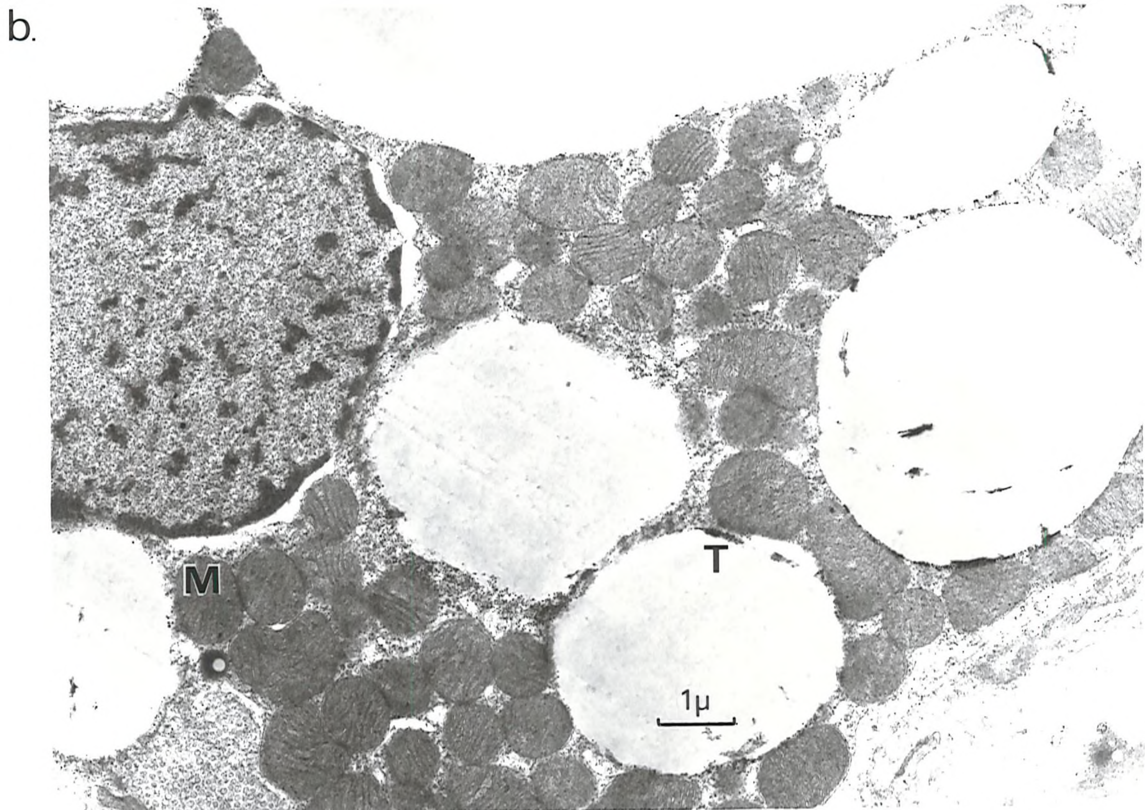


Fig 3.2 Electron micrographs of BAT of 5 week old adrenalectomised lean (a) and obese (b) rats

BAT was removed from the adrenalectomised rats 7 days after surgery and processed as described in section 2.15. Magnification x 15,000

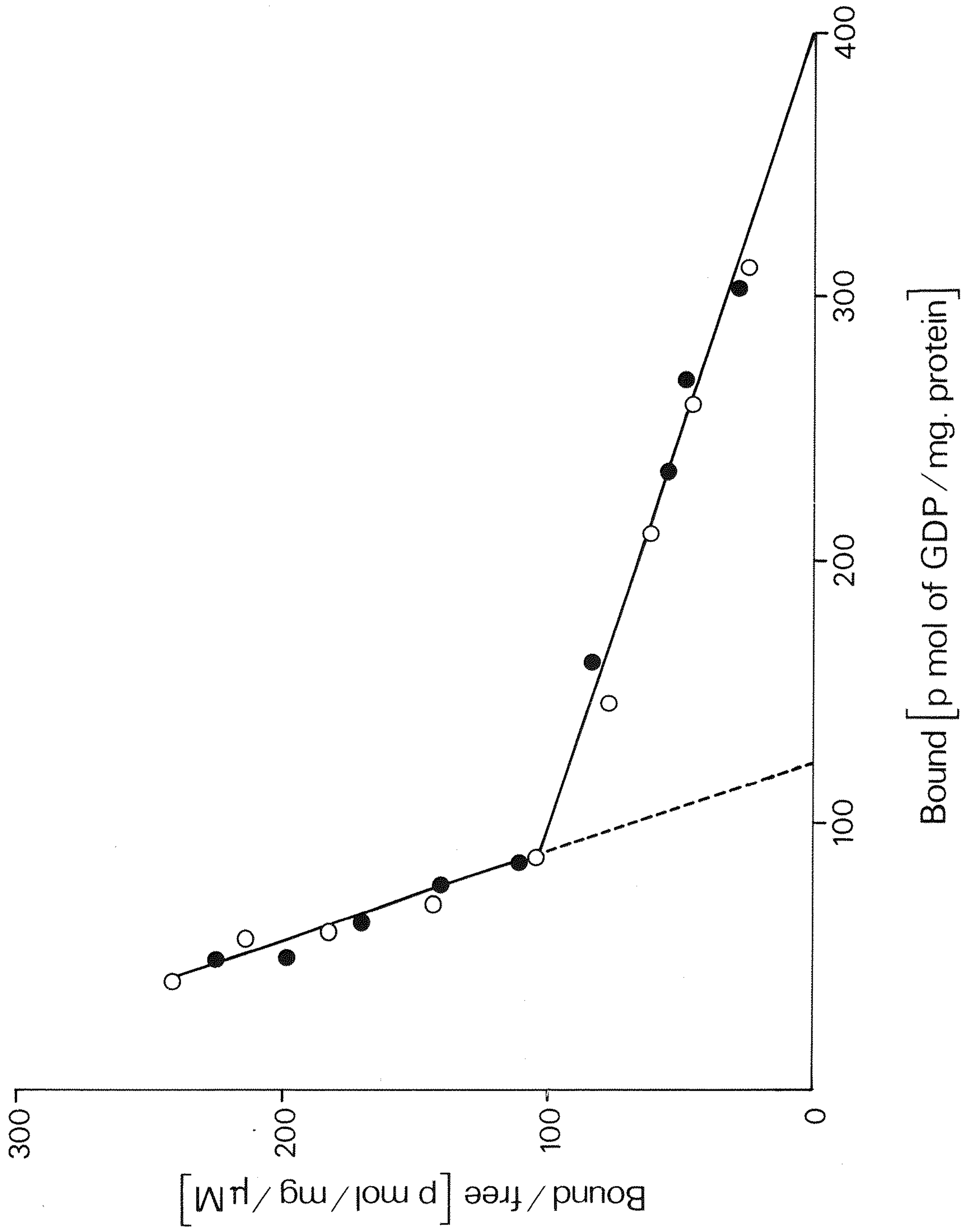
M = Mitochondrion

T = Triglyceride droplet

FIG. 3.2.1 *SCATCHARD PLOT OF [^{3}H]-GDP BINDING TO BAT MITOCHONDRIA OF ADRENALECTOMISED LEAN AND OBESE (fa/fa) RATS*

4 lean (O) and 4 fa/fa (●) rats were adrenalectomised and maintained as described in Section 2.7, for 7 days. Rats were 5-6 weeks of age on sacrifice. BAT from each group was pooled and mitochondria prepared as described in Section 2.9. Scatchard analysis was performed using GDP concentrations ranging from 20 μM to 0.05 μM, other details of assay were as stated in Section 2.10.

---- extrapolation for B_{max} of high affinity binding sites.



Bound / free [p mol / mg / μM]
0
100
200
300
Bound [p mol of GDP / mg. protein]
100
200
300
400

rats, was increased from 85 and 255 pmol/mg (Section 3.1, Fig. 3.1.7) in the 'intact' obese, to 120 and 400 pmol/mg after adrenalectomy, for high and low affinity binding sites respectively), such that the Scatchard plots were indistinguishable from the lean adrenalectomised values.

3.2.3 *Effect of food intake on BAT function*

Obese (fa/fa) rats were pair-fed with the food intake of the adrenalectomised fa/fa rat, to eliminate the possibility that the changes observed in BAT following adrenalectomy were secondary to the reduced food intake. Table 3.2.2 shows that food intake of fa/fa rats was decreased by over 40% after adrenalectomy and that despite this, these rats gained weight at a faster rate than the pair-fed 'intact' fa/fa rats. However, none of the changes in BAT observed after adrenalectomy i.e. increased protein content, succ.cyt.c.O-R activity and GDP binding to BAT mitochondria, were observed in the 'intact' fa/fa rats pair fed with adrenalectomised fa/fa rats.

As the lean rat showed a small (20%) reduction in food intake following adrenalectomy lean 'intact' rats were pair fed to the intake of adrenalectomised lean rats. After 7 days of pair feeding no significant changes in BAT wet weight, protein content, or succ.cyt.c.O-R activity, were observed 0.28 ± 0.03 and 0.26 ± 0.02g for wet weight; 24.2 ± 2.1 and 22.6 ± 1.7mg for protein; 1.66 ± 0.26 and 1.3 ± 0.17μmol cyt.c. reduced/min/tissue for lean control and lean pair-fed groups respectively). BAT mitochondrial GDP binding was significantly reduced in the pair-fed group when compared to both the intact lean and adrenalectomised lean group (Fig. 3.2.2). This demonstrates that a reduced food intake in the 'intact' lean rat reduced BAT mitochondrial GDP binding compared to the <u>ad lib</u>. fed 'intact' lean rat. This reduction in BAT mitochondrial GDP binding is not observed in the adrenalectomised lean rat. In the 'intact' fa/fa rat, restricting food intake has no effect on BAT mitochondrial GDP binding.

3.2.4 *Effect of replacing drinking water with saline on BAT mitochondrial GDP binding in lean and fa/fa rats*

Table 3.2.3 shows that BAT mitochondrial GDP binding remains unchanged in lean and fa/fa rats after saline substitution. This result indicates that the exchange of water for saline after adrenalectomy, is

TABLE 3.2.2

EFFECT OF RESTRICTING FOOD INTAKE ON GDP BINDING TO BAT MITOCHONDRIA OF OBESE (fa/fa) RATS

	Adrenalectomised	Control	
	Fed ad. libitum	Pair-fed	Fed ad. libitum
Food intake (kJ/day)	108.2 ± 3.8	108.2 ± 3.8	184.1 ± 10.3†††
Initial body weight (g)	79.6 ± 6.1	81.9 ± 4.2	83.9 ± 0.9
Body weight (% increase/week)	15.7 ± 3.2	8.1 ± 2.1*	28.5 ± 1.2†††
Brown adipose tissue:			
Wet weight (g)	0.35 ± 0.04	0.45 ± 0.05	0.48 ± 0.10
Protein (mg)	25.1 ± 3.1	15.4 ± 0.7*	18.9 ± 2.9
Succ.cyt.c.O-R activity (µmol/min/depot)	1.96 ± 0.24	0.72 ± 0.13***	1.42 ± 0.24†
[^{3}H]-GDP binding (pmol/mg protein)	280.6 ± 26.5	135.6 ± 15.8***	167.2 ± 7.1

Obese (fa/fa) rats (5 weeks of age) were adrenalectomised and maintained as described in Section 2.7. Control fa/fa rats were either fed ad libitum or pair-fed to adrenalectomised fa/fa rats as described in Section 2.4, for 7 days. BAT protein, succ.cyt.c.O-R activity, and mitochondrial GDP binding assays were determined as described in Section 2.11, 2.12 and 2.10 respectively.

Values represent means ± S.E.M. for 4 animals in each group. * $p < 0.05$, *** $p < 0.001$, compared with adrenalectomised fed ad libitum; † $p < 0.05$, ††† $p < 0.001$, compared with pair-fed control group.

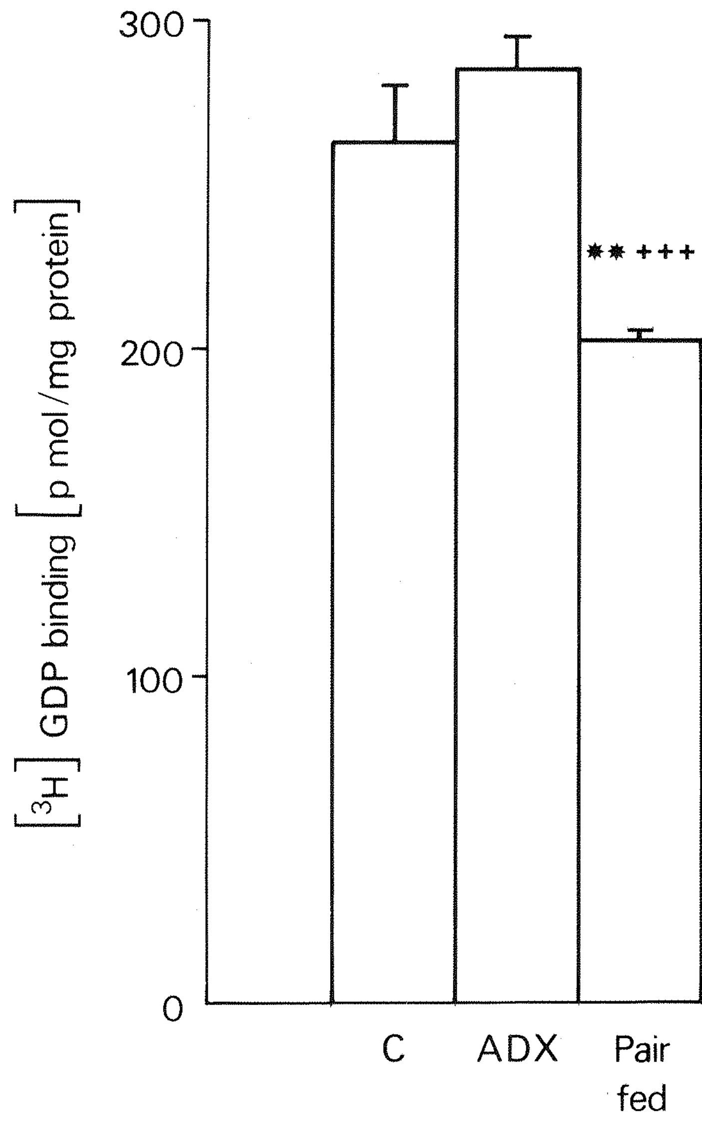
FIG. 3.2.2 *EFFECT OF RESTRICTING FOOD INTAKE ON BAT MITOCHONDRIAL GDP BINDING IN LEAN RATS*

4-5 week old lean rats were adrenalectomised (ADX), as described in Section 2.7. 'Intact' rats were allowed to eat ad libitum (C) or were pair-fed to the food intake of the adrenalectomised lean rat, as described in Section 2.4. All treatments were continued for 7 days. BAT mitochondria were prepared and GDP binding assays performed, as described in Section 2.9 and 2.10 respectively.

Values represent means ± S.E.M. for 4 animals in each group;

** $p < 0.01$, compared with lean control group;

††† $p < 0.001$, compared to adrenalectomised lean group.



300
200
100
0
[3H] GDP binding [p mol/mg protein]
** +++
C
ADX
Pair fed

TABLE 3.2.3

EFFECT OF REPLACEMENT OF DRINKING WATER WITH 0.9% SALINE SOLUTION, ON BAT MITOCHONDRIAL GDP BINDING IN LEAN AND OBESE (fa/fa) RATS

	BAT Mitochondrial [^{3}H] GDP Binding (pmol/mg protein)	
	Control	Saline
Lean	254.8 ± 20.2	286.6 ± 23.6
Obese (fa/fa)	132.0 ± 15.2***	142.0 ± 12.2***

4 lean and 4 fa/fa rats were housed individually, and given either water or 0.9% (w/v) saline solution as drinking fluid for 7 days. BAT mitochondria were prepared and GDP binding assays performed, as described in Sections 2.9 and 2.10 respectively.

Values represent means ± S.E.M. for 4 animals in each group

*** $p < 0.001$, compared with lean group.

unlikely to be responsible for the changes observed in BAT of adrenalectomised fa/fa rats.

3.2.5 *Time course of the changes in BAT mitochondrial GDP binding after adrenalectomy of lean and fa/fa rats*

The binding of GDP to BAT mitochondria of fa/fa rats has been shown to be normalised 7 days after adrenalectomy. The time course of the effect is shown in Fig. 3.2.3. In lean rats BAT mitochondrial GDP binding remained relatively unchanged during the first 7 days. After 28 days there was a slight decrease in GDP binding. However, after 28 days the rats were 8 weeks of age and this may account for the slight reduction (Section 3.1). In contrast, fa/fa rats showed a rapid increase in BAT mitochondrial GDP binding in the 24 h after adrenalectomy, and this was followed by a slower rate of increase over the next 6 days. Again, 4 weeks after adrenalectomy there was a slight fall in GDP binding, but this value was still almost 3 times obese (fa/fa) control levels.

3.2.6 *Effect of adrenalectomy on BAT mitochondrial GDP binding in lean and fa/fa rats of different ages*

The age dependence of the effects of adrenalectomy seen in the fa/fa rat was investigated by measuring BAT mitochondrial GDP binding in animals adrenalectomised between the ages of 4 and 10 weeks. After adrenalectomy BAT mitochondrial GDP binding in lean rats remained within the range observed for the 'intact' lean group at all ages (Fig. 3.2.4), it reached a maximum at 6 weeks of age and then slowly declined until the age of 10 weeks. The adrenalectomised fa/fa rat showed greatly increased BAT mitochondrial GDP binding at all ages when compared to the 'intact' fa/fa rat. Maximum GDP binding in the fa/fa rat also occurred at 6 weeks of age with a slow decline thereafter. Adrenalectomy was not attempted after 10 weeks of age, as the fa/fa rat is massively obese and adrenalectomies are very difficult to perform.

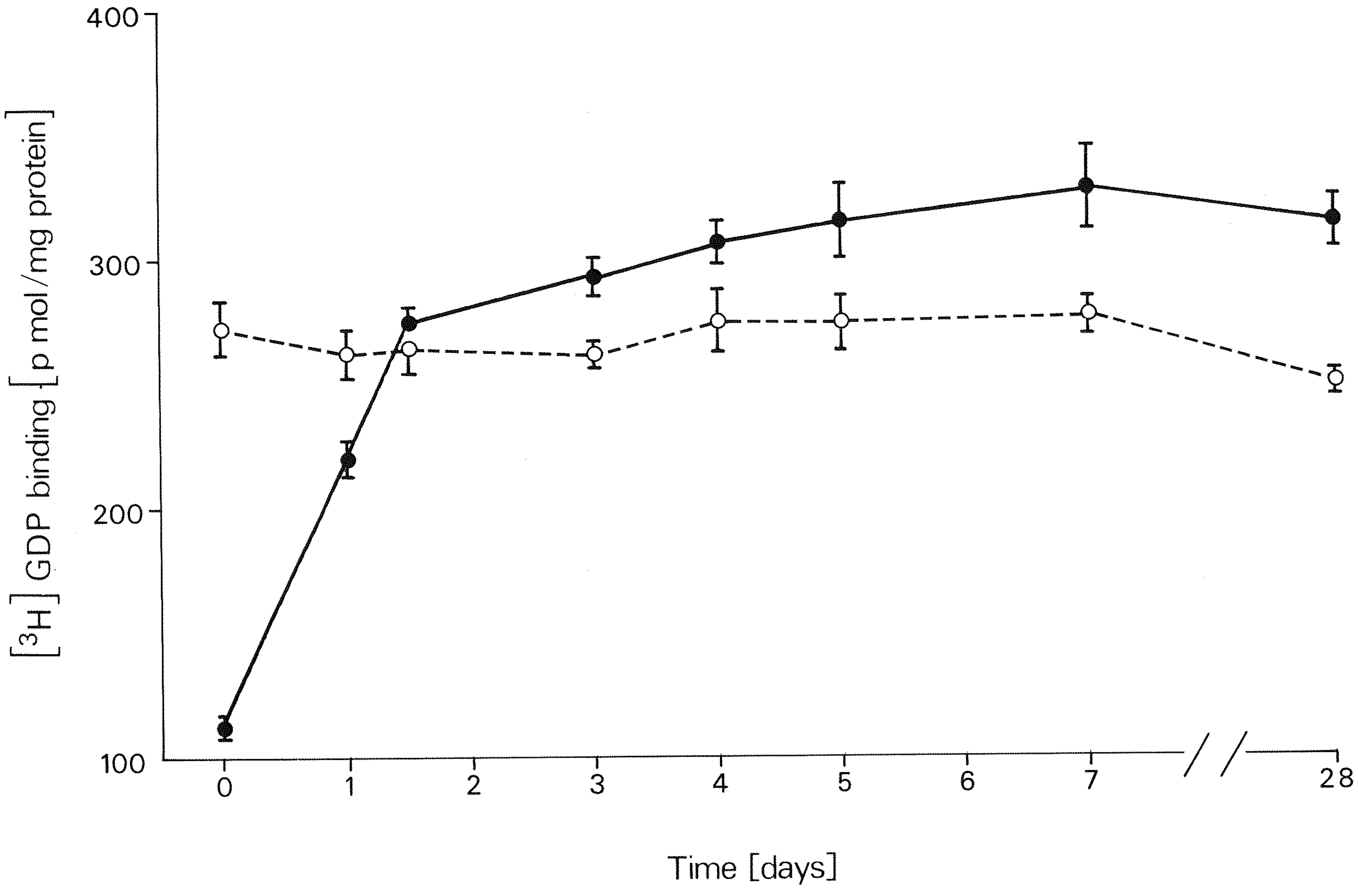
3.2.7 *Effect of aldosterone and corticosterone replacement to adrenalectomised lean and fa/fa rats*

Adrenalectomy removes both mineralocorticoid and glucocorticoid hormones. Hence, adrenalectomised rats were subjected to aldosterone and corticosterone replacement (Table 3.2.4). Aldosterone replacement had

FIG. 3.2.3 *TIME COURSE OF THE CHANGES IN BAT MITOCHONDRIAL GDP BINDING AFTER ADRENALECTOMY OF LEAN AND OBESE (fa/fa) RATS*

4-5 week old lean (O) and fa/fa (●) rats were adrenalectomised at zero time, and maintained as described in Section 2.7, and sacrificed at the times indicated. BAT was prepared, and mitochondrial GDP binding assays performed as described in Section 2.9 and 2.10 respectively. Rats sacrificed 28 days after adrenalectomy, were 8 weeks of age.

Values represent means ± S.E.M. for 4 rats at each time point, for each group.



[3H] GDP binding [p mol/mg protein]
400
300
200
100
0
1
2
3
4
5
6
7
28
Time [days]

FIG. 3.2.4 EFFECT OF ADRENALECTOMY ON BAT MITOCHONDRIAL GDP BINDING OF LEAN AND OBESE (fa/fa) RATS, AT DIFFERENT AGES

Lean and fa/fa rats were adrenalectomised or sham-operated at 3, 5, 7 and 9 weeks of age, and maintained as described in Section 2.7. Rats were sacrificed 7 days after adrenalectomy, at the ages indicated. BAT mitochondria were prepared and GDP binding assays performed as described in Section 2.9 and 2.10 respectively.

Values represent means ± S.E.M. for 4 animals at each time point.

Key to Groups

□--- Lean control

○--- Lean adrenalectomised

■—— Obese control

●—— Obese adrenalectomised

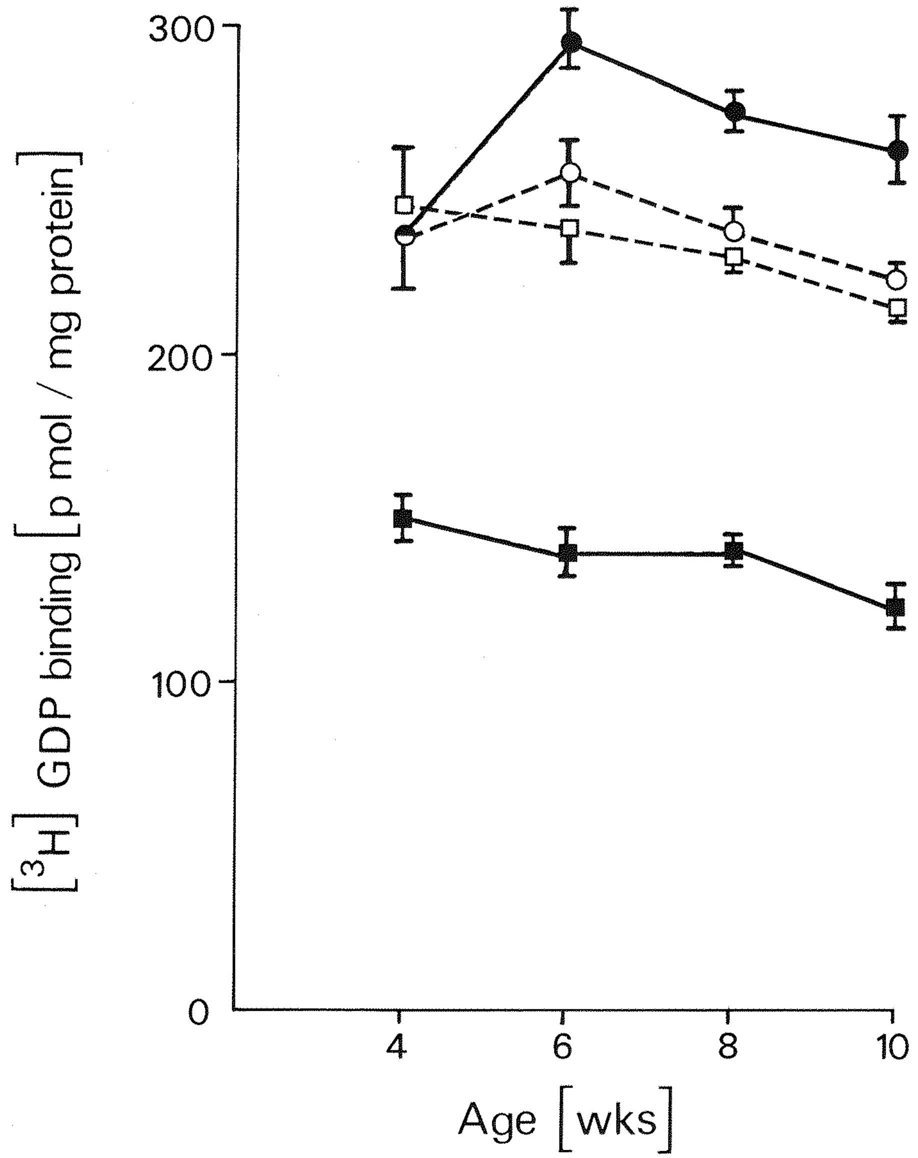

[3H] GDP binding [p mol / mg protein]
300
200
100
0
4
6
8
10
Age [wks]

TABLE 3.2.4

EFFECT OF ALDOSTERONE AND CORTICOSTERONE ON BAT OF ADRENALECTOMISED LEAN AND OBESE (fa/fa) RATS

	Brown adipose tissue		
Group	Protein (mg)	Succ.cyt.c.O-R activity (μmol/min/depot)	[3H]-GDP Binding (pmol/mg protein)
Intact			
Lean	28.0 ± 2.2	2.02 ± 0.21	260.0 ± 22.0
Obese	19.6 ± 2.6	1.21 ± 0.24*	140.0 ± 16.2**
Adrenalectomised			
Lean	24.2 ± 2.4	1.61 ± 0.16	252.2 ± 16.1
Obese	25.4 ± 2.0	1.66 ± 0.22	260.0 ± 25.0ΔΔ
Adrenalectomised + aldosterone			
Lean	24.0 ± 3.6	1.66 ± 0.20	270.2 ± 12.1
Obese	24.4 ± 1.8	1.62 ± 0.05	254.4 ± 2.4
Adrenalectomised + corticosterone			
Lean	22.4 ± 2.6	1.46 ± 0.10	197.0 ± 22.0
Obese	19.4 ± 2.6	1.28 ± 0.10	150.2 ± 14.1††
Intact + corticosterone			
Lean	24.2 ± 2.2	1.68 ± 0.20	172.1 ± 13.2ΔΔ
Obese	18.4 ± 2.1	1.50 ± 0.18	152.0 ± 21.2

5-6 week old lean and fa/fa rats were injected daily with either corticosterone or vehicle (1 mg/100g body weight) as described in Section 2.8.2, for 5 days. Adrenalectomised lean and fa/fa rats were injected with either aldosterone (10 μg/100g body weight) corticosterone (1 mg/100g body weight) or vehicle as described in Section 2.8.1 and 2.8.2 respectively, beginning on day 1 after adrenalectomy and continuing for 5 days. BAT protein, succ. cyt.c.O-R activity and mitochondrial GDP binding were assayed as described in Sections 2.11, 2.12 and 2.10 respectively.
Values represent means ± S.E.M. for 5 animals in each group. *$p < 0.05$, ** p 0.01, compared with equivalent lean group; †† $p < 0.01$, compared with equivalent adrenalectomised control group; ΔΔ $p < 0.01$, compared with equivalent intact control group.

no effect on BAT protein content, succ.cyt.c.O-R activity and BAT mitochondrial GDP binding in either lean or fa/fa adrenalectomised rats. Replacement of corticosterone to the adrenalectomised lean rat causes a slight reduction in BAT succ. cyt.c.O-R activity and mitochondrial GDP binding; however, this result does not reach statistical significance. The adrenalectomised fa/fa rat treated with corticosterone showed a small, not statistically significant, decrease in BAT protein, and succ.cyt.c.O-R activity; but mitochondrial GDP binding was reduced by over 40%, back to the values observed in the fa/fa 'intact' controls.

The effect of chronic corticosterone treatment on 'intact' lean and fa/fa rats is also shown in Table 3.2.4. A 34% reduction in BAT mitochondrial GDP binding was observed in the lean rat, whereas no change was observed in the fa/fa rat, so that the levels of GDP binding in both groups were similar after corticosterone treatment.

The lean rat showed an acute response to corticosterone. After a single s.c. injection (1 mg/100g body weight), BAT mitochondrial GDP binding was significantly decreased (258 ± 19.7 and 150 ± 9.5 pmol GDP bound/mg protein for lean control and corticosterone treated, respectively), when measured 8 h after treatment. No such reduction was observed in the fa/fa rat (136 ± 10.2 and 128 ± 16.6 pmol GDP bound/mg protein, for fa/fa control and corticosterone treated, respectively).

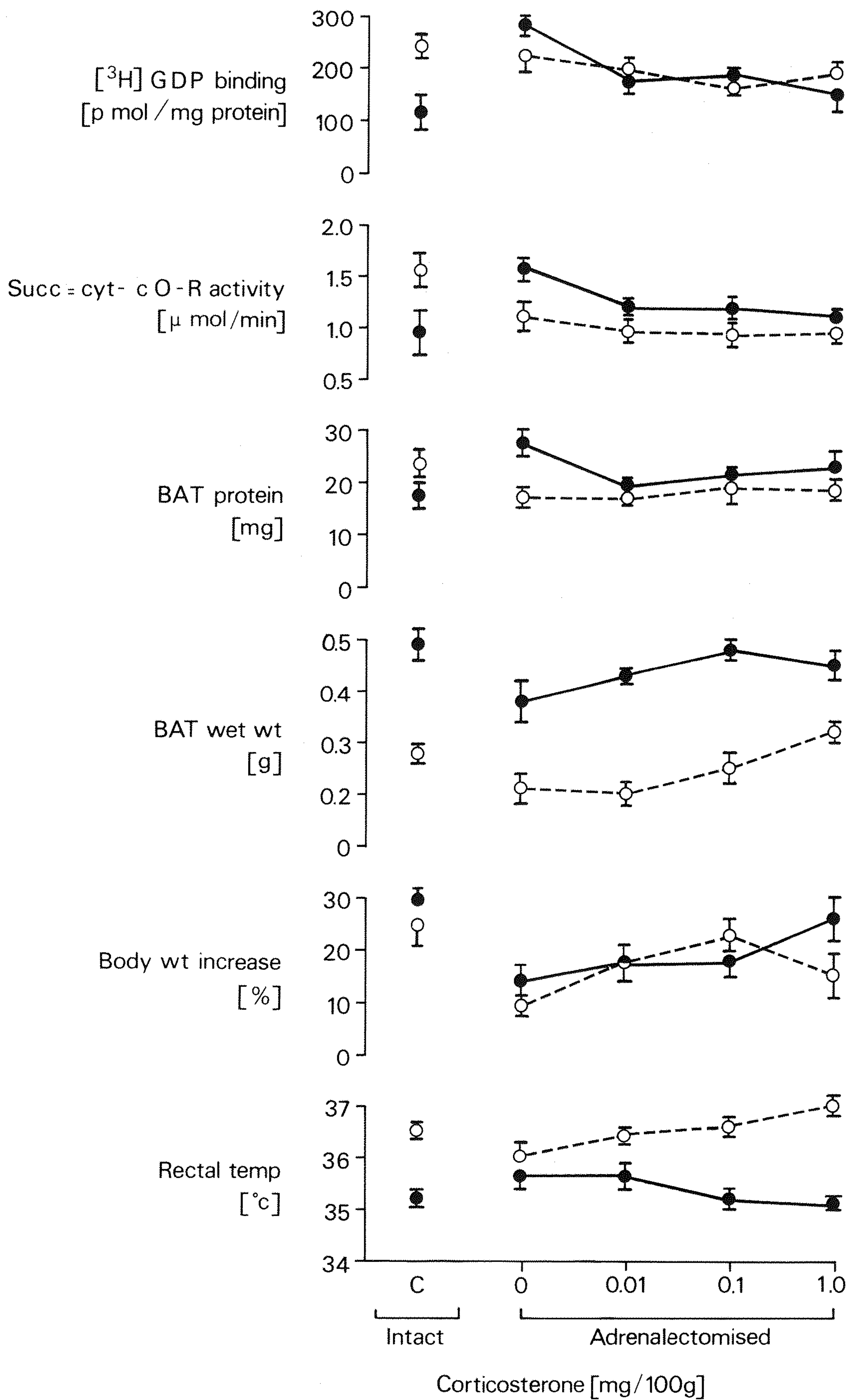
The serum concentrations of corticosterone were measured (Section 2.8.2) in young (5-6 week old) lean and fa/fa rats, and found to be similar (0.85 ± 0.15 and 0.72 ± 0.10 ng corticosterone per ml of serum, for lean and fa/fa rats, respectively) in both groups.

Fig. 3.2.5 shows the dose dependence of the effects of corticosterone replacement to adrenalectomised lean and fa/fa rats. Adrenalectomy of lean rats was associated with a small fall in rectal temperature, a slower rate of body weight gain and reduced BAT wet weight, protein content and succ.cyt.c.O-R activity. Corticosterone replacement increased rectal temperature, body weight gain and BAT weight in a dose dependent manner, towards levels seen in the intact animal, whereas BAT protein content and succ.cyt.c.O-R activity were unaffected. BAT mitochondrial GDP binding was not altered after adrenalectomy in the lean rat, but was slightly decreased in a dose dependent fashion with corticosterone replacement. In the fa/fa rats, adrenalectomy resulted in an increase in rectal temperature, BAT protein content, succ.cyt.c.O-R activity

FIG. 3.2.5 *EFFECT OF CORTICOSTERONE ON BODY WEIGHT, RECTAL TEMPERATURE AND BAT OF LEAN AND OBESE (fa/fa) RATS*

Lean (O) and fa/fa (●) rats were adrenalectomised or sham-operated (C) at 5 weeks of age, and maintained as described in Section 2.7. Adrenalectomised rats were allowed to recover for 24h and then injected with corticosterone or vehicle as described in Section 2.8.2, at the doses shown, for 7 days. Body weight gain is expressed as percentage increase per week, and rectal temperatures were recorded at the end of the period of treatment. BAT protein, succ.cyt.c.O-R activity (expressed as μmol/min/depot) and GDP binding assays were performed as described in Section 2.11, 2.12 and 2.10 respectively.

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



[^{3}H] GDP binding
[p mol / mg protein]
300
200
100
0
Succ - cyt - c O - R activity
[μ mol/min]
2.0
1.5
1.0
0.5
BAT protein
[mg]
30
20
10
0
BAT wet wt
[g]
0.5
0.4
0.3
0.2
0
Body wt increase
[%]
30
20
10
0
Rectal temp
[°c]
37
36
35
34
C
0
0.01
0.1
1.0
Intact
Adrenalectomised
Corticosterone [mg/100g]

and BAT mitochondrial GDP binding. It also resulted in a decreased rate of body weight gain and a decrease in BAT weight. With corticosterone replacement all these criteria returned towards values seen in the intact fa/fa animal, in a dose dependent manner. Although the protein content of BAT in adrenalectomised fa/fa rats was decreased to values seen in intact fa/fa rats by the lowest dose of corticosterone (0.1 mg kg/day), higher doses slightly increased protein content of the BAT.

3.2.8 *Effect of adrenalectomy on serum triiodothyronine (T_3) concentration in lean and fa/fa rats*

Serum T_3 levels were lower in 5 week old fa/fa rats than in lean animals, but increased after adrenalectomy to values observed in the lean control rats (Table 3.2.5). Adrenalectomy also resulted in an increase in serum T_3 in lean rats. Corticosterone (1 mg/100g per day) replacement to adrenalectomised rats decreased serum T_3 levels to or below those of their respective control groups.

3.2.9 *Effect of thyroid hormone treatment on lean and fa/fa rats*

As the previous table (3.2.5) indicates, the levels of serum T_3 are low in the fa/fa rat, but are raised to normal after adrenalectomy. To examine the effect of additional thyroid hormone, lean and fa/fa rats were treated with 0.03% thyroid powder (TP) given as a dietary supplement. Fig. 3.2.6 shows that after 6 days of TP treatment the rate of increase in body weight was reduced in both lean and fa/fa rats (although the result only reached statistical significance in the fa/fa group), despite an unchanged energy intake (86.0 ± 2.3 and 78.2 ± 2.8 kJ/day for lean rats, and 100 ± 11.3 and 96.9 ± 4.3 kJ/day for fa/fa rats, for control and TP-treated rats respectively). Despite this, BAT wet weight was elevated by 22% in lean rats and 21% in fa/fa rats. TP-treated lean rats showed a 30% reduction in BAT mitochondrial GDP binding (Fig. 3.2.6) whereas the level in the fa/fa rat was unchanged from the untreated fa/fa control group. Serum insulin concentration was unchanged in the TP-treated lean group (Table 3.2.6) but serum free fatty acids were increased by 40% when compared to the lean control group. The fa/fa rat was hyperinsulinemic in comparison with the lean rat, and treatment with TP resulted in a significant decrease in serum insulin levels. Serum free fatty acids were similar in the fa/fa and

TABLE 3.2.5

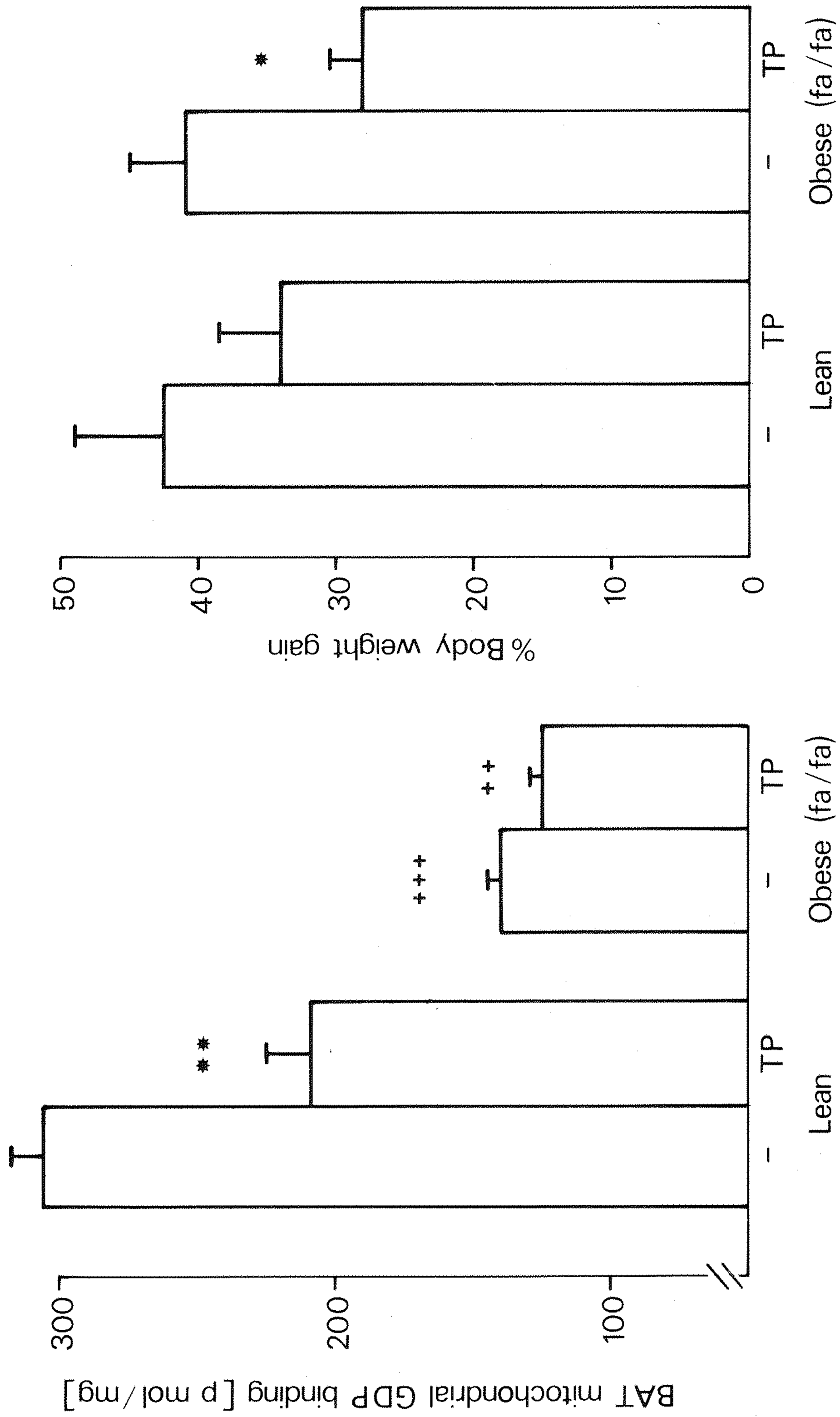
SERUM TRIIODOTHYRONINE CONCENTRATIONS IN 5-6 WEEK OLD ADRENALECTOMISED LEAN AND OBESE (fa/fa) RATS

	Serum triiodothyronine (ng/ml)		
Group	Lean	Obese (fa/fa)	P
Control	0.98 ± 0.03 (15)	0.68 ± 0.04 (15)	0.001
Adrenalectomised	1.70 ± 0.28 (4)*	1.06 ± 0.04 (6)***	0.05
Adrenalectomised + corticosterone	0.75 ± 0.06 (4)	0.60 ± 0.07 (6)	N.S.

Rats were adrenalectomised or sham operated at 4-5 weeks of age, then injected with either corticosterone (1 mg/100g per day), or vehicle for 7 days as described in Section 2.8.2. Serum triiodothyronine levels were measured as described in Section 2.14.5. Values represent means ± S.E.M. for the numbers of animals shown in parenthesis. * $p < 0.05$, *** $p < 0.001$, compared with respective control group; NS not significant.

FIG. 3.2.6 EFFECT OF THYROID HORMONE ON BODY WEIGHT AND BAT MITOCHONDRIAL [^{3}H]-GDP BINDING IN LEAN AND OBESE (fa/fa) RATS

4 week old lean and fa/fa rats were housed individually and given either a standard chow diet, or chow supplemented with 0.03% thyroid powder (TP), as described in Section 2.8.3, for 6 days. Rats were weighed daily during this period. BAT mitochondrial GDP binding was determined as described in Section 2.10.
Body weight gain was expressed as percentage body weight gain for the 6 day period.
Values represent means ± S.E.M. for 4 animals in each group. * $p < 0.05$, ** $p < 0.01$, compared with equivalent control group; †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent lean group.



BAT mitochondrial GDP binding [p mol / mg]
300
200
100
% Body weight gain
50
40
30
20
10
0
–
TP
Lean
Obese (fa / fa)

TABLE 3.2.6

EFFECT OF THYROID HORMONE ON SERUM INSULIN AND FREE FATTY ACID CONCENTRATIONS IN LEAN AND OBESE (fa/fa) RATS

	Serum	
	Insulin (ng/ml)	Free Fatty Acids (μmol/ℓ)
Lean control	2.28 ± 0.42	0.324 ± 0.030
Lean + T.P.	1.11 ± 0.24	0.456 ± 0.013**
Obese (fa/fa) control	24.30 ± 4.39††	0.388 ± 0.020
Obese (fa/fa) + T.P.	11.40 ± 2.61*††	0.616 ± 0.072*

4 week old lean and fa/fa rats were housed individually and fed either a standard chow diet or chow plus 0.03% thyroid powder (T.P.), as described in Section 2.8.3, for 6 days. Serum insulin and free fatty acid concentrations were determined as described in 2.14.

Values represent means ± S.E.M. for 4 animals in each group.
*$p < 0.05$, ** $p < 0.01$, compared with equivalent control group;
†† $p < 0.01$, compared with equivalent lean group.

lean control rats (Table 3.2.6), and treatment with TP resulted in a 60% increase in the fa/fa rat, to levels similar to that of the TP-treated lean group.

3.2.10 *Effect of induction of diabetes with streptozotocin on BAT of lean and fa/fa rats*

The young (5 week old) fa/fa rat is already hyperinsulinemic when compared to the lean rat (Table 3.2.6). Serum insulin levels decreased after adrenalectomy to levels observed in lean rats (2.49 ± 0.49 and 6.22 ± 2.72 μU insulin/ml serum, for adrenalectomised lean and fa/fa rats respectively). Experimental diabetes was induced with streptozotocin to determine whether the fall in serum insulin could be responsible for the increased BAT thermogenesis observed in adrenalectomised fa/fa rats.

Table 3.2.7 shows the expected rise in blood glucose (a characteristic of diabetic rats), in lean and fa/fa rats. Lean diabetic rats showed a small but statistically significant decrease in food intake accompanied by a reduced rate of body weight gain when compared to the control lean group. BAT wet weight was decreased in the diabetic lean rat and protein content was decreased by 45%. However, BAT mitochondrial GDP binding (per mg of mitochondrial protein) was unchanged when compared to the lean control group. Total tissue binding was reduced in the diabetic lean rat indicating a reduced mitochondrial population.

The fa/fa diabetic rat showed a 30% reduction in food intake and a 65% decrease in the rate of body weight gain. BAT wet weight however, was not reduced in the fa/fa diabetic rat and protein content was slightly but not significantly reduced, compared to the control fa/fa rat. BAT mitochondrial GDP binding in the diabetic fa/fa rat was significantly reduced when compared to lean control and diabetic rats, but unchanged from the control fa/fa group. Total tissue binding in the diabetic fa/fa rat was also unchanged from the fa/fa control values.

The levels of BAT mitochondrial GDP binding in all groups in the present experiment were low in comparison with previous values. However the housing temperature was slightly increased (25°C, instead of the normal 23°C) and the animals used in this experiment were all 8 weeks of age on sacrifice (Table 3.2.7). This combined increase in age and

TABLE 3.2.7

EFFECT OF STREPTOZOTOCIN INDUCED DIABETES ON BODY WEIGHT AND BAT OF LEAN AND OBESE (fa/fa) RATS

	Control		Streptozotocin	
	Lean	Obese (fa/fa)	Lean	Obese (fa/fa)
Blood glucose (mg/100 ml)	92.0 ± 4.0	95.0 ± 3.0	190.0 ± 12.0††	194.0 ± 26.0†
Food intake (kJ/day)	164.1 ± 7.2	210.7 ± 8.6*	134.5 ± 6.2†	149.5 ± 9.6††
Initial body weight (g)	130.2 ± 2.6	191.0 ± 4.2***	129.7 ± 3.2	189.2 ± 5.0***
Body weight gain (% increase/week)	23.4 ± 2.6	46.7 ± 9.2	15.7 ± 0.3†	16.4 ± 1.2†††
Brown adipose tissue:				
Wet wt (g)	0.35 ± 0.02	0.87 ± 0.04***	0.22 ± 0.02†	0.81 ± 0.16***
Protein (mg)	44.0 ± 4.2	52.0 ± 6.0	24.2 ± 3.6†	40.0 ± 3.0*
[^{3}H]-GDP binding (pmol/mg protein)	187.6 ± 6.2	94.2 ± 12.2 **	183.6 ± 8.9	89.0 ± 8.2**
Total tissue [^{3}H]GDP binding (pmol/tissue)	1590 ± 162	557 ± 106**	1101 ± 102	631 ± 108*

7 week old lean and fa/fa rats were housed individually and injected with a single dose of streptozotocin (8 mg/100g body weight) or vehicle as described in Section 2.8.4. Rats were then maintained for 7 days at 25°C, during which time food intake and body weights were monitored. Serum glucose, BAT protein and GDP binding were assayed as described in 2.14.3, 2.11 and 2.10 respectively. Total tissue binding was calculated based on 100% mitochondrial recovery. Values represent means ± S.E.M. for 3 animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with equivalent lean group; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent control group.

temperature effects may have resulted in the reduced levels of BAT mitochondrial GDP binding.

3.2.11 Discussion

The observations reported in this section confirmed earlier findings (Section 3.1) that BAT mitochondrial GDP binding was decreased in fa/fa rats and suggested that this defect was central to the development of obesity in Zucker rats. The present results indicate that the reduced activity of the proton conductance pathway in BAT of fa/fa rats may be rectified by adrenalectomy, such that 7 days post-operatively, the number of binding sites had returned to the levels observed in lean rats. This increase in purine nucleotide binding in adrenalectomised fa/fa rats is consistent with an increased thermogenesis, since similar parallel increases in binding and thermogenesis have been reported in both cold-adapted and cafeteria-fed lean rats *(Sundin & Cannon 1980; Brooks et al., 1982)*. Until recently, only one class of GDP-binding site had been observed in BAT mitochondria *(Nicholls 1976b; Sundin & Cannon, 1980; Brooks et al., 1982)*. However, *Bryant et al. (1983)* have now shown that Scatchard analysis of GDP binding to lean rat BAT mitochondria revealed two distinct types of binding site, low capacity high affinity (Kd 0.052 μM) and high capacity, low affinity (Kd 2.56 μM) sites. Cold-adaptation or cafeteria feeding, increased binding capacity of the low affinity site, due to an increased number of the GDP binding sites, without any change in affinity for the ligand. The present Scatchard analyses also indicate the presence of 2 classes of binding sites, with high and low affinities for GDP. The Kd of the lower affinity site (between 2.9 and 3.3 μM for lean and fa/fa rats, control and adrenalectomised) was similar to those reported by *Bryant et al. (1983)*. However, the high affinity site had an increased Kd (0.26 μM for control lean and fa/fa rats and 0.36 μM for adrenalectomised lean and fa/fa rats) when compared to the values reported by the above authors (approx. 0.05 μM). This variation may be due to strain and age differences in the animals used. Another consideration must be the graphical method of analysis used to determine the Kd in the present experiments. This is less accurate than the computer aided analysis, as the presence of low affinity sites would interfere with the calculation of Kd for the high affinity sites. The increase in BAT mitochondrial

GDP binding seen in the adrenalectomised fa/fa rat appeared to be largely due to an increased binding capacity of the low affinity binding site, rather than any change in affinity for GDP. To a smaller extent an increased binding capacity is observed for the high affinity sites, however, as the proportion of high affinity sites was low, the contribution to the overall increase in BAT mitochondrial GDP binding would be minimal.

An increase in BAT mitochondrial GDP binding was observed within 24 h of adrenalectomy in the fa/fa rat. This suggests that the acute increase in BAT thermogenesis may be due to an 'unmasking' of GDP binding sites, already present in the mitochondrial membrane. The acute increase in BAT mitochondrial GDP binding in cold-exposed rats, cannot be inhibited by protein synthesis inhibitors and has also been ascribed to an 'unmasking' of GDP binding sites *(Desautels et al., 1978; Desautels & Himms-Hagen, 1979)*. Similarly the fa/fa rat responds acutely to a cold stimulus (Section 3.3) with an increase in BAT mitochondrial GDP binding. Thus, the functional capacity of BAT must, to a large extent, be intact in the fa/fa rat, and the defect may lie in the control of the tissue. The acute increase in mitochondrial GDP binding 24 h after adrenalectomy of the fa/fa rat, was followed by a slower increase in binding during the next 7 days. This presumably represented an increased synthesis of the 32K protein, accompanying the hypertrophy of BAT. Preliminary radioimmunoassay experiments *(Holt, York and Cleeter, unpublished observations)* using antibody provided by Dr. M. Ashwell (Dunn Research Institute, Cambridge) have suggested that there is a reduced concentration of the 32K protein in BAT mitochondria of fa/fa rats, when compared to lean rats, and that this is restored to normal 7 days after adrenalectomy. However, further experiments will be needed to confirm the involvement of protein synthesis in the slower secondary response in adrenalectomised fa/fa rats. Adrenalectomy resulted in a large increase in BAT mitochondrial GDP binding, even at 10 weeks of age when the rat was massively obese. This again suggests that the functional capacity of BAT remains intact, and that it is the stimulation of the tissue which is defective in the 'intact' fa/fa rat.

The morphological changes in BAT which followed adrenalectomy of fa/fa rats were also consistent with an improved thermogenesis. The

size of the lipid droplets were reduced and the mitochondria assumed the ultrastructural characteristics of those in BAT of lean and lean adrenalectomised rats.

The improvement in BAT function which followed adrenalectomy of fa/fa rats was prevented by corticosterone but not aldosterone replacement. Thus, despite the requirement for corticosterone, in a permissive capacity, to maintain BAT thermogenic response to cold exposure *(Fellenz et al., 1982)*, it would appear that glucocorticoids are either directly or indirectly responsible for the impairment in BAT function of fa/fa rats. As serum corticosterone values were similar in lean and fa/fa intact rats, it is difficult to reconcile the above suggestion. However, the BAT of the adrenalectomised fa/fa rat appeared to be more responsive to the lowest replacement dose of corticosterone used, than the lean adrenalectomised animal, suggesting that the fa/fa rat may be more sensitive to circulating corticosterone. This suggestion can be supported by other observations *(Yukimura et al., 1978)* which showed that corticosterone replacement to adrenalectomised fa/fa rats, increased food intake and body weight gain when compared to the corresponding lean animals.

It is possible that corticosterone may inhibit fa/fa rat BAT directly, by acting at the mitochondrial level. Early work suggested that cortisone treatment to young lean rats *(Hahn et al., 1969; Skala & Hahn, 1971)* reduced the oxidative capacity of BAT mitochondria, but the lipolytic response to noradrenaline in vitro remained intact. *Skala & Hahn (1971)* suggested that the fat accumulation in BAT after cortisone treatment was due to a reduced ability to oxidise fatty acids. As treatment with corticosterone reduced BAT mitochondrial GDP binding in lean 'intact' rats in the present study, it is possible that corticosterone may thus be limiting the rate of oxidation of fatty acids, and therefore controlling the metabolic activity of the tissue. This may be supported by the observation that mice treated chronically with corticosterone *(Galpin et al., 1983)* show a reduced BAT oxidative capacity and reduced BAT mitochondrial GDP binding. However, as NST is not impaired in corticosterone treated mice *(Galpin et al., 1983)*, and in cold exposed fa/fa rats (Section 3.3), it is most likely that the glucocorticoids have an indirect, rather than a direct effect on BAT in these animals.

It is possible that the increase in BAT mitochondrial GDP binding, observed in the adrenalectomised fa/fa rat, may be due to the increase in ACTH secretion which follows removal of corticosterone on adrenalectomy *(Yukimura et al., 1978)*. ACTH is known to increase blood flow to BAT *(Smith & Horwitz, 1969)* and preliminary results in our laboratories *(York et al., unpublished observations)* indicate that ACTH treatment increases BAT mitochondrial GDP binding and increases rectal temperature in fa/fa rats. This effect appears to be greater in the fa/fa rat than in the lean rat. The possible role for ACTH in BAT thermogenesis of the fa/fa rat will be discussed more fully in Chapter 4.

Reduction of serum insulin by streptozotocin treatment did not improve BAT thermogenesis in the (normally hyperinsulinemic) fa/fa rat. The diabetic lean animal showed an overall reduction in BAT mitochondrial GDP binding capacity, and since the protein content of BAT was markedly reduced, this probably reflected a reduction in the mitochondrial population. This observation confirms other reports *(Seydoux et al., 1983; Agius et al., 1981; Rothwell & Stock, 1981a; Rothwell et al., 1983c)* of an insulin requirement for normal BAT thermogenesis. *Seydoux et al. (1983)* have recently shown that lean rats made diabetic with streptozotocin, demonstrated a specific decrease in the rate of β oxidation of fatty acids in BAT. They suggest that this decrease in fatty acid degredation may become rate limiting for the tissue. Unlike lean rats, fa/fa rats *(Rothwell et al., 1983c)* failed to respond to insulin or carbohydrate with an increase in oxygen consumption (which could be blocked by the β antagonist propranolol). This, along with the present observations of an unchanged BAT thermogenesis in the streptozotocin-treated fa/fa rat, suggests that the fa/fa rat is insensitive to the thermogenic effects of insulin.

The SNS is thought to be the principal regulator of BAT thermogenesis *(Landsberg & Young, 1983)* and noradrenaline turnover and content of BAT have been shown to be reduced in fa/fa rats when compared to the lean rat *(York & Marchington, 1984)*. Support for the present findings that adrenalectomy increases BAT thermogenesis in fa/fa rats, has been obtained recently from studies of noradrenaline turnover rates *(York & Marchington, 1984)*. Adrenalectomy restored the impaired noradrenaline turnover in BAT of fa/fa rats to that observed in lean rats, indicating a normalisation of sympathetic activity following

removal of corticosterone. Conversely, BAT noradrenaline turnover was reduced in corticosterone-treated lean 'intact' rats *(York & Marchington, 1984)*. Adrenalectomy did not result in an increase in BAT thermogenesis in lean rats. This may result from a coincident reduction in sympathetic activity due to reduced food intake observed in these animals, following adrenalectomy. Restriction of food intake is known to reduce noradrenaline turnover in BAT of lean rats *(Young et al., 1982)*. This suggestion is also supported by the present observation of a reduced BAT mitochondrial GDP binding in lean food-restricted rats. Removal of corticosterone in the lean rat by adrenalectomy, may release an inhibitory effect on the SNS activity in BAT, but the effect is counteracted by a reduced food intake. Restricting the food intake of the fa/fa rat did not reduce BAT mitochondrial GDP binding. Therefore, unlike the lean rat, the fa/fa rat does not appear to alter BAT thermogenesis in response to changes in food intake. (This statement will be supported by further evidence in Section 3.3). As adrenalectomy restores BAT mitochondrial GDP binding to normal, despite a markedly reduced food intake, it is possible that corticosterone may be suppressing DIT in the fa/fa rat.

The present results demonstrate that adrenalectomy reduced food intake and lowered the rate of weight gain in fa/fa rats, to values observed in lean rats. Recent parallel energy balance studies *(Marchington et al., 1983)*, confirm that young fa/fa rats have a reduced energy expenditure when compared to lean rats. Adrenalectomy did not significantly influence energy balance in lean rats, but restored the energetic efficiency and body energy gain of fa/fa rats, to the level of lean animals.

Serum T_3 levels are reduced in fa/fa rats but were raised to normal values after adrenalectomy. It is difficult to associate the significance of hypothyroidism in the fa/fa rat *(Autissier et al., 1980)* with a reduced thermogenesis, as thyroid hormones are only required permissively for BAT thermogenesis *(Fellenz et al., 1982; Himms-Hagen, 1983c)* and fa/fa rats respond normally to cold (Section 3.3). Additional thyroid hormone did not increase BAT mitochondrial GDP binding in the fa/fa rat, and in the lean rat GDP binding was actually decreased compared to control values. A similar finding has also been reported by *Sundin (1981)* who suggested that a thyroid-induced elevation of heat production in other tissues, reduced the requirement for NST in BAT, presumably via a reduced SNS activity which is associated with

hyperthyroidism *(Gibson, 1981; Axelrod, 1975; Tedesco et al., 1977)*. The TP-treated fa/fa rat did not show any improvement in BAT function. However, the increased serum free fatty acids and reduction in serum insulin may indicate an increased sympathetic stimulation to organs other than BAT *(Levin et al., 1982)*. Also a restriction in body weight gain, despite an unchanged food intake, suggests that energetic efficiency is reduced in fa/fa rats after TP treatment. Other workers *(Levin et al., 1982)* are in agreement with the above findings and show a reduced body weight gain and partial correction of the hyperinsulinemia in the fa/fa rat. These authors observed that the reduced noradrenaline turnover in BAT of fa/fa rats was not improved by treatment with thyroid hormone. However, they also demonstrate that noradrenaline turnover was enhanced in other tissues (aorta and heart) which they suggest may be responsible for the increased rectal temperature and improved cold tolerance observed.

Studies on thyroid function in fa/fa rats have suggested that the hypothalamic regulation of pituitary TSH secretion might be impaired *(Bray & York, 1971b; York et al., 1972)*, resulting either in a fall in serum thyroxine (T_4) as found in older rats *(Martin et al., 1978; Autissier et al., 1980)*, or as reported in the present experiments, a fall in serum T_3 in young fa/fa rats. Glucocorticoids are known to influence thyroid function at a number of sites, including the inhibition of peripheral deiodination of T_4 to T_3 *(Chopra et al., 1975)* and the inhibition of TSH secretion in response to TRH *(Wilber & Utiger, 1969; Pamenter & Hedge, 1980)*, which would decrease thyroid hormone secretion. Peripheral deiodination mechanisms are also responsive to sympathetic activation, and the apparent depression in sympathetic tone in fa/fa rats *(Levin et al., 1982)* may contribute to the abnormal peripheral deiodination of T_4 noticed in other studies of obesity *(Pittman et al., 1972; Ingbar & Galton, 1975; Bray et al., 1976; Autissier et al., 1980)*. BAT has a high activity of 5'-deiodinase, the enzyme responsible for the conversion of T_4 to T_3 *(Leonard et al., 1983)*, and recently the activity of this enzyme has been shown to be under adrenergic control *(Silva & Larsen, 1983)*. Glucocorticoids may be suppressing the diet-related sympathetic activation of BAT 5'-deiodinase, and it is possible that a low activity of this enzyme may contribute to the low serum T_3 levels observed in fa/fa rats. This is supported by

the present observation, that T_3 levels in the fa/fa rat are increased after adrenalectomy, when BAT noradrenaline content and turnover are also increased to normal lean values *(York & Marchington, 1984)*. Other forms of rodent obesity are known to be prevented by adrenalectomy. The development of obesity in the ob/ob mouse has been shown to be corrected after adrenalectomy *(Solomon et al., 1977)*. Results presented in Table 3.2.8 demonstrate that the attenuated level of BAT mitochondrial GDP binding in the ob/ob mouse, which has been associated with the loss of both diet and cold related BAT thermogenesis *(Himms-Hagen & Desautels, 1978; Trayhurn et al., 1982; Himms-Hagen, 1983a,b)* was significantly elevated after adrenalectomy. This suggests that adrenal glucocorticoids may directly or indirectly inhibit BAT function in ob/ob mice. However, unlike the fa/fa rat, serum corticosterone levels are elevated in ob/ob mice from an early age *(Dubuc, 1976)*. The development of hypothalamic obesity in rats induced by ventromedial hypothalamic (VMH) lesions (a condition associated with reduced BAT mitochondrial GDP binding) *(Seydoux et al., 1982)*, was prevented by adrenalectomy *(Bruce et al., 1982)*, and restored with administration of corticosterone. VMH-lesioned animals also demonstrate a normal thermogenic response to cold but not to diet *(Hogan et al., 1982)*. It is thus possible that the corticosterone-dependent inhibition of diet related sympathetic activity may be a common cause of a number of rodent obesities.

TABLE 3.2.8

THE EFFECT OF ADRENALECTOMY ON RECTAL TEMPERATURE AND BROWN ADIPOSE TISSUE FUNCTION IN LEAN AND OBESE (ob/ob) MICE

	Lean		Obese	
	Control	Adrenalectomised	Control	Adrenalectomised
Weight gain (%/week)	15.4 ± 3.2	7.9 ± 2.1	28.6 ± 4.2†	6.2 ± 1.6***
Rectal temperature (°C)	34.0 ± 0.1	34.6 ± 0.4	33.4 ± 0.1††	34.8 ± 0.6
Brown Adipose Tissue:				
Wet wt (g)	0.15 ± 0.02	0.12 ± 0.02	0.38 ± 0.02†††	0.28 ± 0.02
Protein (mg)	10.0 ± 1.2	10.3 ± 0.9	10.5 ± 1.6	9.8 ± 1.2
Succ.cyt.c.O-R (μmol/min/depot)	0.70 ± 0.06	0.74 ± 0.03	0.70 ± 0.03	0.78 ± 0.04
[^{3}H]-GDP binding (pmol/mg)	166.0 ± 10.1	149.2 ± 17.2	102.6 ± 11.2††	263.4 ± 16.2***

5 week old lean and obese (ob/ob) mice were adrenalectomised or sham operated, and maintained on 0.9% (w/v) saline or water as drinking fluid respectively, for 7 days. Initial body weights were 24.2 ± 1.5g for lean and 28.0 ± 1.0g for obese mice, prior to adrenalectomy. BAT protein content, succ. cyt.c.O-R activity and mitochondrial GDP binding was determined as described in Section 2. Values represent means ± S.E.M. for 6 animals in each group. *** $p < 0.001$, compared with control obese mice, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$, compared with control lean mice.

SECTION 3.3 The Effect of Temperature and Overfeeding on BAT Thermogenesis in the Obese (fa/fa) Rat

The previous sections (3.1 and 3.2) have demonstrated that BAT thermogenesis is defective in the fa/fa rat from an early age, and that this impairment is corrected by adrenalectomy. Since BAT thermogenesis may be enhanced by both thermal and nutritional signals, the attenuated level of BAT mitochondrial GDP binding in fa/fa rats could reflect a lack of response to either environmental housing temperature and/or to food intake.

In the present section, experiments are described in which the capacity of the fa/fa rat for non-shivering thermogenesis (NST) and diet-induced thermogenesis (DIT) are examined, by housing at a range of environmental temperatures and by inducing a higher food intake, using a voluntary sucrose feeding regimen. Results suggest that adrenal corticoid secretion may suppress the diet related activation of BAT.

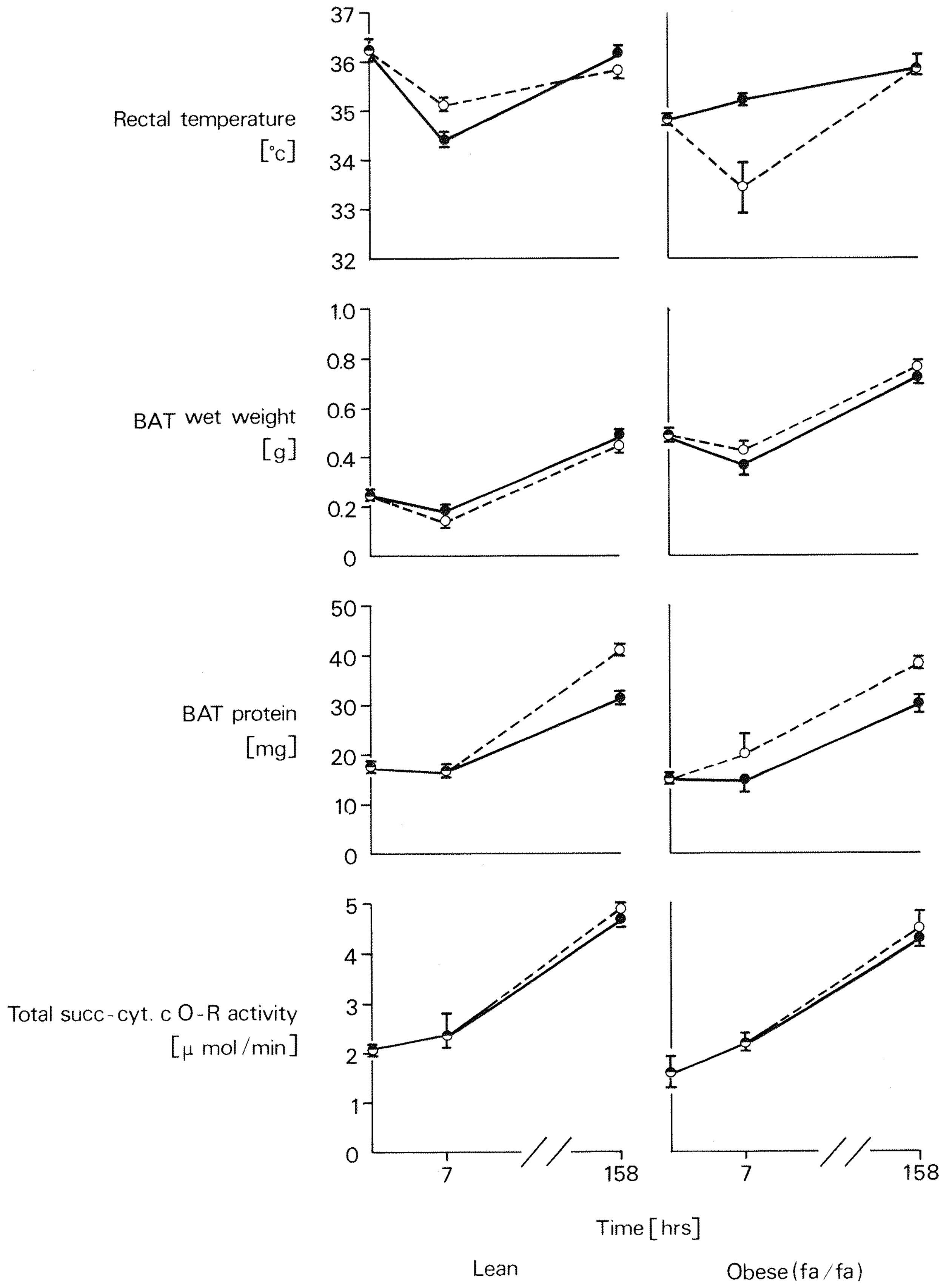
3.3.1 Effect of environmental temperature on BAT of lean and fa/fa rats

The response of 5 week old lean and obese (fa/fa) rats to housing at different environmental temperatures is shown in Fig. 3.3.1 and Fig. 3.3.2. Rectal temperatures of lean rats were reduced after housing at $4^{o}C$ for 7 h but had returned to normal values by 7 days. An increase in BAT thermogenesis was evident within 7 h of cold exposure as indicated by an increase in mitochondrial GDP binding. BAT wet weight was slightly lower after 7 h at $4^{o}C$ although protein content and mitochondrial succ.cyt.c.O-R activity remained at normal levels, indicative of the mobilisation of lipid stores. After 7 days at $4^{o}C$, BAT protein and succ.cyt.c.O-R activity were elevated in the lean rat, indicative of an increase in mitochondrial population. BAT mitochondrial GDP binding (per mg. mitochondrial protein) was increased by 95% and total tissue binding by over 260%. Similar acute and chronic responses to housing at $4^{o}C$ were observed in the obese (fa/fa) rats, with the exception that rectal temperature was significantly elevated above that of control animals (housed at $23^{o}C$) after 7 days. In these animals, both the binding of GDP per mg of mitochondria (230% increase) and the total tissue mitochondrial GDP binding (400% increase), were

FIG. 3.3.1 EFFECT OF SHORT TERM EXPOSURE AND ACCLIMATION TO 14°C AND 4°C ON BAT AND RECTAL TEMPERATURE OF LEAN AND OBESE (fa/fa) RATS

4-5 week old lean and fa/fa rats were housed at either 23°C (◒), 14°C (●) or 4°C (O), for the times indicated. BAT protein and succ.cyt.c.O-R activity (expressed as μmol/min/depot) were measured as described in Section 2.11 and 2.12 respectively. Rectal temperatures were taken at the end of the temperature treatments.

Values represent means ± S.E.M. for 6 animals at each time point.

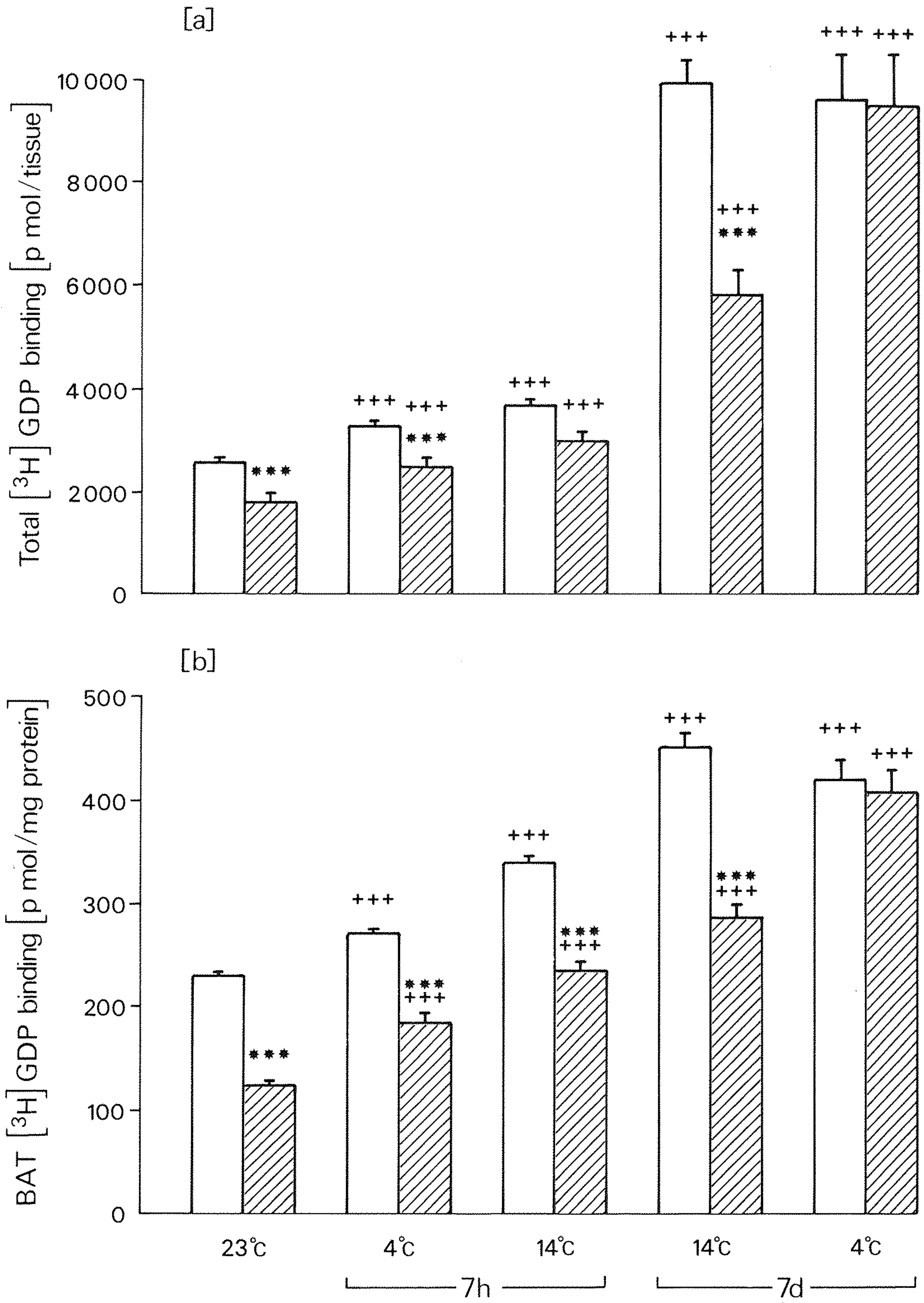


Rectal temperature
[°c]
37
36
35
34
33
32
BAT wet weight
[g]
1.0
0.8
0.6
0.4
0.2
0
BAT protein
[mg]
50
40
30
20
10
0
Total succ-cyt. c O-R activity
[µ mol/min]
5
4
3
2
1
0
7
158
7
158
Time [hrs]
Lean
Obese (fa/fa)

FIG. 3.3.2 EFFECT OF SHORT TERM EXPOSURE AND ACCLIMATION TO 14°C AND 4°C ON BAT MITOCHONDRIAL GDP BINDING IN LEAN AND OBESE (fa/fa) RATS

4-5 week old lean (open bars) and fa/fa (cross-hatched bars) rats were exposed for 7 hours (7h) or acclimated for 7 days (7d) at either 14°C or 4°C, as described in Section 2.6. Control rats were housed at 23°C. BAT mitochondria were prepared and GDP binding assays performed as described in Section 2.9 and 2.10, respectively; (a) total BAT GDP binding based on 100% mitochondrial recovery; (b) BAT mitochondrial GDP binding (pmol/mg protein).

Values represent means ± S.E.M. for 6 animals in each group.
*** $p < 0.001$, compared with equivalent lean group, ††† $p < 0.001$ compared with equivalent group at 23°C.


[a]
Total [3H] GDP binding [p mol/tissue]
10 000
8000
6000
4000
2000
0
[b]
BAT [3H] GDP binding [p mol/mg protein]
500
400
300
200
100
0
23℃
4℃
14℃
14℃
4℃
7h
7d

increased from the depressed control values up to those observed in lean animals housed at 4°C, and therefore reflected a larger response than that observed in the lean rats.

The response of lean rats to housing at 14°C was qualitatively and quantitatively similar to that of lean rats housed at 4°C, with the exception that the initial fall in rectal temperature (7 h) was less severe. Changes in BAT mitochondrial content and GDP binding were also similar in the two groups, although total BAT protein showed a relatively smaller increase in rats housed at 14°C. Obese (fa/fa) rats also responded to housing at 14°C with changes in BAT. However, in these rats, the increase in BAT mitochondrial GDP binding, per mg mitochondrial protein, was less pronounced (130% increase) after 7 days acclimatization, although mitochondrial content, as indicated by succ.cyt.c.O-R activity, showed a similar increase to that observed in lean rats housed at 4 and 14°C, and fa/fa rats housed at 4°C. In addition, the acute increase in BAT thermogenesis, together with the normal physical response to cold exposure, were clearly sufficient to raise rectal temperatures in fa/fa rats housed at 14°C for only 7 h.

The normal housing temperature of rats (23°C) is below their thermoneutral temperature (28-30°C), thus they would be under cold stress even at these temperatures. To investigate whether the reduced level of BAT mitochondrial GDP binding observed in fa/fa rats housed at 23°C was due to an impaired response to mild cold, rats were housed for 7 days at 30-32°C (Fig. 3.3.3 and Table 3.3.1). Under these conditions, rectal temperatures were elevated, and BAT mitochondrial GDP binding was reduced (by approximately 75%), in both lean and fa/fa rats, without any significant loss of mitochondrial succ.cyt.c.O-R activity. However, even at these thermoneutral temperatures, the BAT mitochondrial GDP binding of fa/fa rats was only 63% of that in lean rats. The decrease in total tissue binding in both lean and fa/fa rats, on housing at thermoneutral temperature, could be accounted for almost entirely by the reduction observed in BAT mitochondrial GDP binding per mg of mitochondrial protein. This suggests there is no significant loss of mitochondria, which is supported by the similar levels of succ.cyt.c.O-R activity in animals housed at the two temperatures. This observed reduction in binding in lean and fa/fa rats housed at 30-32°C may reflect either a masking of binding sites present in the BAT mitochondrial membrane, or a reduction in the concentration of 32K protein.

FIG. 3.3.3 *EFFECT OF ACCLIMATION TO 30-32°C ON ENERGY INTAKE, BODY WEIGHT AND RECTAL TEMPERATURE OF LEAN AND OBESE (fa/fa) RATS*

4 to 5 week old lean and fa/fa rats were housed individually and acclimated to either 23°C (open bars) or 30/32°C (cross-hatched bars) for 7 days, as described in Section 2.6. Food intake, body weight and rectal temperature were recorded daily throughout this period. Body weight gain is expressed as percentage increase for the 7 day period.

Values represent means ± S.E.M. for 8 animals in each group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with equivalent control group; †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent lean group.

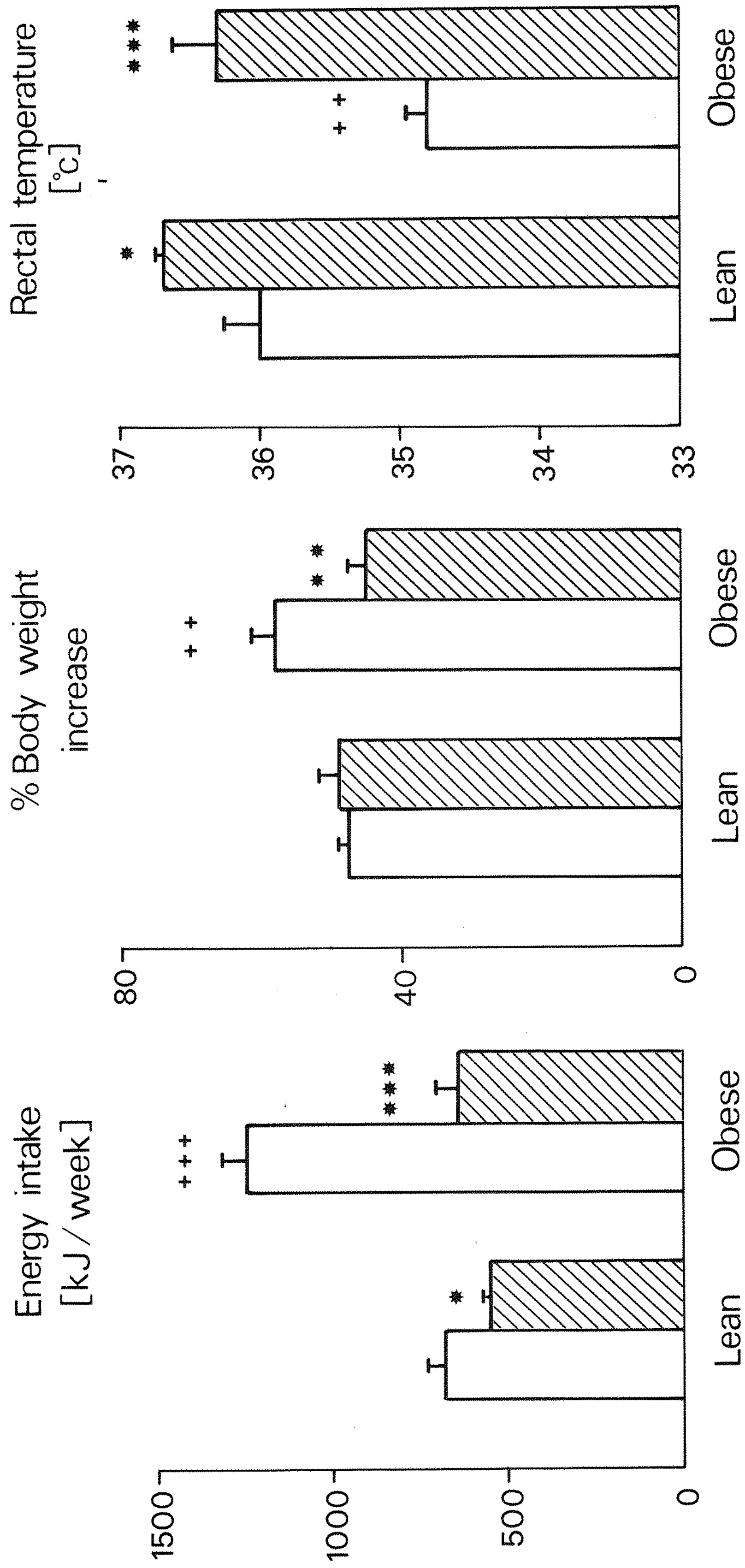

Energy intake
[kJ / week]
1500
1000
500
0
Lean
Obese
% Body weight
increase
80
40
0
Lean
Obese
Rectal temperature
[°C]
37
36
35
34
33
Lean
Obese

TABLE 3.3.1

EFFECT OF HOUSING AT 30-32°C ON BAT OF LEAN AND OBESE (fa/fa) RATS

	Lean		Obese	
	23°C	30/32°C	23°C	30/32°C
Brown adipose tissue:				
Wet wt (g)	0.26 ± 0.02	0.31 ± 0.02	0.48 ± 0.02†††	0.63 ± 0.04**, †††
Protein (mg)	35.6 ± 2.4	27.4 ± 2.6*	22.1 ± 0.2†††	21.0 ± 2.1
Succ.cyt.c.O-R activity (μmol/min/depot)	2.52 ± 0.22	2.27 ± 0.16	2.40 ± 0.21	2.05 ± 0.10
[^{3}H]-GDP binding (pmol/mg protein)	197.3 ± 9.0	51.8 ± 1.1***	115.0 ± 10.6†††	27.3 ± 4.5***, †††
Total [^{3}H]-GDP binding (pmol/tissue)	1611 ± 202	430 ± 94***	1027 ± 164†	159 ± 32***, ††

4 to 5 week old lean and obese (fa/fa) rats were housed individually and maintained at either 23°C or 30-32°C for 7 days. BAT protein, succ.cyt.c.O-R activity and mitochondrial GDP binding were assayed as described in Sections 2.11, 2.12 and 2.10 respectively. Total tissue binding was based on 100% mitochondrial recovery. Values represent means ± S.E.M. for 8 animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with control group at room temperature; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent lean group.

Five week old fa/fa rats, housed at 23°C, were hyperphagic (Table 3.3.2). During acclimation to 14°C food intake was increased to similar levels in both lean and fa/fa rats. Food intake in lean rats acclimated to 4°C was increased, but to a lesser extent than that of rats housed at 14°C. This difference in food intake may explain the apparently similar values of BAT mitochondrial GDP binding in rats acclimated to 14 or 4°C. In contrast, fa/fa rats housed at 4°C maintained a similar level of food intake to that of fa/fa rats housed at 14°C. Housing at thermoneutral temperatures had a small, but significant effect on the food intake of lean rats (Fig. 3.3.3), however, it abolished the characteristic hyperphagia of fa/fa rats.

These changes in food intake and BAT thermogenesis were associated with changes in weight gain. Since all animals were of similar weight at the start of the study, weight changes are expressed as percentage gain. Thus weight gain was increased in rats housed at 14°C but depressed at 4°C (Table 3.3.2) in both lean and fa/fa rats and also in fa/fa rats housed at 30-32°C (Fig. 3.3.3). Body composition changes were not measured in this study.

3.3.2 Effect of acclimation to 4°C on BAT mitochondrial GDP binding in lean and fa/fa rats, of 5 and 10 weeks of age

Levels of BAT mitochondrial GDP binding in 5 week old lean and fa/fa rats were increased, to similar values, after exposure to a cold environment (4°C) for 7 days (Fig. 3.3.4). Older (10 week old) lean rats showed a similar increase in mitochondrial GDP binding to young (5 week) lean rats. However, in the 10 week-old fa/fa rat, the increase in BAT mitochondrial GDP binding was markedly diminished compared with both younger fa/fa rats and lean rats. This apparent decrease in thermogenic response was reflected in the lower rectal temperatures of the 10 week-old fa/fa rats (35.50 ± 0.04^{o}C for cold acclimated young fa/fa group, compared with 34.50 ± 0.26^{o}C for 10 week old, cold acclimated fa/fa group).

TABLE 3.3.2

ENERGY INTAKE AND BODY WEIGHT CHANGE IN LEAN AND OBESE (fa/fa) RATS, ACCLIMATED TO VARIOUS HOUSING TEMPERATURES

		Housing Temperature		
		23°C	14°C	4°C
Energy intake (kJ/week)	Lean	516 ± 79	1596 ± 210†††	1183 ± 145†††
	Obese	1183 ± 81***	1645 ± 145††	1715 ± 152*,††
Body weight gain (% increase/week)	Lean	34.7 ± 2.3	48.6 ± 2.3†††	27.6 ± 11.2
	Obese	39.8 ± 2.0	48.7 ± 2.2††	24.2 ± 3.2†††

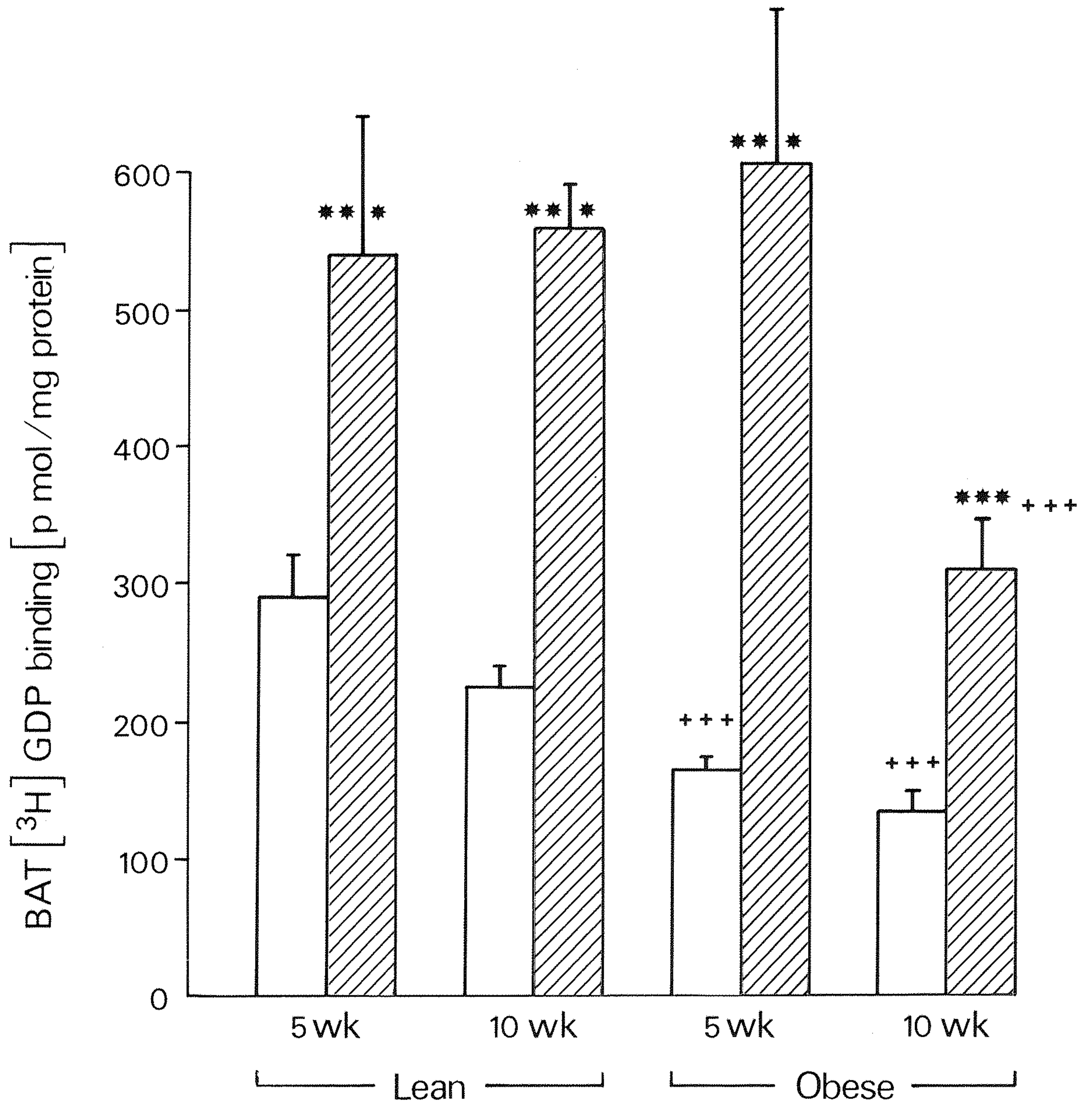
5 week old lean and fa/fa rats were acclimated to 14°C or 4°C for 7 days, as described in Section 2.6. Food intake and body weight was recorded daily throughout this period. Control animals were maintained at 23°C

Values represent means ± S.E.M. for 6 animals in each group.
* $p < 0.05$, *** $p < 0.001$, compared to equivalent lean group;
†† $p < 0.01$, ††† $p < 0.001$, compared to control group at 23°C.

FIG. 3.3.4 EFFECT OF ACCLIMATION TO 4°C ON BAT MITOCHONDRIAL GDP BINDING, IN YOUNG (5 WEEK OLD) AND ADULT (10 WEEK OLD) LEAN AND OBESE (fa/fa) RATS

Lean and fa/fa rats were housed individually at either 23°C (open bars) or 4°C (cross-hatched bars), for 7 days, as described in Section 2.6. BAT mitochondria were prepared and mitochondrial GDP binding assays performed, as described in Sections 2.9 and 2.10 respectively.

Values represent means ± S.E.M. for 6 animals in each group; *** $p < 0.001$, compared with equivalent control group at 23°C; ††† $p < 0.001$, compared with equivalent lean group.



BAT [^{3}H] GDP binding [p mol / mg protein]
600
500
400
300
200
100
0
5 wk
10 wk
5 wk
10 wk
Lean
Obese

3.3.3 *Effect of sucrose feeding in lean and fa/fa rats*

The thermogenic response to cold appeared to be relatively normal in the fa/fa rat, and at thermoneutrality (30°C) the fa/fa rat BAT mitochondrial GDP binding remained lower than in the lean animal. These observations suggested a failure of BAT to respond not to cold, but to diet.

The effect of overfeeding on BAT thermogenesis in the fa/fa rat was examined (Table 3.3.3). After 7 days of sucrose feeding, despite only a small increase in energy intake in lean rats fed with additional sucrose, BAT protein content was increased (however, there was no corresponding increase in succ.cyt.c.O-R activity), and there was an almost two-fold increase in BAT mitochondrial GDP binding. An increased rectal temperature was consistent with an increased thermogenesis in the lean rat fed with sucrose. The fa/fa control rat was clearly hyperphagic at 5 weeks of age. When additional sucrose was offered, a compensatory decrease in solid food intake was noticed, but the total energy intake was still increased. Inguinal fat pad weight was elevated in the sucrose fed fa/fa group, and no increase in either BAT protein content or total succ.cyt.c.O-R activity was observed. Despite the sucrose fed fa/fa rat being hyperphagic when compared to the lean sucrose fed rat, BAT mitochondrial GDP binding was unchanged from control values, indicating that the fa/fa rat was unable to exhibit the normal thermogenic response to overfeeding. This lack of thermogenic response was reflected in the low rectal temperature.

The effect of overfeeding was then examined in weanling (20 day old) lean and fa/fa rats, which had never previously been fed solid food. Table 3.3.4 shows that energy intake was doubled in the lean rat fed with additional sucrose, and that this extra intake resulted from an increase in chow, as well as the extra energy obtained from the sucrose solution. Despite this increased energy intake, the rate of body weight gain was reduced, and the weight of the inguinal fat pad was unchanged. BAT wet weight was unaltered in the sucrose-fed lean rats, although the protein content and succ.cyt.c.O-R activity were increased. The binding of GDP to BAT mitochondria was increased by more than 50% after sucrose feeding in the lean rats, and this increase in thermogenesis in response to diet, was reflected in an increased rectal temperature. Obese (fa/fa) rats consumed the same amount of

TABLE 3.3.3

EFFECT OF SUPPLEMENTARY SUCROSE FEEDING ON 5 WEEK OLD LEAN AND OBESE (fa/fa) RATS

	Lean		Obese (fa/fa)	
	Chow only	Additional sucrose	Chow only	Additional sucrose
Food intake:				
Chow (kJ/day)	99.7 ± 2.6	87.3 ± 1.7	133.0 ± 2.3†††	78.7 ± 1.7††
Sucrose (kJ/day)		27.0 ± 1.6		71.5 ± 2.0†††
Total (kJ/day)	99.7 ± 2.6	114.3 ± 3.6**	133.0 ± 2.3†††	150.0 ± 2.6***,†††
Initial body weight (g)	68.3 ± 2.5	66.1 ± 1.6	64.7 ± 1.2	66.6 ± 3.8
Body weight gain (% increase)	38.2 ± 3.2	40.9 ± 2.7	55.1 ± 3.2†††	48.6 ± 2.7
Inguinal fat pad weight (g)	0.54± 0.03	0.59± 0.05	1.54± 0.09†††	1.91± 0.06***,†††
Brown adipose tissue:				
Wet wt (g)	0.28± 0.02	0.29± 0.02	0.41± 0.02†††	0.45± 0.02†††
Protein (mg)	32.0 ± 2.2	40.0 ± 2.0*	24.2 ± 3.6	25.2 ± 2.8†††
Succ.cyt.c.O-R activity (μmol/min/depot)	2.06± 0.25	2.04± 0.13	1.76± 0.14	1.79± 0.15
[^{3}H]-GDP binding (pmol/mg protein)	224.9 ± 9.2	436.0 ± 7.8***	150.1 ± 7.6†††	141.2 ±17.7†††
Rectal temperature (^{O}C)	36.8 ± 0.2	37.5 ± 0.1**	35.4 ± 0.2†††	35.1 ± 0.1†††

Lean and obese (fa/fa) aged 4 weeks were housed individually and fed either a standard chow diet or chow plus a 35% (w/v) sucrose drinking solution, as described in Section 2.3, for 7 days. Body weights were recorded daily, throughout this period. On sacrifice inguinal fat pads were dissected out and weighed. BAT protein, succ.cyt.c.O-R activity and mitochondrial GDP binding were assayed as described in Section 2.11, 2.12, 2.9 and 2.10 respectively. Rectal temperatures were recorded after the 7 day feeding period.
Values represent means ± S.E.M. for 6 animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with control (non sucrose fed) group; †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent lean group.

TABLE 3.3.4

EFFECT OF SUPPLEMENTARY SUCROSE FEEDING ON WEANLING LEAN AND OBESE (fa fa) RATS

	Lean		Obese (fa/fa)	
	Chow only	Additional sucrose	Chow only	Additional sucrose
Food intake:				
Chow (kJ/day)	63.6 ± 2.4	88.0 ± 2.0***	75.7 ± 1.9†††	43.4 ± 1.6***,†††
Sucrose (kJ/day)		38.0 ± 2.4		38.7 ± 2.3
Total (kJ/day)	63.6 ± 2.4	126.0 ± 4.2***	75.7 ± 1.9†††	81.1 ± 3.2†††
Initial body weight (g)	48.6 ± 2.0	47.6 ± 2.2	48.2 ± 2.8	48.6 ± 2.7
Body weight gain (% increase/week)	54.2 ± 2.4	43.3 ± 1.5**	47.4 ± 1.6†	51.5 ± 2.6†
Inguinal fat pad wt (g)	0.50± 0.02	0.54± 0.03	1.46± 0.07†††	1.80± 0.09***,†††
Brown adipose tissue:				
Wet wt (g)	0.32± 0.02	0.30± 0.05	0.40± 0.03†	0.49± 0.01***,†††
Protein (mg)	32.0 ± 2.4	39.8 ± 2.2*	26.8 ± 3.6	24.6 ± 2.2†††
Succ.cyt.c.O-R activity (μmol/min/depot)	2.20± 0.01	2.50± 0.03***	1.86± 0.03†††	1.84± 0.06†††
[^{3}H]-GDP binding (pmol/mg protein)	266.0 ±13.6	411.0 ±13.2 ***	170.1 ± 8.6†††	172.2 ± 8.0†††
Rectal temperature (^{o}C)	36.2 ±0.1	37.0 ± 0.2**	35.1 ± 0.2†††	35.9 ± 0.3†

Lean and fa/fa weanling (20 days of age) rats were housed individually and fed either standard chow diet or chow plus a 35% (w/v) sucrose drinking solution, as described in Section 2.3, for 7 days. Body weights were recorded over this period. On sacrifice, inguinal fat pads were dissected out and weighed. BAT protein, succ.cyt.c.O-R activity, BAT mitochondria prepared and GDP binding measured as described in Section 2.11, 2.12, 2.9 and 2.10, respectively. Rectal temperatures were recorded after the 7 day feeding periods.
Values represent means ± S.E.M. for 6 animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with control group; † $p < 0.05$, ††† $p < 0.001$, compared with equivalent lean group.

sucrose as the lean rats but showed a compensatory decrease in chow intake, such that the level of energy intake in the chow fed and sucrose fed fa/fa groups were similar. Although the rate of weight gain was not elevated in the sucrose fed fa/fa rat, the weight of the inguinal fat pad was increased. In contrast with the lean rats, no significant changes were observed in BAT protein content, succ.cyt.c.O-R activity, BAT mitochondrial GDP binding, or rectal temperatures in fa/fa rats after sucrose feeding.

To examine whether DIT (like NST) could be induced acutely, lean and fa/fa rats were offered additional sucrose for 8 h (Table 3.3.5). Over this period, the lean rat increased its energy intake by 50% when offered sucrose and BAT mitochondrial GDP binding was increased by 60%. The increase in total binding per depot was due to an increase in binding per mg of mitochondrial protein, rather than any increase in mitochondrial population. The acute increase in BAT thermogenesis in the lean rat was reflected in an increased rectal temperature. Although the fa/fa rat increased its total energy intake by a smaller 25%, when compared to the lean rat, it was still hyperphagic and took 55% of its total intake in the form of sucrose. Despite this, the fa/fa rat showed no increase in BAT mitochondrial GDP binding, and this failure to induce a thermogenic response to overfeeding with sucrose was reflected in an unchanged rectal temperature.

3.3.4 Effect of cold and diet on serum T_3 and insulin concentration in lean and fa/fa rats

Since NST and DIT are known to be associated with an increase in serum thyroid hormone concentrations *(Himms-Hagen, 1983c)* and insulin levels are moderated by NST and DIT *(Rothwell & Stock, 1981a; Rothwell & Stock 1983b)*, the effects of cold acclimation and overfeeding with sucrose on serum T_3 and insulin concentrations, were examined in lean and fa/fa rats (Table 3.3.6). Both cold acclimation and overfeeding elevated T_3 levels in lean rats, when BAT mitochondrial GDP binding was also known to be elevated, indicating an increased thermogenesis in these animals. Serum insulin however did not increase significantly in the lean rat on cold acclimation or sucrose feeding, despite a marked hyperphagia. Serum T_3 levels were decreased in fa/fa rats when compared to the lean group but increased after cold acclimation to values observed in lean controls. This is consistent with a normal increase in BAT mitochondrial GDP binding observed in the

TABLE 3.3.5

THE SHORT TERM EFFECT OF OVERFEEDING WITH SUCROSE ON BAT IN LEAN AND OBESE (fa/fa) RATS

	Lean		Obese (fa/fa)	
	Chow only	Additional sucrose	Chow only	Additional sucrose
Food intake:				
Chow (kJ/8h)	112.3 ± 9.4	110.6 ± 7.8	142.2 ± 2.4††	98.8 ± 4.4***
Sucrose (kJ/8h)		58.0 ± 7.3		78.8 ± 2.2†
Total (kJ/8h)	112.3 ± 9.4	168.6 ± 8.2***	142.2 ± 2.3††	177.6 ± 5.2***
Brown adipose tissue:				
Protein (mg)	30.2 ± 5.7	28.3 ± 1.6	26.6 ± 1.2	25.4 ± 2.4
Succ.cyt.c.O-R activity (μmol/min/depot)	2.86 ± 0.35	2.99 ± 0.33	2.01 ± 0.30	2.12 ± 0.21
[^{3}H]-GDP binding (pmol/mg protein)	208.0 ± 8.0	332.0 ± 10.1***	136.0 ± 4.8†††	142.0 ± 10.2 †††
Total [^{3}H]-GDP binding (pmol/tissue)	3211 ± 208	5458 ± 276***	2001 ± 116	2110 ± 87†††
Rectal temperature (°C)	36.0 ± 0.1	37.1 ± 0.2***	35.3 ± 0.2†	35.1 ± 0.1†††

Lean and obese (fa/fa) rats aged 5-6 weeks were given either chow or chow plus an additional 35% (w/v) sucrose drinking solution, as described in Section 2.3 for 8h (from 24.00 until 08.00h) and energy intake was recorded during this period. BAT protein, succ.cyt.c.O-R activity and mitochondrial GDP binding were assayed as described in Section 2.11, 2.12, and 2.10 respectively. Total binding was calculated based on 100% mitochondrial recovery. Rectal temperatures were recorded after the 8h feeding period.
Values represent means ± S.E.M. for 6 animals in each group. *** $p < 0.001$, compared with control (non sucrose-fed) group; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$, compared with equivalent lean group.

TABLE 3.3.6

EFFECT OF COLD ACCLIMATION AND SUCROSE OVERFEEDING ON SERUM T_3 AND INSULIN CONCENTRATION IN LEAN AND OBESE (fa/fa) RATS

	T_3 (ng/ml)		Insulin (ng/ml)	
	Lean	Obese (fa/fa)	Lean	Obese (fa/fa)
Control (23°C)	0.92±0.03	0.71±0.05**	1.82±0.38	14.52±2.97***
Cold acclimated (4°C)	1.16±0.06††	1.04±0.05†††	2.40±0.26	12.85±2.94**
Sucrose-fed	1.35±0.06†††	0.79±0.01***	3.59±1.40	22.19±1.06***,†

4-5 week old lean and obese (fa/fa) rats were housed individually at either 23°C or 4°C for 7 days, as described in Section 2.6; or housed at 23°C and offered a 35% (w/v) sucrose solution in addition to chow, as described in Section 2.3, for 7 days. All rats offered additional sucrose consumed 10-20% more metabolisable energy than control groups, fed with chow only. Serum insulin and T_3 concentrations were measured as described in Section 2.14.

Values represent means ± S.E.M. for 8 animals in each group.

** $p < 0.01$, *** $p < 0.001$, compared to lean group; † $p < 0.05$,
†† $p < 0.01$, ††† $p < 0.001$, compared to appropriate control group at 23°C.

young fa/fa rat (see Fig. 3.3.4). However, after sucrose feeding no such increase in serum T_3 levels were observed. Serum insulin was elevated in the fa/fa rat when compared to the lean rat, so displaying the characteristic hyperinsulinemia seen after weaning in this mutant. After cold acclimation, when BAT thermogenesis was increased, insulin levels of the fa/fa rat were unchanged despite a large increase in energy intake (see Table 3.3.2). However, insulin levels were elevated on sucrose feeding, potentiating the hyperinsulinemic condition.

3.3.5 Effect of sucrose feeding in adrenalectomised lean and fa/fa rats

Adrenalectomy restored the depressed level of BAT mitochondrial GDP binding, observed in fa/fa rats, to lean values (Section 3.2), and restored energetic efficiency to normal *(Marchington et al., 1983)*. It was of interest therefore to examine whether the increased thermogenesis seen in adrenalectomised fa/fa rats, was due to an improvement in the ability to regulate thermogenesis in response to dietary intake. Table 3.3.7 shows that after adrenalectomy, the food intake was similar in both lean and fa/fa rats, and that both groups significantly increased their daily energy intake when a sucrose drinking solution was available. The percentage increase in body weight was higher in adrenalectomised lean rats fed with sucrose when compared with the chow fed controls. The adrenalectomised fa/fa rat had an increased rate of body weight gain compared to the lean rat, and this was not significantly increased on sucrose feeding. The inguinal fat pad weights, however, were significantly increased in both groups of sucrose fed, adrenalectomised rats. BAT wet weight, total protein content and succ.cyt.c.O-R activity were all increased in adrenalectomised lean rats after sucrose feeding; however, the increase in BAT mitochondrial GDP binding per mg of mitochondrial protein was smaller than that observed in sucrose fed, intact lean rats (Table 3.3.3), being increased by only 17% although total tissue binding was markedly elevated (increased by 46%), as a result of an increased mitochondrial population. An increase in BAT protein content and succ.cyt.c.O-R activity was also observed in the adrenalectomised fa/fa rat fed with sucrose. The adrenalectomised fa/fa rat showed a 36% increase in BAT mitochondrial GDP binding after 7 days of sucrose feeding, and a two-fold increase in total tissue binding, also indicating a large increase in mitochondrial population.

TABLE 3.3.7

EFFECT OF FEEDING ADDITIONAL SUCROSE ON FOOD INTAKE, WEIGHT GAIN AND BAT OF ADRENALECTOMISED LEAN AND OBESE (fa/fa) RATS

	Lean		Obese (fa/fa)	
	Chow only	Additional sucrose	Chow only	Additional sucrose
Food intake:				
Chow (kJ/day)	73.0 ± 0.3	93.6 ± 2.4***	72.0 ± 0.7	73.0 ± 3.2†††
Sucrose (kJ/day)	-	19.4 ± 3.5	-	31.5 ± 5.4
Total (kJ/day)	73.0 ± 0.3	113.0 ± 5.0***	72.0 ± 0.7	104.5 ± 5.6***
Initial body weight (g)	92.8 ± 2.1	92.4 ± 4.4	89.6 ± 8.9	84.9 ± 13.0
Body weight gain (% increase)	8.5 ± 2.2	24.8 ± 2.0***	14.9 ± 3.8	20.2 ± 5.3
Inguinal fat pad weight (g)	0.40 ± 0.05	0.43 ± 0.10	1.55 ± 0.17†††	1.67 ± 0.42†††
Rectal temperature (°C)	35.6 ± 0.1	36.6 ± 0.1***	35.3 ± 0.1	36.3 ± 0.2***
Brown adipose tissue:				
Wet wt (g)	0.25 ± 0.02	0.39 ± 0.03***	0.37 ± 0.02†††	0.39 ± 0.06
Protein (mg)	32.0 ± 1.4	42.2 ± 1.8***	28.7 ± 2.4	44.5 ± 1.8***
Succ.cyt.c.O-R activity (μmol/min/depot)	1.62 ± 0.15	2.22 ± 0.20*	1.54 ± 0.12	2.17 ± 0.15**
[^{3}H]-GDP binding (pmol/mg protein)	276.0 ± 12.2	324.0 ± 13.0 *	240.0 ± 20.0	327.0 ± 18.9 **
Total [^{3}H]-GDP binding (pmol/tissue)	2101 ± 96	3071 ± 202***	1846 ± 113	3840 ± 253***,†

4 week old lean and obese (fa/fa) rats were adrenalectomised and maintained as described in Section 2.7. After recovery, rats were fed either standard chow diet, or chow plus a 0.35% (w/v) sucrose drinking solution, as described in Section 2.3, for 7 days. Food intake and body weight was recorded throughout this period. Rectal temperatures were taken immediately before sacrifice. BAT protein, succ.cyt.c.O-R activity and mitochondrial GDP binding were assayed as described in Section 2.11, 2.12 and 2.10 respectively.
Values represent means ± S.E.M. for 6 animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with control (non sucrose fed) group; † $p < 0.05$, ††† $p < 0.001$, compared with equivalent lean group.

3.3.6 Discussion

Young (5-6 week old) fa/fa rats housed at normal environmental temperatures show a reduced BAT mitochondrial GDP binding, despite an increased food intake, when compared to the lean rat, indicative of a reduced BAT thermogenesis. Housing at or above thermoneutral temperature (30-32°C) abolishes the requirement for the adaptive cold induced thermogenesis, but young fa/fa rats still showed a reduced BAT thermogenesis when compared to lean rats. These results are similar to those shown by *Triandafillou & Himms-Hagen (1983)*, and suggest a failure of BAT to respond not to cold but to diet.

Overfeeding was induced by allowing animals access to sucrose solutions. Sucrose feeding is a relatively simple manipulation of the diet, that can easily be quantified. In addition, sucrose feeding is known to enhance sympathetic activity and to increase the turnover of noradrenaline in BAT *(Young & Landsberg, 1977b)*; this increase in sympathetic activity is thought to be principally responsible for the elevated DIT in these animals *(Landsberg & Young, 1981; Rothwell & Stock, 1980a)*.

Weanling lean rats showed an increase in total energy intake when offered additional sucrose, but did not gain a significant amount of inguinal adipose tissue. These results are similar to those found by others *(Kanarek & Marks-Kaufmann, 1979)*, who showed a significant increase in energy intake when weanling rats were given sucrose, but an increase in body fat was not observed until the rats were over 7 weeks of age. Since weanling lean rats select to maintain a constant protein intake in relation to total energy *(Musten et al., 1974)*, the hyperphagia observed on sucrose feeding, may be interpreted as an attempt to keep the ratio of protein intake to total energy intake constant. Similarly, lean rats fed a low protein diet, become hyperphagic in an attempt to maintain their protein intake *(Musten et al., 1974; Castonguay et al., 1982)*. Obese (fa/fa) rats do not appear to regulate protein in this way *(Castonguay et al., 1982)* and when offered a diet consisting of three separate macronutrient sources, i.e. cornstarch, casein and cornoil, fa/fa rats selected to increase the fat component and reduced their protein intake.

The present results demonstrate that weanling fa/fa rats fed with additional sucrose, showed little change in total energy intake.

However, almost 50% of the total intake was from the sucrose component and it resulted in an increased accumulation of body fat. This compensatory reduction in chow intake and increased accumulation of body fat in the fa/fa rat resembles similar observations in older lean rats, where there was also an increase in energetic efficiency when sucrose was given in the diet *(Kanarek & Orthen-Gambill, 1982; Hallfrisch et al., 1978, 1981)*. Sucrose feeding markedly elevated plasma insulin levels in the young fa/fa rat, which may lead to over-stimulation of adipose tissue and increased lipid accumulation.

In both weanling and 5 week old lean rats, chronic sucrose feeding induced hyperphagia, and the increase in BAT protein content and total succ.cyt.c.O-R activity (indicating mitochondrial proliferation) resembled the changes reported in cold acclimated lean rats *(Desautels & Himms-Hagen, 1979)*. The propoposal that DIT and NST have a similar mechanism of heat production in BAT *(Rothwell & Stock, 1980a)* and can be monitored by an increase in purine nucleotide binding to BAT mitochondria *(Himms-Hagen et al., 1981)*, is substantiated in the present work. DIT also appears to be able to evoke an acute response in BAT mitochondrial GDP binding in lean rats, similar to the 'unmasking' effect observed in the acutely cold exposed rat *(Desautels et al., 1978)*.

In contrast to lean rats, fa/fa rats failed to respond to either acute or chronic sucrose overfeeding, with any increase in BAT mito-chondrial GDP binding, BAT growth, or rectal temperature. Since the fa/fa rat also showed a large increase in inguinal fat deposition, these results suggested that the fa/fa rat failed to respond to sucrose feeding with an increased BAT thermogenesis, and therefore failed to dissipate the extra energy consumed as heat. This defect may contribute to the increased energetic efficiency seen in the fa/fa rat and the resulting obesity.

Recent work has also shown *(Rothwell et al., 1982a, 1983b)* that fa/fa rats show a reduced metabolic response to a normal meal when compared to the lean rat. The failure of the fa/fa rat to respond to food, is supported by the observation that 10-12 week old fa/fa rats exhibited a lower oxygen consumption than lean rats *(Kaplan, 1979b)*, at a thermoneutral temperature ($30^{o}C$), when NST is not required.

Similarly, it has been shown that fa/fa rats fail to respond to 'cafeteria' overfeeding *(Triandafillou & Himms-Hagen, 1983)* with an increase in BAT mitochondrial GDP binding. *Young et al. (1980)*

reported that, unlike lean rats, fa/fa animals fail to exhibit DIT when fed a low protein, high carbohydrate diet, suggesting that the high efficiency and excessive weight gain in the fa/fa rat is partly due to an inability to alter energy expenditure, in response to changes in food intake. In the present experiments, the compensatory reduction in chow, seen (more markedly) in the fa/fa rat on sucrose feeding, makes the diet essentially low in protein. Low protein diets are known to stimulate BAT thermogenesis in lean animals *(Rothwell et al., 1982c)*. The failure to exhibit DIT in the fa/fa rat is also seen in the rat made obese by VMH lesioning *(Seydoux et al.,* 1982). This animal also exhibits a reduced BAT mitochondrial GDP binding, but in this case, it is reversed by food restriction. The lesioned animal also exhibits obesity when hyperphagia is absent *(Han, 1967, Bernardis & Goldman, 1976, VanderTuig et al., 1982)*, due to an increased efficiency of energy utilisation and a reduced energy expenditure.

A reduced activity of BAT thermogenesis in response to diet, has also been observed in the ob/ob mouse and the db/db mouse *(Thurlby & Trayhurn, 1980; Trayhurn & James, 1978; Trayhurn & Fuller, 1980; Trayhurn et al., 1982)*; however, unlike the fa/fa rat, these animals show a markedly reduced cold tolerance, and fail to increase BAT mitochondrial GDP binding on cold exposure *(Trayhurn & James, 1978; Himms-Hagen & Desautels, 1978; Hogan & Himms-Hagen, 1980)*, which is also indicative of a defective BAT thermogenesis.

The lack of diet related stimulation of BAT in the fa/fa rat may lead to a gradual involution of the tissue, which would contribute towards the age-dependent loss of non-shivering thermogenic capacity.

Hyperinsulinemia is a characteristic of the weaned fa/fa rat *(Bray & York, 1979; Godbole et al., 1978)* and is associated with hyperphagia, increased lipogenesis *(Godbole & York, 1978)*, and increased energetic efficiency *(Chan & Stern, 1981, 1982; Chan et al., 1982)*. However, there is evidence to suggest a higher insulin requirement in rats exhibiting DIT and NST *(Agius et al., 1981; Portet, 1981)*. When serum insulin concentration is low, as in the case of the streptozotocin-diabetic rat, 'cafeteria' feeding failed to produce the normal thermogenic response *(Rothwell & Stock, 1981a)*, suggesting an insulin requirement for BAT thermogenesis. In the present experiment sucrose feeding resulted in hyperphagia in lean and fa/fa rats, but despite this only

the fa/fa rats showed an increase in serum insulin, suggesting that in animals capable of resisting obesity by switching on BAT thermogenesis, an increasing glucose utilisation *(Smith, 1978; Portet, 1981; McCormack, 1982)* may maintain insulin homeostasis.

The present results show that at lower environmental temperatures, the fa/fa rat activates BAT thermogenesis and can survive at temperatures as low as 4°C. The first 24 hours of cold exposure is not thought to be accompanied by an increase in the synthesis of the 32K protein in BAT mitochondria *(Desautels et al., 1978; Desautels & Himms-Hagen, 1979)*. The acute increase in GDP binding is thought to involve an 'unmasking' of existing binding sites present in the mitochondrial membrane. Since lean and fa/fa rats increased BAT mitochondrial GDP binding by the same proportion when acutely exposed to either 14°C or 4°C, the unmasking response in the fa/fa rat appears to be functionally normal.

Chronic exposure to 4°C produces changes in fa/fa BAT thermogenesis quantitatively similar to the lean rat, reflecting a completely normal adaptive response. This normal response to cold is substantiated by others *(Armitage et al., 1981a,b)*, who showed a completely normal increase in energy expenditure with decreasing environmental temperature in fa/fa rats. The above workers also confirmed that fa/fa rats did not appear to suffer at temperatures down to 5°C during the 10 day duration of exposure. However, the present findings do suggest that the response of fa/fa rats, on chronic exposure to more moderate temperatures (14°C), is reduced in comparison to lean rats.

In comparative experiments with the white rat *(Pospisilova & Jansky, 1976)*, the specific activity of mitochondrial cytochrome oxidase (when related to wet weight of BAT) increases disproportionately with decreasing temperature, i.e. the greatest increase of cytochrome oxidase activity (in BAT homogenates) is seen at mildly reduced temperatures. Lowering the adaptational temperature further does not significantly increase the specific activity of the enzyme. Total activity, however, increases with the growth of BAT seen on cold acclimation. The present experiments show a similar trend in succ.cyt.c.O-R activity, for the lean rats. The BAT mitochondrial succ.cyt.c.O-R activity of the fa/fa rat responds identically with that of the lean rat to decreasing environmental temperature, suggesting that there is no defect in the

activation of the mitochondrial oxidative enzymes. However, since the increase in BAT mitochondrial GDP binding is not as high as expected when the fa/fa rat is acclimated to 14°C (despite a normal increase in mitochondrial content, as indicated by succ.cyt.c.O-R activity), it appears that there is a defect in either the synthesis or 'unmasking' of the 32K protein, at this temperature, which is not apparent at 4°C.

BAT thermogenesis appears to be age dependent in fa/fa rats, being normal at 5 weeks, but depressed by 10 weeks of age. These results agree with others *(Trayhurn et al., 1976; Triandafillou & Himms-Hagen, 1983)*, which have shown a marked hypothermia on exposure to cold in older fa/fa rats, along with a defective BAT thermogenesis. That fa/fa rats show a normal response to cold, but not to diet, may explain the more moderate increase in BAT mitochondrial GDP binding seen in fa/fa rats when exposed to 14°C. At this temperature both lean and fa/fa groups exhibit hyperphagia, and as the lean rat would be responding to both a dietary increase and to cold, this would result in an additive effect on BAT thermogenesis. Such a synergistic effect has been noticed in 'cafeteria' fed rats, which exhibit improved cold tolerance *(Rothwell & Stock, 1980a)*, and in cold acclimated rats which are 'cafeteria' fed *(Rothwell et al., 1982a)*. The present results however, show that at 4°C, the food intake of the lean rat is reduced when compared to that of the fa/fa rat; so the dietary induced component of thermogenesis may be modified, leading to the similar levels of BAT mitochondrial GDP binding observed in lean and fa/fa rats.

The present experiments indicate that hyperinsulinemia in cold acclimated fa/fa rats does not interfere with the normal functional capacity of BAT thermogenesis. The requirement for insulin in NST can be demonstrated in streptozotocin-diabetic rats *(Seydoux et al., 1983)*, in which cold tolerance and noradrenaline stimulated metabolic rate are reduced. The present experiments demonstrate that, despite a large increase in energy intake on cold acclimation in lean and fa/fa rats, the insulin levels remain unchanged, suggesting that the increase in NST in the fa/fa rat may be accompanied by an improved insulin status. The relatively normal response to cold in the fa/fa rat is similar to that of the VMH lesioned rat, in which a decreased BAT mitochondrial GDP binding *(Seydoux et al., 1982)*, and a reduced turnover of nor-adrenaline *(VanderTuig et al., 1982)*, are reversed on cold acclimation.

As exposure to the cold is known to increase sympathetic activity *(Young & Landsberg, 1979)*, the defect seen in the VMH lesioned rat is suggested to be a consequence of an absence of stimulation of sympathetic activity by diet rather than by cold.

Adrenalectomy (discussed in Section 3.2) was shown to normalise both BAT mitochondrial GDP binding and energetic efficiency *(Marchington et al., 1983)* in fa/fa rats. The present study demonstrated that adrenalectomy normalised the thermogenic capacity of BAT and restored the adaptive response to sucrose overfeeding. This increased thermogenesis was reflected in an increased rectal temperature. In confirmation of these results, *Marchington et al.,(1983)* demonstrated that adrenalectomised fa/fa rats displayed similar increases in oxygen consumption, after a 50kJ Complan meal, as did lean rats, therefore showing a normal response to food. This was in contrast to the attenuated increase observed in intact fa/fa rats.

The mechanism by which adrenalectomy restores BAT function of fa/fa rats is not clear. Corticosterone must be involved, either directly or indirectly, in the suppression of BAT mitochondrial GDP binding, since when corticosterone was replaced to adrenalectomised fa/fa rats, GDP binding was depressed back towards levels observed in intact fa/fa animals. Also, intact lean rats given corticosterone show a reduction in BAT mitochondrial GDP binding (Section 3.2). These results suggest that the initial increase in energetic efficiency, which leads to obesity in fa/fa rats, may result from a glucocorticoid-dependent inhibition of diet related BAT thermogenesis. Such a role for glucocorticoids has been supported by the report of depressed BAT mitochondrial GDP binding in corticosterone treated mice *(Galpin et al., 1983)*, and also by studies on the BAT thermogenic response of adult rats to cafeteria feeding, which was reduced when compared to younger rats, but was restored after adrenalectomy *(Rothwell et al., 1984)*.

If corticosterone does inhibit BAT thermogenesis, it is unlikely to inhibit BAT directly (although BAT does possess corticosterone receptors *(Feldman, 1978)*), since the tissue shows a relatively normal increase in mitochondrial GDP binding in response to cold. Similarly, corticosterone-treated mice show a normal acute response to cold *(Galpin et al., 1983)*. Corticosterone is also required, in permissive amounts, for the normal thermogenic response to cold exposure *(Fellenz et al., 1982)*; and since glucocorticoid levels increase, at least

transiently, during cold exposure *(Deavers & Musacchia, 1979)*, it appears unlikely that corticosterone would inhibit diet-induced BAT thermogenesis directly. Corticosterone may be inhibiting the diet related sympathetic activation of thermogenesis in BAT of fa/fa rats.

The SNS is thought to be the principal regulator of BAT thermogenesis. Noradrenaline turnover, an index of sympathetic activity, has been shown to be low in fa/fa rats housed at normal laboratory temperatures, when compared to lean rats *(Levin et al., 1981a, b; York & Marchington, 1984)*. Noradrenaline content of BAT in fa/fa rats is lower than in lean animals *(Levin et al., 1981a; Triandafillou & Himms-Hagen, 1983; York & Marchington, unpublished observations)*. The ability of fa/fa rats to withstand cold environments, is indicative of a normal functional capability; a normal cold induced increase in BAT noradrenaline turnover supports these findings *(York & Marchington, unpublished observations)*. However, on sucrose over-feeding, when noradrenaline turnover was increased in BAT of lean rats, there was no such increase in the BAT of young fa/fa rats *(York & Marchington, unpublished observations)*. The reduced capacity for NST in older fa/fa rats may be due to gradual involution of BAT, due to lack of sympathetic stimulation. This reasoning is in keeping with the observation of a reduced blood flow to BAT in older fa/fa rats in comparison with lean animals *(Wickler et al., 1982)*. BAT of older fa/fa rats also has reduced noradrenaline content and turnover *(Triandafillou & Himms-Hagen, 1983; Levin et al., 1981a)* in comparison with younger fa/fa rats.

Thyroid hormones are also required in permissive amounts for normal BAT thermogenesis, and are thought to act by regulating the sensitivity to noradrenaline stimulation *(Leblanc & Villemaire, 1970; Fellenz et al., 1982; Triandafillou et al., 1982)*. The present results show that serum triodothyronine (T_3), the metabolically active form of thyroid hormone *(Hardeveld et al., 1979a,b; Scammel et al., 1981)*, was decreased in 5 week old fa/fa rats. This report differs from some previous reports on older fa/fa rats, in which serum T_3 was normal, but thyroxine levels were decreased *(Martin et al., 1978; Autissier et al., 1980)*. The significance of the fall in serum T_3 to the impaired BAT function is problematical, since fa/fa rats show a relatively normal response to cold exposure and thyroid hormones are only required in permissive

amounts for BAT thermogenesis *(Fellenz et al., 1982; Sellers et al., 1974)*.

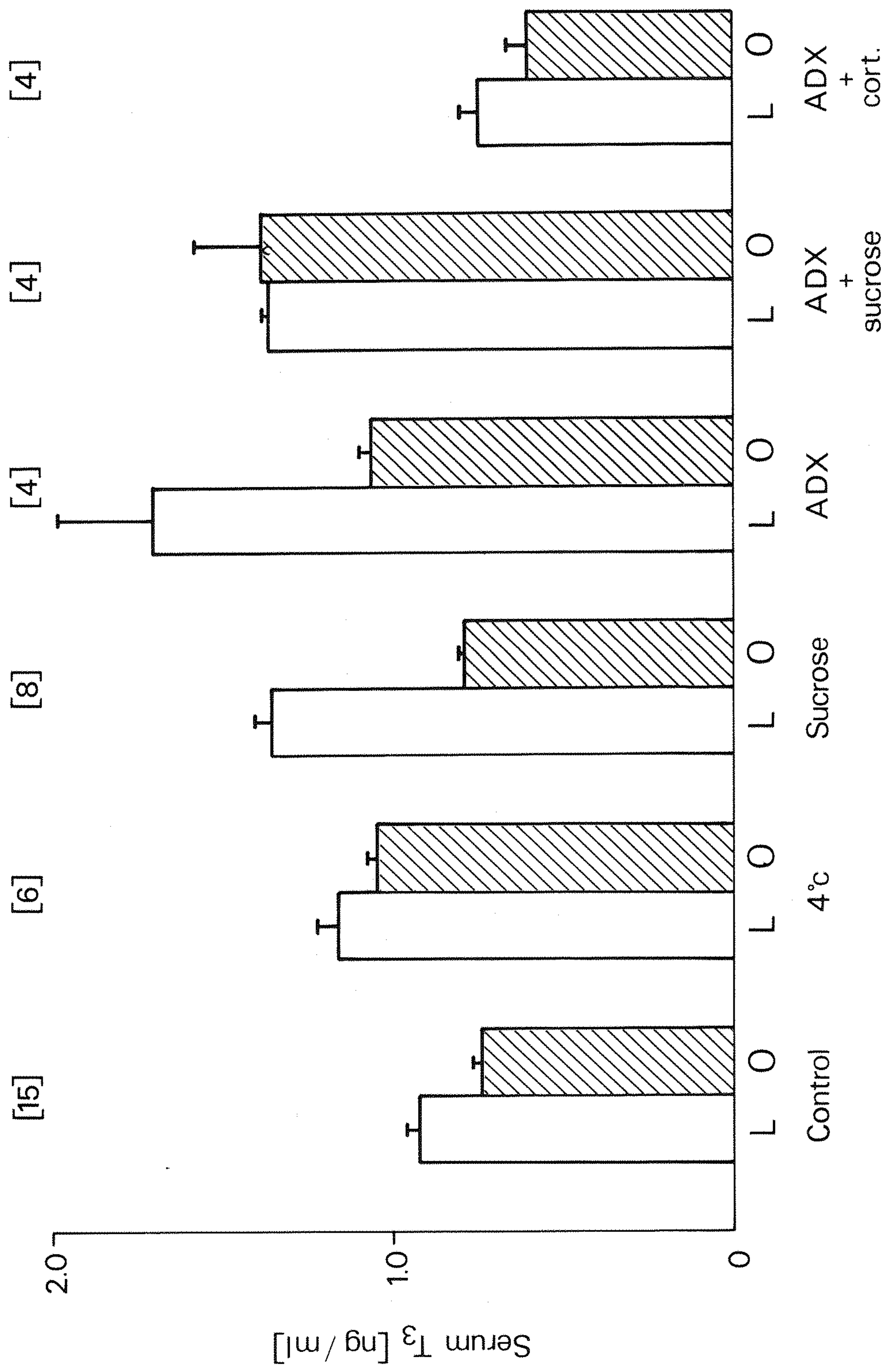
The serum T_3 levels may be related to the sympathetic tone of BAT, as is shown by the data re-presented in Fig. 3.3.5. Levels of serum T_3 are high following adrenalectomy in fa/fa rats, when noradrenaline turnover was increased *(York & Marchington, 1984)*. Serum T_3 levels were increased on cold acclimation of fa/fa rats and after sucrose feeding of adrenalectomised fa/fa rats. Under these conditions, BAT thermogenesis, noradrenaline turnover *(York & Marchington, unpublished observations)*, and energetic efficiency *(Marchington et al., 1983)* were returned to levels observed in lean rats. Neither these parameters, nor serum T_3 levels were effected when intact fa/fa rats were sucrose fed. Corticosterone replacement to adrenalectomised rats, which is associated with a reduced BAT thermogenesis (Section 3.2) and noradrenaline turnover *(York & Marchington, 1984)*, also resulted in a reduction of serum T_3 levels. The coincidence of increased serum T_3 levels with normalised mitochondrial function, and increased sympathetic activity of BAT, suggests that the sympathetically controlled deiodination of T_4 to T_3 by 5'-deiodinase *(Silva & Larson, 1983)*, which has a high activity in BAT *(Leonard et al., 1983)*, may be important in regulating serum T_3 levels. Increasing T_3 levels in BAT on sympathetic stimulation, may lead to an increased sensitivity of BAT to noradrenaline, a phenomenon noticed in hyperthyroid rats *(Hsieh et al., 1966)*.

Although the fa/fa rat was able to show an acute response to cold exposure, BAT mitochondrial GDP binding did not reach the level observed in the lean rat. This shortfall could be due, either to a reduced sympathetic stimulation, or to a lower capacity for GDP binding, (perhaps due to a lower concentration of the 32K protein). Attempts to measure the relative amount of the 32K protein in mitochondria, by SDS PAGE, have not detected any differences between lean and fa/fa rats *(Triandafillou & Himms-Hagen, 1983)*. However, preliminary studies using a radioimmunoassay technique *(Holt, York & Cleeter, unpublished observations)* to measure the quantity of the 32K protein in BAT mitochondria, have suggested that the concentration of the 32K protein is reduced in fa/fa rats compared to lean, and that this difference is corrected after adrenalectomy. Antibody for these experiments was kindly provided by Dr. M. Ashwell (The Dunn Research Institute, Cambridge).

FIG. 3.3.5 EFFECT OF COLD ACCLIMATION, SUCROSE FEEDING, ADRENALECTOMY AND CORTICOSTERONE ON SERUM T_3 LEVELS IN LEAN AND OBESE (fa/fa) RATS

4-6 week old lean (open bars) and fa/fa (cross-hatched bars) rats were either 'untreated' (control group); acclimated to $4^{o}C$; fed an additional (35%) sucrose drinking solution; adrenalectomised; adrenalectomised and fed with additional (35%) sucrose solution; or adrenalectomised and injected daily with corticosterone (1 mg/100g/day). All treatments were continued for 7 days. Serum T_3 concentrations were determined as described in Section 2.14.5. Values represent means ± S.E.M. for number of animals shown in parentheses.

This data has been re-presented from earlier sections (with the omission of statistical analysis).



Serum T_3 [ng / ml]
2.0
1.0
0
[15]
[6]
[8]
[4]
[4]
[4]
L O
L O
L O
L O
L O
L O
Control
4°c
Sucrose
ADX
ADX + sucrose
ADX + cort.

SECTION 3.4 Sympathetic Regulation of BAT in the Obese (fa/fa) Rat

Previous experiments have shown that the thermogenic response to diet-related signals was absent in fa/fa rats. However, the BAT thermogenic response to environmental temperature remained intact. Adrenalectomy prevented the development of obesity, and was associated with a restoration of diet-related BAT mitochondrial thermogenic function. These changes were prevented by corticosterone replacement. As the SNS has been identified as the principal regulator of BAT function *(Foster & Frydman, 1978; Rothwell & Stock, 1979a; Himms-Hagen et al., 1981)*, it was possible that this corticosterone-dependent loss of diet-related function in fa/fa rats, resulted from inhibition of the sympathetic stimulation of the tissue. Preliminary experiments, designed to investigate this hypothesis, are described in this section. The results suggest that adrenal glucocorticoids may inhibit the diet-related sympathetic stimulation of BAT in the fa/fa rat.

3.4.1 Effect of noradrenaline on BAT of lean and fa/fa rats

Chronic treatment with noradrenaline (30 μg/100g body wt/d for 7 d) decreased the rate of body weight gain in both lean and fa/fa rats, although food intake remained unaltered from control values (Table 3.4.1). Noradrenaline increased BAT protein content and, to a small extent, BAT mitochondrial GDP binding (per mg of mitochondrial protein) in lean rats. However, total tissue GDP binding was increased by over 80%, reflecting a large increase in mitochondrial population (also indicated by the increase in BAT succ.cyt.c. O-R activity). The fa/fa rat treated with noradrenaline also showed an increased BAT protein content. GDP binding, in the fa/fa rat (per mg of mitochondrial protein), was increased by 83% and total tissue binding by 95% to reach values observed in the lean rat. The increase in mitochondrial population of BAT in the fa/fa rat, treated chronically with noradrenaline, was also indicated by the increase in succ.cyt.c.O-R activity.

The time course of the response of BAT mitochondrial GDP binding to a single injection of noradrenaline (50 μg/100g body weight) was examined (Fig. 3.4.1). GDP binding rose sharply in both lean and fa/fa rats after noradrenaline injection, and reached maximum values

TABLE 3.4.1

EFFECT OF CHRONIC NORADRENALINE TREATMENT ON LEAN AND OBESE (fa/fa) RATS

	Control		Noradrenaline	
	Lean	Obese (fa/fa)	Lean	Obese (fa/fa)
Food intake (kJ/day)	114.0 ± 6.2	164.2 ± 5.6***	112.2 ± 4.8	141.3 ± 8.8*
Initial body weight (g)	110.0 ± 2.1	121.0 ± 3.6	109.6 ± 1.8	118.0 ± 2.7
Body weight gain (% increase /week)	32.0 ± 2.0	36.0 ± 2.2	22.0 ± 2.6†	21.1 ± 1.8†††
Brown adipose tissue:				
Protein (mg)	27.0 ± 1.2	22.2 ± 1.4*	33.2 ± 1.4††	26.6 ± 1.3*
Succ.cyt.c.O-R activity (pmol/min/depot)	2.0 ± 0.2	1.8 ± 0.2	3.0 ± 0.3†	2.5 ± 0.2†
[^{3}H]-GDP binding (pmol/mg protein)	220.0 ± 10.6	120.0 ± 15.6***	266.0 ± 12.2†	220.0 ± 16.6††
Total [^{3}H]-GDP binding (pmol/depot)	2000 ± 146	1280 ± 176*	3633 ± 194†††	2800 ± 190*,†††

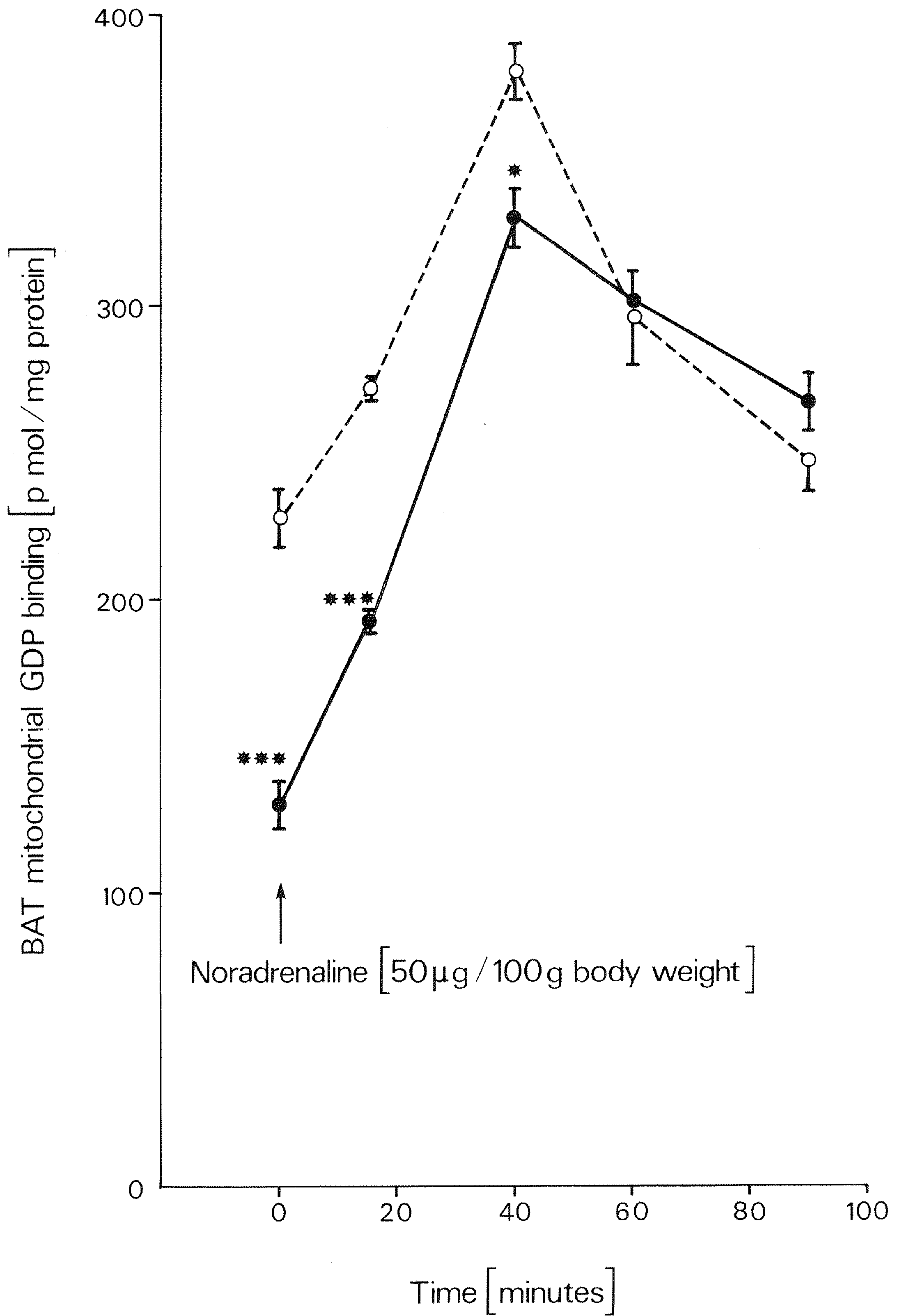
6 week old lean and obese (fa/fa) rats were injected twice daily (at 09.00 and 17.00h) with either noradrenaline (30 μg/100g body weight) or saline vehicle, as described in Section 2.8.5, for 7 days. Rats were not injected on the morning of sacrifice. BAT protein content, succ.cyt.c.O-R activity and GDP binding were assayed as described in Section 2.11, 2.12 and 2.10 respectively. Body weights were measured daily during the experiment. Total tissue binding was calculated based on 100% mitochondrial recovery.

Values represent means ± S.E.M. for 5 animals in each group. * $p < 0.05$, *** $p < 0.001$, compared to equivalent lean group; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$, compared to control group.

FIG. 3.4.1 *TIME COURSE OF THE EFFECT OF NORADRENALINE TREATMENT ON BAT MITOCHONDRIAL [^{3}H]-GDP BINDING OF LEAN AND OBESE (fa/fa) RATS*

5-6 week old lean (O) and fa/fa (●) rats were injected (s.c.) with a single dose of noradrenaline or vehicle as described in Section 2.8.5, at zero time, and sacrificed at the times indicated. BAT mitochondrial GDP binding assays were performed as described in Section 2.10.

Values represent means ± S.E.M. for 4 animals at each time point, * $p < 0.05$, *** $p < 0.001$, compared with lean group at each time point.


BAT mitochondrial GDP binding [p mol / mg protein]
400
300
200
100
0
***
***
*
Noradrenaline [50 µg / 100 g body weight]
0
20
40
60
80
100
Time [minutes]

after 40 minutes. Subsequently, mitochondrial GDP binding fell rapidly in the lean rat, and approached the initial values by 90 minutes. In contrast, mitochondrial GDP binding in the fa/fa rat treated with noradrenaline, declined only slowly from its peak value, such that 90 minutes later, GDP binding was still two-fold higher than the initial fa/fa rat value. Fig. 3.4.2 shows these results expressed as percentage increase in BAT mitochondrial GDP binding. The noradrenaline treated lean rat gave a maximal 67% increase in GDP binding after 40 minutes, whereas the fa/fa rat, treated with noradrenaline, clearly showed a more rapid initial rate of increase. This reached a maximal 153% over the fa/fa rat control value, 40 minutes after administration of noradrenaline. These results show, that BAT of fa/fa rats does not have an impaired capacity to respond to direct stimulation by noradrenaline, indeed the response is proportionally greater in the fa/fa rat than in the lean rat. However, the maximum level of GDP binding did not reach that of the lean rat.

3.4.2 *Effect of noradrenaline on BAT mitochondrial GDP binding in corticosterone treated lean rats*

In order to determine whether corticosterone treatment impaired the thermogenic response to noradrenaline, lean control and corticosterone treated rats were injected with noradrenaline (50 μg/100g body weight), and BAT mitochondrial GDP binding was measured. The results shown in Table 3.4.2 indicate that the inhibition of BAT mitochondrial GDP binding, seen after chronic corticosterone treatment (Section 3.2) does not lead to a suppression of the acute response to noradrenaline. BAT mitochondrial GDP binding was increased by 43% and 47% for the vehicle and corticosterone treated lean rats respectively, after noradrenaline injection. However, in corticosterone treated rats, the level of GDP binding per mg of mitochondrial protein attained after noradrenaline injection was still reduced, when compared with the control lean rats. This suggested that chronic corticosterone treatment may have reduced the capacity of BAT mitochondria for GDP binding, by reducing the concentration of the 32K protein. However, it is possible, that larger doses of noradrenaline might have increased GDP binding to the same maximal levels in both groups.

FIG. 3.4.2 TIME COURSE OF THE INCREASE IN BAT MITOCHONDRIAL GDP BINDING AFTER TREATMENT WITH NORADRENALINE IN LEAN AND OBESE (fa/fa) RATS

Experimental details are the same as those described in the legend for Fig. 3.4.1. The increase in GDP binding, after treatment with noradrenaline, is expressed as a percentage increase over control (untreated) values.

*** $p < 0.001$, compared with lean group at each time point.

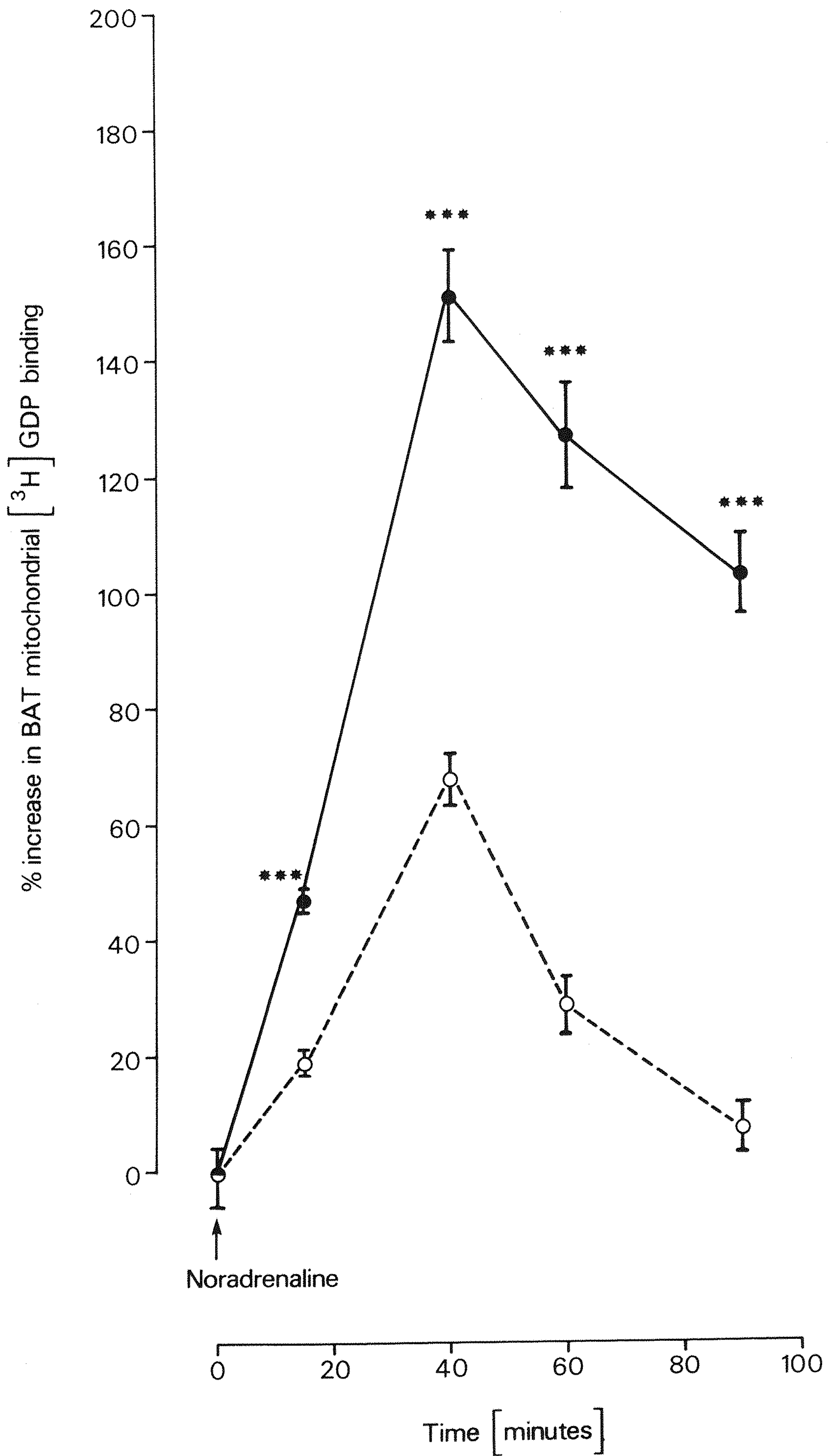

200
180
160
140
120
100
80
60
40
20
0
% increase in BAT mitochondrial [3H] GDP binding
***
***
***
***
Noradrenaline
0
20
40
60
80
100
Time [minutes]

TABLE 3.4.2

THE EFFECT OF CORTICOSTERONE ON NORADRENALINE STIMULATION OF BAT GDP BINDING IN LEAN RATS

	BAT Mitochondrial [^{3}H] GDP Binding (pmol/mg protein)	
Noradrenaline	Lean	Lean + Corticosterone
-	300.1 ± 10.6	233.1 ± 10.1††
+	428.0 ± 22.2**	343.0 ± 17.5**†

4 week old lean rats were injected s.c. with corticosterone (1 mg/100g body weight) or vehicle, as described in Section 2.8.2, daily for 7 days. A single dose of noradrenaline (50 μg/100g body weight) or vehicle (as described in Section 2.8.5) was given 40 minutes before sacrifice. BAT mitochondria were prepared and GDP binding assays performed as described in Section 2.9 and 2.10 respectively.

Values represent means ± S.E.M. for 4 animals in each group.
** $p < 0.01$, compared to equivalent group not treated with noradrenaline;
† $p < 0.05$, †† $p < 0.01$, compared to equivalent lean control.

3.4.3 *Effect of the β antagonist propranalol on BAT mitochondrial GDP binding in lean and fa/fa rats*

As noradrenaline is thought to be responsible for mediating the thermogenic response in BAT (predominantly through a β receptor) it was of interest to examine to what extent the β antagonist, propranalol, would inhibit BAT mitochondrial GDP binding in lean and fa/fa rats. Table 3.4.3 showed BAT mitochondrial GDP binding in lean rats to be reduced by almost 40% after propranalol treatment, to approach values observed in the 'control' fa/fa rat. The dose of propranalol used completely blocked the response to exogenous noradrenaline in both lean and fa/fa rats. The fa/fa rat treated with propranalol did not show a significant reduction in BAT mitochondrial GDP binding. This suggested that BAT of fa/fa rats was not being actively stimulated by the SNS.

3.4.4 *Effect of β blockade with propranalol on BAT mitochondrial GDP binding in adrenalectomised lean and fa/fa rats*

Table 3.4.4 shows that BAT mitochondrial GDP binding in adrenalectomised lean rats was reduced by over 40% after treatment with propranalol, to reach levels similar to those seen in the propranalol treated intact lean rats (Table 3.4.3). Adrenalectomy increased BAT mitochondrial GDP binding of fa/fa rats by almost 80% only 24 h after adrenalectomy. This increase was prevented by treatment with propranalol, suggesting that sympathetic activity was increased in the adrenalectomised fa/fa rat.

3.4.5 *Effect of β blockade with propranalol, to adrenalectomised lean and fa/fa rats fed with additional sucrose*

It has been reported in Section 3.3, that the fa/fa rat failed to increase BAT mitochondrial GDP binding in response to supplementary sucrose feeding. Following adrenalectomy, however, this response was restored, with similar increases in GDP binding in lean and fa/fa rats. To examine if this increase was sympathetically mediated, lean and fa/fa rats were adrenalectomised, then after recovery, fed with additional sucrose for a 15 h period, and at the same time treated with propranalol. Fig. 3.4.3 shows that the lean adrenalectomised rats increased energy intake by 80% when offered additional sucrose.

TABLE 3.4.3

EFFECT OF TREATMENT WITH PROPRANALOL ON BAT MITOCHONDRIAL GDP BINDING IN LEAN AND OBESE (fa/fa) RATS

	BAT [^{3}H]-GDP binding (pmol/mg protein)		
	Control	Propranalol	Propranalol + Noradrenaline
Lean	313.0 ± 11.8	190.9 ± 10.9†††	169.7 ± 15.7
Obese (fa/fa)	158.9 ± 7.7***	143.2 ± 8.7**	165.4 ± 9.1

4-5 week old lean and fa/fa rats were injected (s.c.) with either propranalol (2 mg/100g body weight) or vehicle as described in Section 2.8.6, 3 times over a 15h period, at 17.00, 24.00 and 08.00h. The animals were sacrificed 1h after the final injection. Noradrenaline (50 μg/100g body wt), or vehicle was injected (s.c.), as described in Section 2.8.5, 40 minutes before sacrifice. BAT mitochondria were isolated and GDP binding assays performed as described in Section 2.9 and 2.10.

Values represent means ± S.E.M. for 6 animals in each group.
** $p < 0.01$, *** $p < 0.001$, compared to equivalent lean group;
††† $p < 0.001$, compared to control group.

TABLE 3.4.4

EFFECT OF PROPRANALOL TREATMENT ON BAT MITOCHONDRIAL GDP BINDING IN ADRENALECTOMISED LEAN AND OBESE (fa/fa) RATS

	BAT [^{3}H]-GDP Binding (pmol/mg protein)		
	Control	Adrenalectomised	Adrenalectomised + Propranalol
Lean	290.0 ± 16.2	314.0 ± 12.2	177.6 ± 4.5
Obese (fa/fa)	146.0 ± 12.2***	258.8 ± 15.0*,†††	168.1 ± 18.8

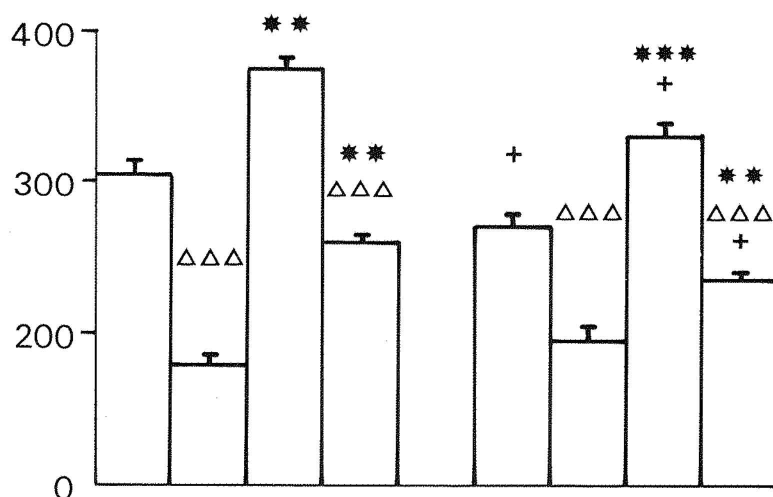
Lean and obese (fa/fa) 4-5 week old rats were adrenalectomised or sham operated and maintained as described in Section 2.7. The adrenalectomised group were injected (s.c.) with either propranalol (2 mg/100g body weight) or vehicle as described in Section 2.8.6, 3 times over a 15h period (beginning immediately after recovery) at 17.00, 24.00 and 08.00h. Animals were sacrificed 1h after the final injection. On sacrifice, the rats had been adrenalectomised for 24h. BAT mitochondria were prepared and GDP binding assays performed, as described in Section 2.9 and 2.10.

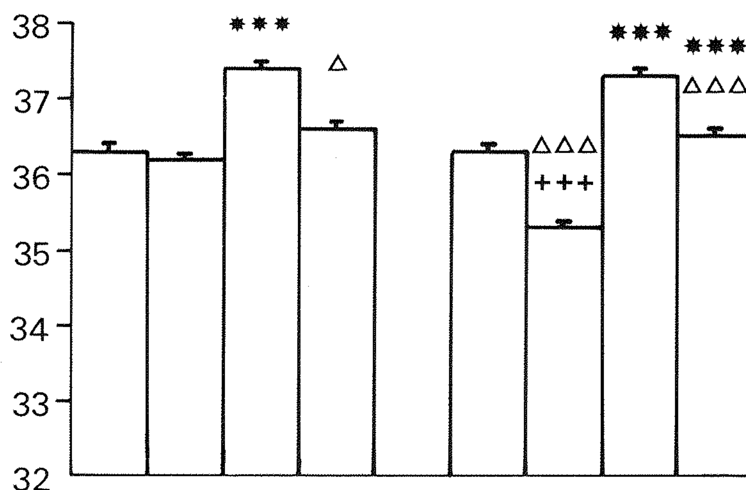
Values represent means ± S.E.M. for 4 animals in each group.
* $p < 0.05$, *** $p < 0.001$, compared to equivalent lean group;
††† $p < 0.001$, compared to control group.

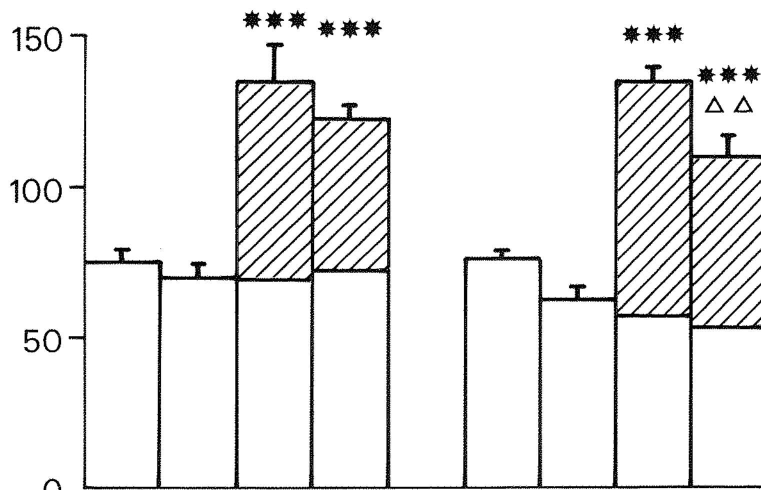
FIG. 3.4.3 EFFECT OF β BLOCKADE WITH PROPRANALOL ON ENERGY INTAKE, RECTAL TEMPERATURE AND BAT MITOCHONDRIAL GDP BINDING IN ADRENALECTOMISED LEAN AND OBESE (fa/fa) RATS

4-5 week old lean and fa/fa rats were adrenalectomised and maintained as described in Section 2.7. After recovery, rats were given either a standard chow diet or chow plus a 35% (w/v) sucrose solution as described in Section 2.3, for 15h. During this time, chow and chow + sucrose fed adrenalectomised animals, were injected s.c. with propranalol (2 mg/100g body weight) or vehicle as described in Section 2.8.6, three times over a 15h period, at 17.00, 24.00 and 08.00h. Animals were sacrificed 1h after the final injection. Food intake was monitored during this 15h period. Rectal temperatures were taken just before sacrifice. BAT mitochondrial GDP binding was assayed as described in Section 2.10.

Values represent means ± S.E.M. for 5 animals in each group; ** $p < 0.01$, *** $p < 0.001$, compared to equivalent group not fed with sucrose; † $p < 0.05$, ††† $p < 0.001$, compared with equivalent lean group, Δ $p < 0.05$, ΔΔ $p < 0.01$, ΔΔΔ $p < 0.001$, compared with equivalent group not treated with propranalol.


BAT [³H] GDP binding [p mol / mg protein]
400
300
200
0
✱✱
✱✱
△△△
△△△
+
△△△
✱✱✱
+
✱✱
△△△
+


Rectal temperature [°c]
38
37
36
35
34
33
32
✱✱✱
△
△△△
+++
✱✱✱
✱✱✱
△△△


Energy intake [kJ / 17·00 – 08·00 h]
150
100
50
0
✱✱✱
✱✱✱
✱✱✱
✱✱✱
△△
Lean control
Lean sucrose
Obese control
Obese sucrose
Propranalol – + – + – + – +

Treatment with propranalol reduced food intake slightly, but not significantly, when compared to the untreated sucrose fed adrenalectomised lean rat. Propranalol also decreased levels of BAT mitochondrial GDP binding in adrenalectomised lean rats fed with additional sucrose. However, the increase in GDP binding observed after sucrose feeding, was also observed when the animals were treated with propranalol. The increased rectal temperature in the sucrose-fed adrenalectomised lean rat, was reduced on treatment with propranalol, reflecting a reduced thermogenesis.

The adrenalectomised fa/fa rat showed the normal increase in BAT mitochondrial GDP binding, which was further enhanced after sucrose feeding (Section 3.2), but in this experiment the levels were slightly reduced when compared to the equivalent lean groups. When propranalol was given to the adrenalectomised sucrose-fed fa/fa rat, the energy intake was again slightly reduced (Fig. 3.4.3), but was comparable to the intake of sucrose-fed adrenalectomised lean rats treated with propranalol. Propranalol decreased levels of GDP binding in adrenalectomised sucrose-fed fa/fa rats. As with the lean rat discussed above, the increase in GDP binding observed after sucrose feeding, was also observed when the animals were treated with propranalol. Rectal temperatures in the fa/fa rat (Fig. 3.4.3), followed the same trends described for GDP binding.

3.4.6 Discussion

The present results indicate that the fa/fa rat shows a normal response to noradrenaline stimulation. BAT thermogenesis was increase- to approach levels observed in the noradrenaline treated lean animal. The maximum effect of noradrenaline stimulation in BAT, was observed after 40 minutes in both lean and fa/fa rats, indicating that the activation of BAT by exogenous noradrenaline is normal in the fa/fa rat. The time course of effects of noradrenaline stimulation, indicated that the elevated level of thermogenesis in the fa/fa rat was maintained over a longer period. This may reflect the reduced turnover of noradrenaline in BAT of the fa/fa rat *(York & Marchington, 1984)*, which would result in a slower clearance of noradrenaline. Acute stimulation of BAT in the fa/fa rat, resulted in a greater proportional increase in GDP binding than in the lean rat, indicating that the concentration of the 32K protein present, is far in excess of that

suggested by the basal level of BAT mitochondrial GDP binding. This acute 'unmasking' of binding sites in fa/fa rat BAT mitochondria has also been demonstrated (Section 3.3) in response to a cold stimulus and after adrenalectomy (Section 3.2), but does not occur in response to dietary stimuli. These observations substantiate the normal functional capacity of BAT and provide further evidence for a regulatory defect. The normal response of BAT of fa/fa rats to noradrenaline stimulation reported in this section, supports other reports *(Rothwell et al., 1981a)* which have shown a normal increase in oxygen consumption in the fa/fa rat, following noradrenaline stimulation. However, despite the large response to noradrenaline in the present experiments, the level of BAT mitochondrial GDP binding in fa/fa rats, did not reach equivalence with that observed in the noradrenaline treated lean rat. A similar lower maximal GDP binding was also observed in the acutely cold exposed fa/fa rat (Section 3.3), and in the short term response to adrenalectomy (Section 3.2). These results would be consistent with a reduced concentration of the 34K protein in fa/fa BAT mitochondrial membranes, probably due to lack of sympathetic stimulation of the tissue under normal environmental conditions. This was largely corrected 7 days after adrenalectomy or after acclimation to $4^{o}C$, when BAT sympathetic activity *(York & Marchington, unpublished observations)* and BAT thermogenesis (Sections 3.2, 3.3) was completely restored to lean values. These chronic responses may include an increased synthesis of the 32K protein, which would result in a further increase in BAT mitochondrial GDP binding to reach values observed in the lean rat. This suggestion needs to be qualified using radioimmunoassay *(Ashwell et al., 1983)* to quantify the amount of 32K protein in BAT mitochondria. As previously mentioned (Section 3.2) preliminary results using a radioimmunoassay technique *(Holt, York & Cleeter, unpublished observations)*, show that the concentration of the 32K protein is reduced in the fa/fa rat, and restored to normal lean levels, 7 days after adrenalectomy.

The fa/fa rat treated chronically with noradrenaline, also exhibited an increased protein and mitochondrial content, which indicated that, as in the lean rat *(Desautels & Himms-Hagen, 1979)*, chronic catecholamine administration is able to reproduce several of the elements of the cold acclimated state. However, in the present experiment, chronic

noradrenaline administration to fa/fa rats did not result in levels of BAT mitochondrial GDP binding equivalent to lean rats treated similarly. This may be expected as noradrenaline injected into rats for several weeks cannot elicit the changes in 32K protein concentration seen in cold acclimated rats *(Desautels & Himms-Hagen, 1979)*. Thus the changes in BAT mitochondrial GDP binding seen in fa/fa rats, after chronic noradrenaline treatment, are most likely to represent the known 'unmasking' effect.

The improvement in BAT thermogenesis in the fa/fa rat chronically treated with noradrenaline, was consistent with a reduced energetic efficiency, as indicated by a decreased rate of body weight gain, despite an unchanged level of food intake. These results, together with the observation in Section 3.3, that BAT in the young fa/fa rat has a normal capacity for NST, imply that the defect in BAT may result from a misregulation of the sympathetic control in response to deitary stimuli. The present results suggest that the higher level of thermogenesis observed in the lean rat is mediated through an increased BAT sympathetic activity, as propranalol reduced BAT mitochondrial GDP binding in lean, but not fa/fa rats. After adrenalectomy, the dietary induced thermogenic response was restored in the fa/fa rat. The increased GDP binding in the adrenalectomised fa/fa rat was abolished by propranalol, suggesting that an elevated sympathetic activity was responsible for this increase. As corticosterone treatment was shown to reduce BAT mitochondrial GDP binding in lean rats, but not to effec- the response to noradrenaline, it seems that glucocorticoids in the fa/fa rat, may be suppressing the sympathetic activation of BAT in response to dietary stimuli. This suggestion is supported by experiments performed on lean mice chronically treated with corticosterone *(Galpin et al., 1983)*, which show a normal increase in BAT mitochondrial GDP binding in response to cold, but a reduced response to 'cafeteria' feeding. In the present experiments, noradrenaline administration to corticosterone treated lean rats, increased BAT mitochondrial GDP binding by the same proportion as it did in untreated lean rats. However, as GDP binding was suppressed in the corticosterone treated lean rat, the level attained after noradrenaline treatment was still lower than that of the control lean group. These results would again be consistent with a reduction in mitochondrial 32K protein content in response to a corticosterone dependent inhibition of sympathetic

stimulation.

The increases in GDP binding, observed following adrenalectomy in the fa/fa rats, were prevented by propranalol. However, the increases in GDP binding after sucrose feeding in lean and fa/fa rats were not blocked by propranalol. This result suggests that the sucrose induced increase in BAT mitochondrial GDP binding after adrenalectomy, is not mediated by β adrenoreceptor in the brown adipocytes. This suggestion is supported by the observation that propranalol treatment of sucrose fed adrenalectomised rats did not completely return the elevated rectal temperatures to values observed in adrenalectomised rats treated with propranalol. These observations may be in accordance with those made on rats treated chronically with noradrenaline (or a specific β agonist, isoproterenol in which the adaptive changes characteristic for cold-induced BAT hypertrophy, can be only partly reproduced (*Barnard et al., 1980*). It is possible, however, that the observed result may reflect incomplete β blockade by propranalol. This is unlikely, since the same treatment was given to the adrenalectomised control rats.

Sucrose feeding of the adrenalectomised fa/fa rat did not increase BAT mitochondrial GDP binding to the level seen in the adrenalectomised lean rat fed additionally with sucrose. This may be expected, since sucrose-feeding commenced as soon as the rats had recovered following adrenalectomy, and rats had been adrenalectomised for only 24 h on sacrifice. In this time, it is unlikely that there has been any significant increase in the mitochondrial 32K protein concentration, so that the capacity for GDP binding in the fa/fa rat, will still be lower than it is in the equivalent lean animal. In contrast, 7 days after adrenalectomy the response to sucrose was completely normalised (Section 3.2). This reasoning would also explain the present finding, that BAT mitochondrial GDP binding in the propranalol treated, sucrose fed, 24 h adrenalectomised fa/fa rat, was lower than in the similarly treated lean rat.

The decreased level of sympathetic activity in BAT of fa/fa rats indicated in the present experiments, has been found by others (*Levin et al., 1980, 1981a*), who demonstrated a reduced noradrenaline content and turnover. The genetically obese (ob/ob) mouse also showed a reduced sympathetic activity in BAT (*Young & Landsberg, 1983*), but unlike the fa/fa rat (*York & Marchington, unpublished observations*),

also showed a decreased noradrenaline turnover on cold exposure when compared to the lean mouse *(Young & Landsberg, 1983)*. However, a normal BAT noradrenaline turnover has also been reported in cold exposed ob/ob mice *(Zaror-Bahrens & Himms-Hagen, 1983)*.

A reduced, sympathetically mediated, dietary response has been observed in BAT of the ob/ob mouse *(Trayhurn et al., 1982)*, the fa/fa rat (Section 3.2) *(Triandafillou & Himms-Hagen, 1983)* and the VMH lesioned rat *(Hogan et al., 1982)*. Adrenalectomy has been shown to prevent the obesity of all these animals *(Solomon et al., 1977; Yukimura & Bray, 1978; Bruce et al., 1982)*. It is thus possible, that a corticosterone-dependent inhibition of diet related sympathetic activity, may be a common cause of a number of rodent obesities. Recent studies of noradrenaline turnover rates *(York & Marchington, unpublished observations)* have shown that the turnover in the fa/fa rat was greatly enhanced by acclimation to $4^{o}C$ for 7 days, consistent with a normal response to cold stress. Furthermore, noradrenaline turnover in BAT of adrenalectomised fa/fa rats, was increased to that of lean rats, indicating a normalisation of BAT sympathetic activity following removal of adrenal corticosterone. Similarly, corticosterone-treated lean rats exhibit a reduced BAT noradrenaline turnover. This provides direct evidence for the hypothesis that corticosterone suppression of BAT function is mediated through an inhibition of sympathetic activity.

SECTION 3.5 Relationship of Defective BAT Function to the Recessive fa Gene

In the previous sections, results have been presented to show that the fa/fa rat is characterised by an impairment of BAT thermogenesis in response to diet. If this defect is primarily responsible for the energy imbalance and obesity of the fa/fa rat, it would be expected to be apparent at an early age and to be closely related to the expression of the defective fa gene. Experiments in Section 3.1 showed that the reduction in BAT mitochondrial GDP binding was already manifest at 10 days of age, when excess body fat is just beginning to accumulate. In this section, experiments on homozygous (Fa/Fa) lean, heterozygous (Fa/fa) lean and homozygous recessive (fa/fa) obese rats are described. They suggest that the defect in BAT function is closely associated with the fa gene.

3.5.1 Effect of fa gene dosage on BAT of the Zucker rat

Table 3.5.1 shows that the body weights of 8 week old fa/fa rats were greater than those of homozygous (Fa/Fa) lean rats, but not significantly greater than those of heterozygous (Fa/fa) lean rats. Inguinal fat pad weights, however, showed a clear dependence on fa gene dosage, being highest in the homozygous obese (fa/fa) group, and lowest in the homozygous lean (Fa/Fa) group. BAT wet weight of Fa/Fa and Fa/fa rats were similar, but lower than those of the obese (fa/fa) rats. Protein content and total tissue succ.cyt.c.O-R activities were similar in all three groups, indicating that the BAT mitochondrial content is unaltered by the fa gene, in rats of this age. However, BAT mitochondrial GDP binding shows a significant gene dependency, being highest in the lean homozygous (Fa/Fa) rats, and intermediate in the heterozygous (Fa/fa) rats.

The fa/fa rat has been reported to have abnormal levels of some hormones and metabolites *(Bray, 1977)*. Therefore, the serum concentrations of insulin, T_3, corticosterone, triglycerides and free fatty acids, were examined to assess whether they show a relationship to the fa gene dosage. Table 3.5.2 shows that in 8 week old Zucker rats, serum free fatty acid concentrations exhibit a fa gene dosage dependency, as do serum T_3 levels. Serum triglyceride levels suggest a gene dosage dependency, although the result is not statistically significant.

TABLE 3.5.1

EFFECT OF fa GENE DOSAGE ON BODY WEIGHT, INGUINAL FAT PAD WEIGHT AND BAT OF 8 WEEK OLD ZUCKER RATS

	Body weight (g)	Inguinal fat pad weight (g)	Brown Adipose Tissue			
			Wet weight (mg)	Protein (mg)	Succ.cyt.c.O-R activity (μmol/min/depot)	[^{3}H]-GDP binding (pmol/mg protein)
Fa/Fa	138.5 ± 9.1	0.61 ± 0.02†	0.44 ± 0.01	41.9 ± 0.4	4.51 ± 0.25	356.3 ± 7.7†
Fa/fa	148.8 ± 8.7	1.11 ± 0.06†	0.43 ± 0.03	42.9 ± 2.2	3.99 ± 0.12	239.7 ± 8.2†
fa/fa	166.7 ± 7.2*	2.63 ± 0.08†	1.10 ± 0.10***	48.6 ± 2.6	4.20 ± 0.08	128.2 ± 8.6†

Lean homozygous (Fa/Fa), heterozygous (Fa/fa) and obese (fa/fa) rats were bred as described in Section 2.2. Animals were sacrificed at 8 weeks of age, and inguinal fat pads dissected out and weighed. BAT protein, succ.cyt.c.O-R activity and mitochondrial GDP binding were determined as described in Section 2.11, 2.12 and 2.10 respectively.

Values represent means ± S.E.M. for 8 animals in each group. † analysis of variance indicated a significant ($p < 0.001$) gene dosage effect. * significantly different from homozygous (Fa/Fa) group ($p < 0.01$).

*** significantly different from homozygous (Fa/Fa) group ($p < 0.001$).

TABLE 3.5.2

EFFECT OF fa GENE DOSAGE ON SERUM METABOLITE AND HORMONE LEVELS IN 8 WEEK OLD ZUCKER RATS

	Lean		Obese
	Fa/Fa	Fa/fa	fa/fa
Free fatty acids (μmol/ℓ)	341 ± 20†	440 ± 25†	508 ± 50†
Triglycerides (mg/dl)	57 ± 4	69 ± 13	81 ± 6
Insulin (ng/ml)	3.8 ± 0.4	5.2 ± 1.2	18.7 ± 0.7*
Triiodothyronine (nmol/ℓ)	1.5 ± 0.1†	1.2 ± 0.1†	0.7 ± 0.1†
Corticosterone (ng/ml)	119 ± 15	125 ± 13	91 ± 10

Lean homozygous (Fa/Fa), heterozygous (Fa/fa) and obese (fa/fa) rats were bred as described in Section 2.2. Animals were sacrificed at 8 weeks of age and blood collected as described in Section 2.2.1. The serum concentrations of metabolites and hormones were determined as described in Sections 2.14.

Values are means ± S.E.M. for 8 animals in each group. † analysis of variance indicated a significant ($p < 0.001$) gene dosage effect; * significantly different from heterozygous lean group ($p < 0.01$).

The fa/fa rat was clearly hyperinsulinemic, but serum insulin levels were similar in both heterozygous (Fa/fa) and homozygous (Fa/Fa) lean rats. Serum levels of corticosterone do not show a relationship to fa gene dosage, and no significant differences were observed between the three groups.

3.5.2 Effect of genotype on 10 day old pre-obese (fa/fa) rats

In 10 day old rats, fa gene dosage effects can be demonstrated most clearly when rats from the same litter are compared. Inter-litter variations obscure the small differences being sought to demonstrate gene dependency. The genotypes of the young rats were determined by measurement of rectal temperatures, and inguinal fat pad weights. Animals with the lowest rectal temperatures and highest fat pad weights were taken to be fa/fa, those with the highest temperatures and lowest fat pad weights were taken to be homozygous (Fa/Fa) lean, and those having intermediate values, were designated as (Fa/fa) heterozygous. Animals within a litter, clearly fell into 3 groups when inguinal fat pad weights were measured. The numbers in each designated group were consistent with Mendelian genetics.

In the 10 day old fa/fa rat, BAT mitochondrial content (as indicated by the total succ.cyt.c.O-R activity), appeared to be normal when compared to that of the Fa/Fa and Fa/fa groups (Fig. 3.5.1). However, BAT mitochondrial GDP binding showed a clear relationship to fa gene concentration; binding in the homozygous (Fa/Fa) lean group was 14% higher than that in the heterozygous (Fa/fa) group and 29% higher than that in the fa/fa group. Total tissue binding reflected these differences.

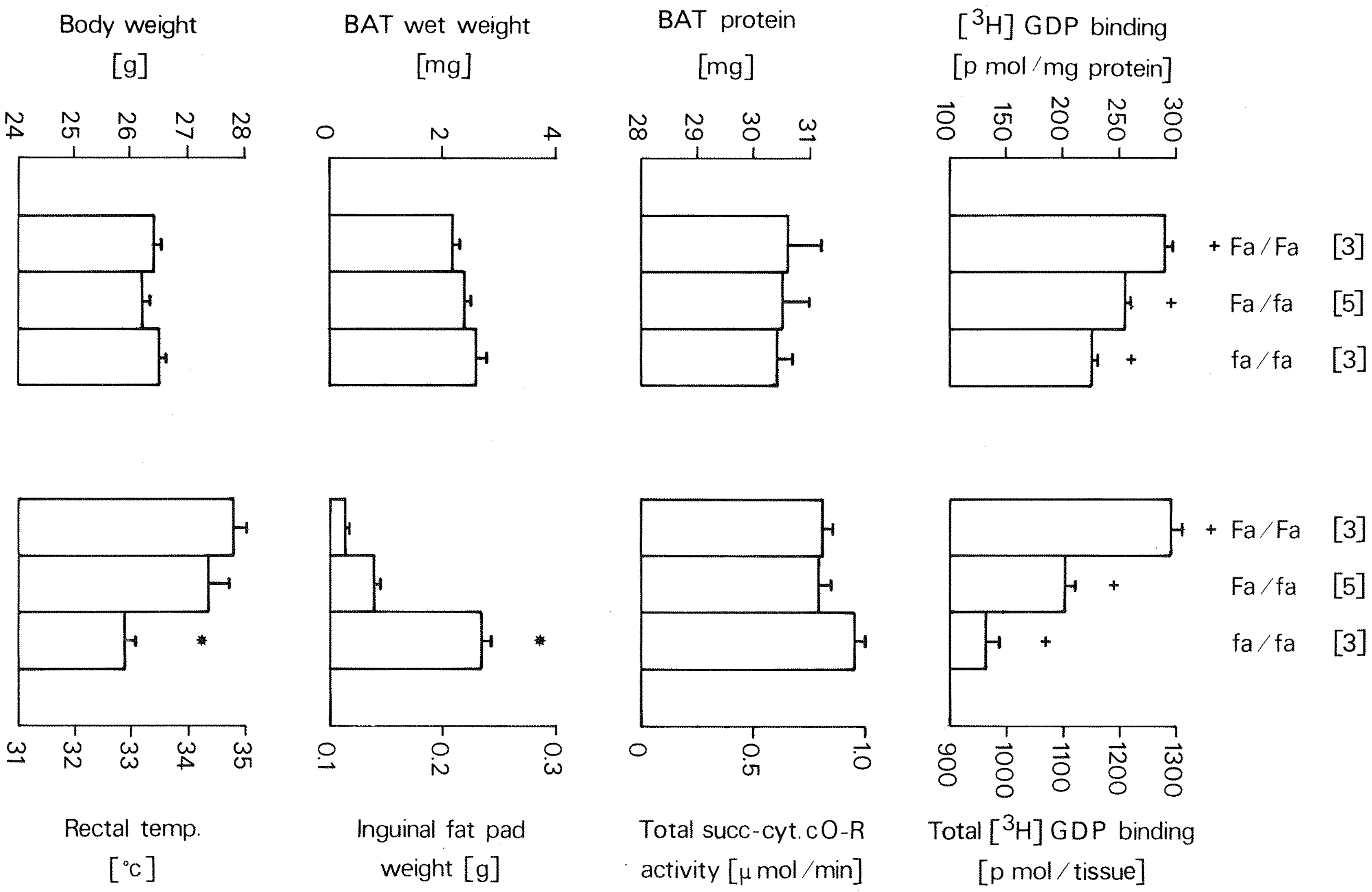
3.5.3 Discussion

BAT thermogenesis normally increases in response to a cold *(Desautels et al., 1978)* or dietary stimulus *(Brooks et al., 1980; Himms-Hagen et al., 1981; Rothwell & Stock, 1979a)*. The results presented earlier (Section 3.3) suggest that the weaned fa/fa rat does not respond to supplementary sucrose feeding, with an appropriate increase in BAT thermogenesis, although their response to cold exposure remains largely intact. It has also been observed *(Rothwell et al., 1981a)* that fa/fa rats exhibit a reduced increase in oxygen consumption after a normal meal, and this indicates that diet-related thermogenesis

FIG. 3.5.1   EFFECT OF GENE DOSAGE ON 10 DAY OLD ZUCKER RATS

One litter (11 rats) of 10 day old Zucker rats were identified as Fa/Fa, Fa/fa and fa/fa genotypes, by measurement of rectal temperature and inguinal fat pad weight, as described in Section 2.5. BAT protein content, succ.cyt.c.O-R activity (expressed as μmol/min/depot), and mitochondrial GDP binding were assayed as described in Section 2.11, 2.12 and 2.10 respectively. Total tissue GDP binding was calculated, based on 100% mitochondrial recovery.

Values represent means ± S.E.M. for the number of animals shown in parenthesis; † analysis of variance indicated a significant ($p < 0.001$) gene-dosage effect; * significantly different from heterozygote (Fa/fa) group ($p < 0.01$).



Body weight
[g]
24
25
26
27
28
BAT wet weight
[mg]
0
2
4
BAT protein
[mg]
28
29
30
31
[^{3}H] GDP binding
[p mol / mg protein]
100
150
200
250
300
+ Fa / Fa [3]
Fa / fa [5]
fa / fa [3]
+
+
31
32
33
34
35
*
Rectal temp.
[°c]
0.1
0.2
0.3
*
Inguinal fat pad
weight [g]
0
0.5
1.0
Total succ-cyt. cO-R
activity [µ mol / min]
900
1000
1100
1200
1300
+
+
Total [^{3}H] GDP binding
[p mol / tissue]
+ Fa / Fa [3]
Fa / fa [5]
fa / fa [3]

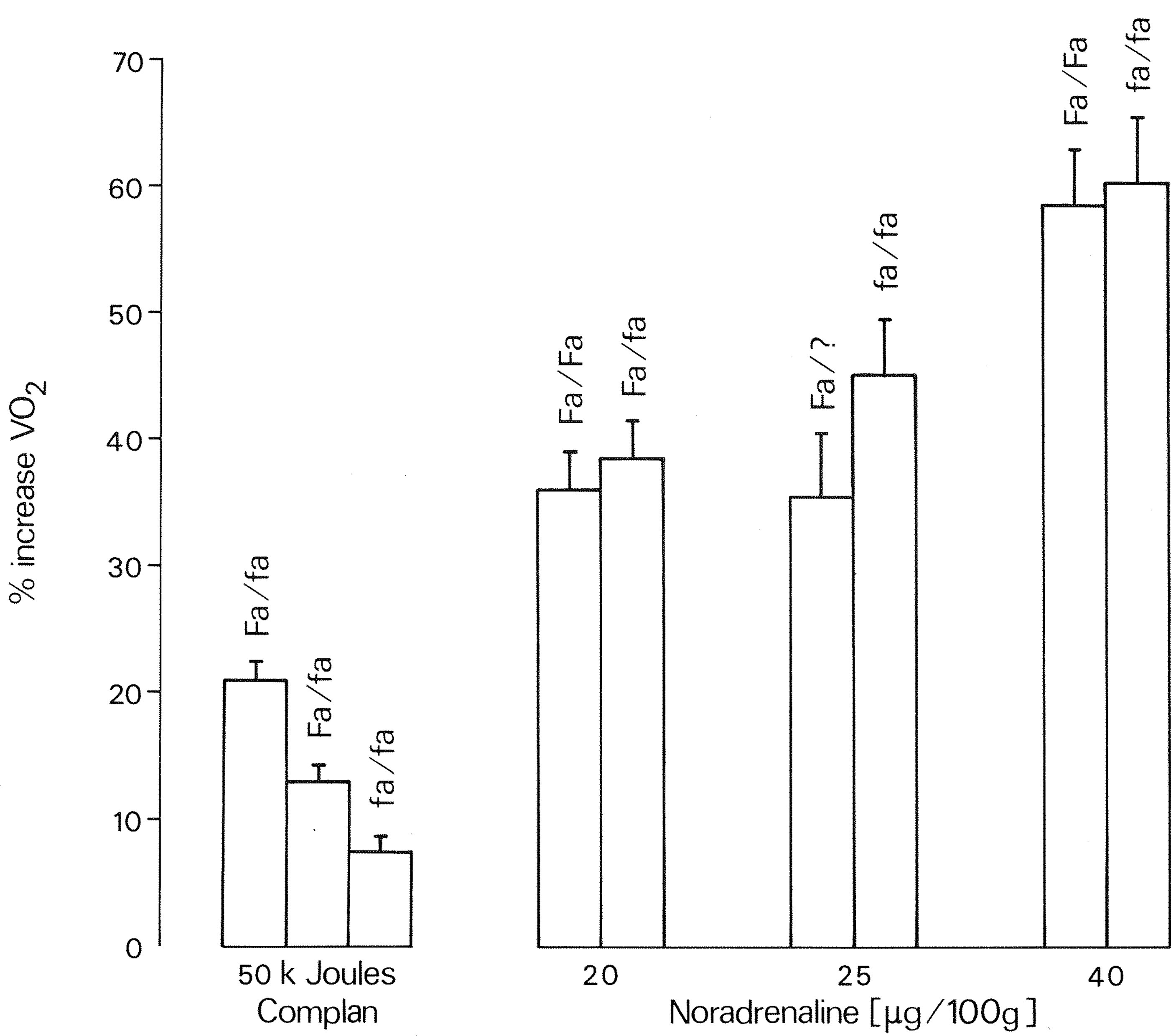
is defective in these animals. Since hyperphagia is absent in the suckling pre-obese Fa/fa rat *(Boulange et al., 1979b)* and oxygen consumption *(Kaplan, 1979b)* and rectal temperature are reduced, it is possible that the primary cause of the energy inbalance observed in suckling fa/fa rats, is a level of BAT thermogenesis inappropriate to the level of dietary intake. The present results demonstrate that BAT mitochondrial GDP binding is reduced in the 10 day old fa/fa rat and that it shows a fa gene dependency. Support for the suggestion that a defective diet related regulation of BAT thermogenesis is closely associated with the gene defect, is shown in Fig. 3.5.2. This work was performed by Dr. N. J. Rothwell and Dr. M. J. Stock, with Zucker rats (Fa/Fa and Fa/fa) from the same litters as those used for the present experiments. The results have been presented in published form *(York et al., 1984)*. The increase in oxygen consumption following a meal also shows a relationship to fa gene dosage; in the homozygous lean (Fa/Fa) there was a 21% increase, compared with a 12.7% increase in the heterozygote lean (Fa/fa) and a 7.2% rise in the obese (fa/fa) rats. Fig. 3.5.2 also shows that the response to exogenous nor-adrenaline treatment was not gene dosage dependent, since a similar increase in oxygen consumption is observed in all three Zucker geno-types. It has already been shown (Section 3.4) that noradrenaline treatment can elicit an acute rise in BAT mitochondrial GDP binding in both lean and fa/fa rats. These results again indicate that the defect in BAT thermogenesis is not due to a defect in BAT itself (an observation which is supported by a normal capacity to respond to cold (Section 3.3)), but to a defect in the regulation of the tissue in response to dietary signals.

The SNS is thought to be the principle regulator of BAT thermo-genesis *(Himms-Hagen, 1976, Landsberg & Young, 1982)*. Noradrenaline turnover, an index of sympathetic activity, is reduced in BAT of fa/fa rats, and increased to lean levels on cold acclimation, but not on sucrose feeding *(York & Marchington, unpublished observations)*. This suggests that the sympathetic innervation of BAT is intact, and that the defect lies in the activation of the SNS in response to diet, which appears to be fa gene dependent. Serum T_3 was reduced in fa/fa rats, and showed a relationship to fa gene dosage. It is difficult to reason the significance of the fall in serum T_3 with the impaired BAT function, since thyroid hormones are only required permissively for BAT

FIG. 3.5.2 THE EFFECT OF A 50 kJOULE COMPLAN MEAN AND NORADRENALINE ON OXYGEN CONSUMPTION (V_{O2}) OF ZUCKER RATS

The values for fa/fa rats fed Complan and for Fa/? and fa/fa rats, injected with noradrenaline at 25 µg/100g body weight, have been previously published *(Rothwell et al., 1983b)*. For other experimental details, see *York et al., 1984*.

Values represent means ± S.E.M. for 7 rats in each group.



% increase VO2
70
60
50
40
30
20
10
0
Fa/fa
Fa/fa
fa/fa
Fa/Fa
Fa/fa
Fa/?
fa/fa
Fa/Fa
fa/fa
50 k Joules
Complan
20
25
40
Noradrenaline [µg/100g]

thermogenesis *(Fellenz et al., 1982)*. Additional thyroid hormone does not increase BAT mitochondrial GDP binding in lean or fa/fa rats (Section 3.2) or in hyperthyroid rats fed a 'cafeteria' diet *(Rothwell et al., 1983a)*. BAT has a high activity of 5'-deiodinase, which is the enzyme responsible for converting thyroxine T_4 to the more active form of the hormone T_3 *(Leonard et al., 1983)*. This enzyme has been shown to be under adrenergic control *(Silva & Larsen, 1983)*. If the defect in the fa/fa rat BAT is a diet-related (fa gene dependent) suppression of SNS activity in this tissue, it is possible that this may result in a reduced activity of the 5'-deiodinase enzyme. This may contribute to the reduced serum T_3 levels observed in the fa/fa rat. The observation that serum T_3 levels of fa/fa rats were normalised when sympathetic stimulation of BAT was enhanced, i.e. in cold acclimated fa/fa rats, and in sucrose-fed adrenalectomised fa/fa rats (Section 3.3), provides further support for this hypothesis. However, measurement of the activity of this enzyme in BAT of fa/fa rats has not beem made.

Hyperinsulinemia is a characteristic trait of the weaned fa/fa rat *(Zucker & Antoniades, 1972; Godbole et al., 1978)*. In this study no significant differences were observed in serum insulin concentrations, between the homozygous (Fa/Fa) and heterozygous (Fa/fa) lean rats at 8 weeks of age. Although hyperphagia is not a requirement for the development of hyperinsulinemia in the fa/fa rat *(Cleary et al., 1980)*, a high food intake in the weaned fa/fa rat does potentiate the condition *(Zucker & Antoniades, 1972)*. As the heterozygous (Fa/fa) lean is not hyperphagic, it is likely that an fa gene dependent expression of abnormal pancreatic function, seen prenatally *(Turkenkopf et al., 1982)*, may be masked by other metabolic and neuro-endocrine factors.

Raised levels of serum free fatty acids in fa/fa rats are thought to be a consequence of increased adipose cell size, which increases cell lipolysis *(Zucker & Antoniades, 1972; Bray, 1977; Bray & York, 1979)*. This increase is not apparent in younger (5-6 week old) rats (Section 3.2; *Zucker & Antoniades, 1972)*. In the present experiments, serum free fatty acid levels exhibit an fa gene dependency. As the weight of the inguinal fat pad has been shown to be closely correlated with fa gene concentration, the adipose cell size may be similarly related *(Bray, 1969b)*; this would explain the observed range of serum free fatty acid concentrations.

Hypertriglyceridemia is present in the fa/fa rat as early as 2 weeks of age despite a normal hepatic triglyceride output *(Boulange et al., 1981)*. Although white adipose tissue LPL activity is elevated at this age, a defective LPL activity is associated with BAT and cardiac and skeletal muscle *(Boulange et al., 1981)*. Therefore, the high levels of serum triglycerides are thought to be a consequence of the decreased clearance rate in the fa/fa rat, which is amplified after weaning, when hepatic output of triglyceride (as very low density lipoprotein) is excessive. The present results show that serum triglycerides exhibit an fa gene dependency, and this may be connected with the decreased activity of LPL in BAT, which has been shown to correlate with the thermogenic activity of this tissue. Cold acclimation and feeding increase BAT LPL activity *(Goubern & Portet, 1981)*, and the activity has been related to the thermogenic role of BAT *(Portet et al., 1974)*. Both the SNS and insulin may participate in the regulation of BAT LPL activity during cold exposure, since noradrenaline produces similar, but less marked, effects in warm-acclimated animals, and insulin partially reverses the cold induced changes in enzyme activity *(Radomski & Orme, 1971)*. It is possible that the reduced LPL activity may be connected with a decreased dietary stimulation of the SNS in the fa/fa rat, and contribute to the fa gene dependent change in serum triglycerides. Glucocorticoids are essential for the expression of the fa gene (Section 3.2; *Yukimura & Bray, 1978; Yukimura et al., 1977)*. The present results showed that serum corticosterone levels were unchanged with fa gene dosage. However, it does appear that the fa/fa rat may show an increased sensitivity to glucocorticoids *(Yukimura et al., 1978)*, and the increase in BAT mitochondrial GDP binding, seen after adrenalectomy in the fa/fa rat, was reversed when a low replacement dose of corticosterone was given (Section 3.2).

Thyroid hormones are known to stimulate corticosterone binding globulin synthesis in the rat *(Labrie et al., 1964)*, thus influencing the proportion of the hormone which is free and active in the plasma. It is possible that the increased sensitivity to corticosterone, seen in the fa/fa rat, may result from a higher circulating level of unbound corticosterone. However, only total serum corticosterone was measured in the present experiments.

CHAPTER 4

SUMMARY AND DISCUSSION

The fa/fa rat has been shown to have a reduced level of BAT mitochondrial GDP binding, indicating that BAT is not fulfilling its normal thermogenic function. The defect was present from a very early age and closely associated with the fa gene. The response of BAT to both noradrenaline and a cold stimulus was largely intact in the young fa/fa rat, but the response to diet was impaired. Adrenalectomy restored the thermogenic response to diet in BAT. The effects of adrenalectomy were reversed by corticosterone. Since the sympathetic activity in BAT of the fa/fa rat has been shown to be reduced, when compared to the lean rat and restored to normal after adrenalectomy *(York & Marchington, 1984)* it was hypothesised that corticosterone may be inhibiting the sympathetic activation of BAT in response to dietary signals in the fa/fa rat.

A large part of the thermogenic activity observed in BAT of the young rat has been shown to be for thermoregulation *(Sundin & Cannon, 1980)*. However, with increasing age, the requirement for thermoregulatory thermogenesis decreases and the dietary induced component becomes more important. The lack of DIT in the fa/fa rat would result in a progressive decrease in sympathetic stimulation of BAT, with increasing age. This may lead to the morphological and structural changes observed and may explain the appearance of an attenuated response to cold in older fa/fa rats.

The mechanism of the inhibitory effect of corticosterone on sympathetic activity is unknown. The absence of any effect of corticosterone on cold-related BAT thermogenesis, suggests that the regulation might be located in the regions of the hypothalamus closely associated with the regulation of feeding and satiety. The hypothalamus receives input from metabolic, hormonal, neurogenic and thermal factors describing the nutritional status of the animal. It is possible that in the fa/fa rat, corticosterone may inhibit one of these sensory inputs describing the state of the internal environment. The fa/fa rat appears to have some defect in the detection of nutrient availability *(Ikeda et al., 1980; Rothwell et al., 1981a; Rothwell et al., 1983c)*. It can produce a metabolic response to exogenous noradrenaline, but not to food or insulin.

Two centres of the hypothalamus are known to be involved in appetite regulation. One situated in the ventromedial hypothalamus (VMH) responsible for producing the sensation of satiety, i.e. the satiety centre, and one situated in the lateral hypothalamus which appears to initiate feeding, i.e. the feeding centre. The areas are, in turn, linked to other areas of the brain which provide regulatory input to them. The VMH is of particular interest because in addition to its role as a satiety centre, it is a centre for control of the SNS *(Ban, 1975)*. Electrical stimulation of this area results in an activation of BAT thermogenesis *(Perkins et al., 1981)*. Lesions of the VMH result in a reduced SNS activity, and a reduction in BAT mitochondrial GDP binding, as well as hyperphagia and obesity *(Seydoux et al., 1982)*. *Hogan et al. (1982)* demonstrated that rats with VMH lesions responded normally to cold, substantiating the suggestion of independent hypothalamic control over NST and DIT. No enhancement of BAT thermogenesis was observed in these hyperphagic animals, which suggested an intact VMH region was necessary for sympathetic mediation of DIT. In the fa/fa rat, corticosterone may inhibit the activation of this area in response to dietary signals, thus reducing the sympathetic outflow to BAT. It is possible that corticosterone could affect the VMH directly, however, receptors for corticosterone have not been reported to be present in this area. Corticosterone receptors have been reported to be present in the hippocampus and septum areas of the brain *(McEwan, 1976)* which may translate effects to the VMH region. The concept of the ventral hypothalamus having two separate control centres is of course an oversimplification. A number of central nervous system tracts associated with the VMH and LH seem to play an important integrative role in the control of feeding and appetite *(Morley, 1980)*. A lesion placed between the lateral and medial hypothalamus produces obesity and hyperphagia, suggesting that a medial-lateral connection carries inhibitory influences from the VMH to the LH *(Albert et al., 1971)*. The link between the two areas appears to be bidirectional. Activation of one inhibits the other, and damage to one releases the inhibition of the other *(Oomura et al., 1969)*. Therefore it is possible that a reduced afferent stimulation of the VMH region, might result in an increased stimulation of areas of the LH, which may elicit the hyperphagia exhibited in the fa/fa rat.

The supposed inhibitory effects of corticosterone in the fa/fa rat may result from indirect effects, possibly through ACTH and β endorphins. Evidence of opiate involvement in the pathophysiology of fa/fa rats and ob/ob mice have been reported (*Margules et al., 1978; Recant et al., 1983b*). Opiates are capable of suppressing the activity of the SNS (*Laubie et al., 1977; Zelis et al., 1974*). The possibility exists therefore, that the increased level of opiate peptides observed in the fa/fa rat may contribute to its reduced sympathetic activity. β-endorphins are also associated with feeding mechanisms. The medial hypothalamus is one of several areas of the brain found to be high in opiate receptor sites (*Cuello, C., 1983*) and injection of β endorphin directly into the VMH has been found to increase food intake in satiated rats. This effect can be blocked by the long-acting opiate antagonist naltrexone (*Grandison & Guidotti, 1977*). High circulating levels of β endorphin and ACTH have also been reported to be present in acid extracts of small intestines of rats (*Orwoll & Kendall, 1980*), and levels of both were increased with fasting, whilst hypothalamic levels were decreased (*Recant et al., 1983a*). This suggests that opiates may be involved in the control of feeding, possibly via these known effects on the pancreas (*Grube et al., 1978; Ipp et al., 1978; Green et al., 1980; Kanter et al., 1980*) and insulin secretion.

In the fa/fa rat, raised levels of β endorphins are found in the pituitary (*Margules et al., 1978*), the hypothalamus and plasma (*Recant et al., 1983b*) when compared to lean animals. The opiate antagonist nalaxone has been shown to suppress food intake, specifically by reducing the fat component of the diet (*Marks-Kaufman & Kanarek, 1981*). When the fa/fa rat is given separate macronutrient sources, it selects to increase the fat component and *Castonguay and Stern (1983)* suggested that endogenous opiates play a role in this selection. The same authors show that adrenalectomised fa/fa rats selectively reduce the fat component of the diet. Since ACTH and β endorphin are known to share a common precursor (*Mains et al., 1977, Nakanishi et al., 1979*), *Castonguay & Stern (1983)* suggest that the elevated levels of ACTH in the adrenal-ectomised rat may act as an opiate antagonist (*Smock & Fields, 1981*) and reduced the β endorphin mediated effects. Similarly β endorphins may compete with ACTH for receptor sites. ACTH has been reported to have an affinity for opiate receptors in the brain (*Terenius, 1976*), and it is

possible that the antagonism results from a direct competition for receptors. Although serum ACTH levels have been reported to be similar in lean and fa/fa rats, and raised to the same level after adrenalectomy *(Yukimura et al., 1978)*, the improved BAT thermogenesis seen after adrenalectomy in the fa/fa rat may involve inhibition of the β endorphin receptors. This hypothesis may be supported by the observation that ACTH treatment increased BAT mitochondrial GDP binding in fa/fa rats, but not in lean controls *(York et al., unpublished observations)*. Hypophysectomy has been shown to attenuate weight gain in fa/fa rats *(Powley & Morton, 1976)*. The cocomittant reduction in β endorphins following hypophysectomy may also contribute to the prevention of excessive weight gain. However, because of the complexity of the opiate peptide system and the sparcity of information available concerning their actions in the fa/fa rat, any suppositions must be regarded as highly speculative.

It is clear that future work should concentrate on identifying the mechanism and sites of action of corticosterone and/or ACTH in regulating BAT thermogenesis in response to diet. In particular, the interrelationships of ACTH and corticosterone with β endorphins in the regulation of food intake and thermogenesis should be investigated.

REFERENCES

AFZELIUS, B. A., (1970): 'Brown adipose tissue, its gross anatomy, histology and cytology', In: Brown Adipose Tissue, Lindberg, O. (Ed.), Elsevier, Holland, 1-32.

AGIUS, L., ROLLS, B., ROWE, E. A. and WILLIAMSON, D. H. (1981): 'Increased lipogenesis in brown adipose tissue of lactating rats fed a cafeteria diet. (The possible involvement of insulin in brown adipose tissue hypertrophy), FEBS Lett. 123, 45-48.

ALBERT, D. J., STORLEIN, L. H., ALBERT, J. G. and MAH, C. J. (1971): 'Obesity following disturbance of the ventromedial hypothalamus: a comparison of lesions, lateral cuts, and anterior cuts', Physiol. Behav. 7, 135-141.

ANAND, B. K. and BROBECK, J. R. (1951): 'Hypothalamic control of food intake in rats and cats', Yale J. Biol. Med. 24, 128-140.

ARMITAGE, G., HARRIS, R. B. S., HERVEY, G. R. and TOBIN, G. (1981a): 'Energy expenditure of Zucker rats in relation to environmental temperature', J. Physiol. 310, 33P.

ARMITAGE, G., HARRIS, R. B. S., HERVEY, G. R. and TOBIN, G. (1981b): 'The part played by variation of energy expenditure in the regulation of energy balance', In: The Body Weight Regulatory System. Normal and Disturbed Mechanisms, L. A. Cioffi et al., (Eds), Raven Press, New York, 137-142.

ASHWELL, M., JENNINGS, G., RICHARD, D., STIRLING, D. M. and TRAYHURN, P. (1983): 'Effect of acclimation temperature on the concentration of the mitochondrial 'uncoupling' protein measured by radio-immunoassay in mouse brown adipose tissue', FEBS Lett. 161, 108-112.

ASSIMACOPOULOS-JEANNET, F. and JEANRENAUD, B. (1976): 'The hormonal and metabolic basis of experimental obesity', J. Clin. Endocrinol. Metabol. 5, 337-365.

AUTISSIER, N., DUMAS, P., LOIREAU, A. and MICHEL, R. (1980): 'Thyroid status and effects of 3 5 3' triiodothyroacetic acid and Fenoproporex in genetically lean and obese female rats', Biochemical Pharmac. 29, 1612-1615.

AXELROD, J. (1975): 'Relationship between catecholamines and other hormones', Recent Prog. Horm. Res. 31, 1-27.

BAN, T. (1975): 'Fibre connections in the hypothalamus and some autonomic functions: Central neural control of eating and obesity', Pharmacol. Biochem. Behav. 3, 3-13.

BANET, M., HENSEL, H. and LIEBERMAN, H. (1978): 'Central control of shivering and non-shivering thermogenesis in the rat', J. Physiol. 283, 569-584.

BARDE, Y. A., CHINET, A. and GIRARDIER, L. (1975): 'Potassium-induced increase in oxygen consumption of brown adipose tissue from the rat', J. Physiol. 252, 523-536.

BARNARD, T., MORY, G. and NECHAD, M. (1980): 'Biogenic amines and the trophic response to brown adipose tissue', In: Biogenic Amines in Development, Parvez, H. and Parvez, S. (Eds), Elsevier, Amsterdam, 391-439.

BARNARD, T. and SKALA, J. (1970): 'The development of brown adipose tissue', In: Brown Adipose Tissue, Lindberg, O. (Ed), Elsevier, Holland, 33-72.

BARNARD, T., SKALA, J. and LINDBERG, O. (1970): 'Changes in interscapular brown adipose tissue of the rat during perinatal and early postnatal development and after cold acclimation. 1. Activities of some respiratory enzymes in the tissue', Comp. Biochem. Physiol. 33, 499-508.

BAZIN, R., ETEVE, D. and LAVAU, M. (1983): 'Impairment of GDP binding to brown adipose tissue mitochondria from very young obese Zucker pups', Fourth International Congress on Obesity, No. 326, 43A.

BAZIN, R. and LAVAU, M. (1982): 'Development of hepatic and adipose tissue lipogenic enzymes and insulinemia during suckling and weaning on to a high fat diet in Zucker rats', J. Lipid Res. 23, 839-849.

BECKER, E. and GRINKER, J. A. (1977): 'Meal patterns in the genetically obese Zucker rat', Physiol. Behav. 18: 685-692.

BELL, G. E. and STERN, J. S. (1977): 'Evaluation of body composition of young obese and lean Zucker rats', Growth 41, 63-80.

BERNARDIS, L. L. and GOLDMAN, J. K. (1976): 'Origin of endocrine-metabolic changes in the weanling rat ventromedial syndrome', J. Neur. Sci. Res. 2, 91-116.

BOULANGE, A., PLANCHE, E. and DE GASQUET, P. (1979a): 'Onset of obesity without hyperphagia in the newborn genetically obese Zucker rat', In: Diabetes and Obesity, (Vague, J. and Vague, Ph.), Excerpta Med., Amsterdam.

BOULANGE, A., PLANCHE, E. and DE GASQUET, P. (1979b): 'Onset of genetic obesity in the absence of hyperphagia during the first week of life in the Zucker rat (fa/fa)', J. Lipid Res. 20, 857-864.

BOULANGE, A., PLANCHE, E. and DE GASQUET, P. (1981): 'Onset and development of hypertriglyceridemia in the Zucker rat (fa/fa)', Metabolism 30, 1045-1052.

BRAY, G. A. (1969a): 'Oxygen consumption of genetically obese rats', Experientia 25, 1100-1101.

BRAY, G. A. (1969b): 'Studies on the composition of adipose tissue from the genetically obese rats', Proc. Soc. Exp. Biol. Med. 131, 1111-1114.

BRAY, G. A. (1976): The Obese Patient, W. B. Saunders Co., Philadelphia.

BRAY, G. A. (1977): 'The Zucker fatty rat: A review', Fed. Proc. 36, 148-153.

BRAY, G. A., FISHER, D. A. and CHOPRA, I. J. (1976): 'Relation of thyroid hormones to body weight', Lancet 1, 1206-1208.

BRAY, G. A. and YORK, D. A. (1971a): 'Genetically transmitted obesity in rodents', Physiol. Rev. 59, 598-646.

BRAY, G. A. and YORK, D. A. (1971b): 'Thyroid function of genetically obese rats', Endocrinology 88, 1095-1099.

BRAY, G. A. and YORK, D. A. (1972): 'Studies on food intake of genetically obese rats', Am. J. Physiol. 223, 176-179.

BRAY, G. A. and YORK, D. A. (1979): 'Hypothalamic and genetic obesity in experimental animals: An autosomal and endocrine hypothesis', Physiol. Rev. 59, 719-809.

BRAY, G. A., YORK, D. A. and SWERLOFF, R. S. (1973): 'Genetic obesity in rats. 1. The effects of food restriction on body composition and hypothalamic function', Metabolism 22, 435-442.

BROOKS, S. L., ROTHWELL, N. J. and STOCK, M. J. (1982): 'Effects of diet and acute noradrenaline treatment on brown adipose tissue development and mitochondrial purine-nucleotide binding', Q. J. Exp. Physiol. Cogn. Med. Sci. 67, 259-269.

BROOKS, S. L., ROTHWELL, N. J., STOCK, M. J., GOODBODY, A. E. and TRAYHURN, P. (1980): 'Increased proton conductance pathway in brown adipose tissue mitochondria of rats exhibiting diet-induced thermogenesis', Nature 286, 274-276.

BRUCE, B. K., KING, B. M., PHELPS, G. R. and VEITA, M. C. (1982): 'Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats', Am. J. Physiol. 243, E152-157.

BRÜCK, K. (1970): 'Non-shivering thermogenesis and brown adipose tissue in relation to age', In: Brown Adipose Tissue, Lindberg, O. (Ed), Elsevier, Holland, 117-154.

BRYANT, K. R., ROTHWELL, N. J., STOCK, M. J. and STRIBLING, D. (1983): 'Identification of two mitochondrial GDP-binding sites in rat brown adipose tissue', Biosci. Rep. 3, 589-598.

BUKOWIECKI, L., FOLLEA, N., LUPIEN, J. and PARADIS, A. (1981): 'Metabolic relationships between lypolysis and respiration in rat brown adipocytes', J. Biol. Chem. 256, 12840-12848.

BUKOWIECKI, L., FOLLEA, N., PARADIS, A. and COLLET, A. (1980): 'Stereospecific stimulation of brown adipocyte respiration by catecholamines via β adrenoreceptors', Am. J. Physiol. 238, E552-563.

BUKOWEICKI, L., FOLLEA, N., VALLIERES, J. and LE BLANC, J. (1978): 'β-adrenergic receptors in brown adipose tissue. Characterization and alterations during acclimation of rats to cold', Eur. J. Biochem. 92, 189-196.

BULYCHEV, A., KRAMAR, R., DRAHOTA, Z. and LINDBERG, O. (1972): 'Role of a specific endogenous fatty acid fraction in the coupling-uncoupling mechanism of oxidative phosphorylation of brown adipose tissue', Exp. Cell. Res. 72, 169-187.

CANNON, B., HEDIN, A. and NEDERGAARD, J. (1982): 'Exclusive occurrence of thermogenin antigen in brown adipose tissue', FEBS Lett. 150, 129-132.

CANNON, B. and LINDBERG, O. (1979): 'Mitochondria from brown adipose tissue: Isolation and properties', Methods Enzymol. 55, 65-78.

CANNON, B. and NEDERGAARD, J. (1979): 'The physiological role of pyruvate carboxylation in hamster brown adipose tissue', Eur. J. Biochem. 94, 419-426.

CANNON, B. and JOHANSSON, B. W. (1980): 'Nonshivering thermogenesis in the newborn', Molec. Aspects Med. 3, 119-223.

CASTONGUAY, T. W., HARTMAN, W. J., FITZPATRICK, E. A. and STERN, J. S. (1982): 'Dietary self-selection and the Zucker rat', J. Nutr. 112, 796-800.

CASTONGUAY, T. W. and STERN, J. S. (1983): 'The effect of adrenalectomy on dietary component selection by the genetically obese Zucker rat', Nutr. Rep. Int. 28, 725-732.

CAWTHORNE, M. A. (1979): 'The use of animal models in the detection and evaluation of compounds for the treatment of obesity', In: Animal Models of Obesity, Festing, M. F. W. (Ed), Macmillan Press, London, 15-37.

CHAN, C. B., PEDERSON, R. A. and BUCHAN, A. M. J. (1983): 'Development of hyperinsulinemia in the Zucker 'fatty' rat', Can. J. Physiol. Pharmacol. 61, AV.

CHAN, C. P., KOONG, L. J. and STERN, J. S. (1982): 'Effect of insulin on fat and protein deposition in diabetic lean and obese rats', Am. J. Physiol. 242, E19-24.

CHAN, C. P. C. and STERN, J. S. (1981): 'Hyperinsulinemia is permissive but not primary in the development of obesity in the genetically obese Zucker rat (fa/fa)', In: The Body Weight Regulatory System: Normal and Disturbed Mechanisms, Cioffi, L. A. et al. (Eds), Raven Press, New York, 65-68.

CHAN, C. P. and STERN, J. S. (1982): 'Adipose lipoprotein lipase in insulin-treated diabetic lean and obese Zucker rats', Am. J. Physiol. 242, E445-450.

CHEN, R. F. (1967): 'Removal of fatty acids from serum albumin by charcoal treatment', J. Biol. Chem. 242, 173-181.

CHINET, A., CLAUSEN, T. and GIRARDIER, L. (1977): 'Microcalorimetric determination of energy expenditure due to active sodium-potassium transport in the soleus muscle and brown adipose tissue of the rat', J. Physiol. 265, 43-61.

CHOPRA, I. J., WILLIAMS, D. E., ORGIAZZI, J. and SOLOMON, D. (1975): 'Opposite effects of dexamethosone on serum concentration of 3, 3' 5' triiodothyronine (rT_3) and 3 3' 5 triiodothyronine (T_3)', J. Clin. Endocrinol. Metab. 41, 911-920.

CLEARY, M. P., VASSELLI, J. R. and GREENWOOD, M. R. C. (1980): 'Development of obesity in Zucker obese (fa/fa) rat in the absence of hyperphagia', Am. J. Physiol. 238, E284-292.

CONKLIN, P. and HEGGENESS, F. W. (1971): 'Maturation of temperature homeostasis in the rat', Am. J. Physiol. 220, 333-336.

COTTLE, W. H. (1970): 'The innervation of brown adipose tissue', In: Brown Adipose Tissue, Lindberg, O. (Ed), Elsevier, Holland, 155-178.

CRETTAZ, M., FREYCHET, P. and JENRENAUD, B. (1978): 'Intracellular defects and insulin resistance in isolated soleus muscles of genetically obese (fa/fa) rats', Diabetes 27, 453A.

CUELLO, C. A. (1983): 'Central distribution of opioid peptides', Br. Med. Bull. 39, 11-16.

DEAVERS, D. R. and MUSACCHIA, X. J. (1979): 'The function of glucocorticoids in thermogenesis', Fed. Proc. 38, 2177-2181.

DEB, S. and MARTIN, R. (1975): 'Effect of exercise and of food restriction on the development of spontaneous obesity in rats', J. Nutr. 105, 543-549.

DEB, S., MARTIN, R. J. and HERSHBERGER, T. V. (1976): 'Maintenance requirement and energetic efficiency of lean and obese Zucker rats', J. Nutr. 106, 191-197.

DEPOCAS, F., ZAROR-BEHRENS, G. and LACELLE, S. (1979): 'Noradrenaline-induced calorigenesis in warm and in cold acclimated rats: effect of desmethylimipramine and normetanephrine', Fed. Proc. 38, 1227.

DESAUTELS, M., ZAROR-BEHRENS, G. and HIMMS-HAGEN, J. (1978): 'Increased purine nucleotide binding, altered polypeptide composition and thermogenesis in brown adipose tissue mitochondria of cold-acclimated rats', Can. J. Biochem. 56, 378-383.

DESAUTELS, M. and HIMMS-HAGEN, J. (1979): 'Roles of noradrenaline and protein synthesis in the cold-induced increase in purine nucleotide binding by rat brown adipose tissue mitochondria', Can. J. Biochem. 57, 968-976.

DESAUTELS, M. and HIMMS-HAGEN, J. (1980): 'Parallel regression of cold-induced changes in ultrastructure, composition and properties of brown adipose tissue mitochondria during recovery of rats from acclimation to cold', Can. J. Biochem. 58, 1057-1068.

DESAUTELS, M. and HIMMS-HAGEN, J. (1981): 'Brown adipose tissue mitochondria of cold acclimated rats: change in characteristics of purine nucleotide control of the proton electrochemical gradient', Can. J. Biochem. 59, 619-625.

DILETTUSO, B. A. and WANGSNESS, P. J. (1977): 'Effect of age on hyperphagia in the genetically obese Zucker rat', Proc. Soc. Expt. Biol. Med. 154, 1-5.

DONATI, R. M., WARNECKE, M. A. and GALLAGHER, N. I. (1963): 'The effect of absolute caloric deprivation on thyroid hormone synthesis and release in the rat', Metabolism 12, 833-836.

DUBUC, P. V. (1976): 'Basal corticosterone levels of young (ob/ob) mice', Horm. Metab. Res. 9, 95-97.

DUNCOMBE, W. G. (1964): 'The colorimetric micro-determination of non-esterified fatty acids in plasma', Clin. Chim. Acta 9, 122-125.

ERSKINE, L. K. (1975): 'Effects of adrenergic blocking agents on the response of brown adipose tissue to neural stimulation', Fed. Proc. 34, 477.

FAHMY, D., REEDS, G. and HILLIER, S. (1975): 'Some observations on the determination of cortisol in human plasma by radioimmunoassa- using antisera against cortisol-3-BSA', Steroids 26, 267-280.

FAIN, J. N., REED, N. and SAPERSTEIN, R. (1967): 'The isolation and metabolism of brown fat cells', J. Biol. Chem. 242, 1887-1894.

FAWCETT, D. W. and JONES, I. C. (1949): 'The effects of hypophysectomy, adrenalectomy and thiouracil feeding on the cytology of brown adipose tissue', Endocrinology 45, 609-621.

FELDMAN, D. (1978): 'Evidence that brown adipose tissue is a glucocorticoid target organ', Endocrinology 103, 2091-2097.

FELLENZ, M., TRIANDAFILLOU, J., GWILLIAM, C. and HIMMS-HAGEN, J. (1982): 'Growth of interscapular brown adipose tissue in cold-acclimated hypophysectomized rats maintained on thyroxine and corticosterone', Can. J. Biochem. 60, 838-842.

FESTING, M. F. W. (1979): 'The inheritance of obesity in animal models of obesity', In: Animal Models of Obesity, Festing, M. F. W. Macmillan Press Ltd., London, 15-38.

FLAIM, K. E., HORWITZ, B. A., HOROWITZ, J. M. (1977): 'Coupling of signals to brown fat: α and β-adrenergic responses in intact rats', Am. J. Physiol. 232, R101-109.

FLAIM, K. E., HOROWITZ, J. M. and HORWITZ, B. A. (1976): 'Functional and anatomical characteristics of the nerve-brown adipose tissue interaction in the rat', Pflugers Arch. 365, 9-14.

FLATMARK, T. and PEDERSEN, J. I. (1975): 'Brown adipose tissue mitochondria', Biochim. Biophys. Acta 416, 53-103.

FOSTER, D. O. and FRYDMAN, M. L. (1978): 'Non shivering thermogenesis in the rat. II: Measurement of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline', Can. J. Physiol. Pharmacol. 56, 110-122.

FOSTER, D. O. and FRYDMAN, M. L. (1979): 'Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats re-evaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by non-shivering thermogenesis', Can. J. Physiol. Pharmacol. 57, 257-270.

FREGLY, M. J., FIELD, F. P., KATOVICH, M. J. and BARNEY, C. C. (1979): 'Catecholamine-thyroid hormone interactions in cold-acclimated rats', Fed. Proc. 38, 2162-2169.

FROHMAN, L. A. (1978): 'The syndrome of hypothalamic obesity', In: Recent Advances in Obesity Research II, (Bray, G. A. - Ed), Newman, London, 133-141.

GALPIN, K. S., HENDERSON, R. G., JAMES, W. P. T. and TRAYHURN, P. (1983): 'GDP binding to brown adipose-tissue mitochondria of mice treated chronically with corticosterone', Biochem. J. 214, 265-268.

GARFINKEL, A. S., NILSSON-EHLE, P. and SCHOTZ, M. C. (1976): 'Regulation of lipoprotein lipase induction by insulin', Biochem. Biophys. Acta. 424, 264-273.

GARROW, J. S. (1978): In: Energy Balance and Obesity in Man, 2nd ed., Elsevier/North-Holland Press, New York.

GIBSON, A. (1981): 'The influence of endocrine hormones on the autonomic nervous system', J. Auton. Pharmacol. 1, 331-358.

GIRARDIER, L. (1977): 'The regulation of the biological furnace of warm blooded animals', Experientia 33, 1121-1122.

GIRARDIER, L. (1981): 'Brown adipose tissue as energy dissipator: a physiological approach', In: Obesity: Pathogenesis and Treatment Enzi, G., Crepaldi, G., Pozza, G., Renold, A. E. (Eds), Academic Press, London, 55-72.

GODBOLE, V. Y., GRUNDLEGER, M. L., PASQUINE, T. A. and THENEN, S. W. (1981): 'Composition of rat milk from day 5-20 of lactation and milk intake of lean and preobese Zucker pups', J. Nutr. 111, 480-487.

GODBOLE, V. and YORK, D. A. (1978): 'Lipogenesis in situ in the genetically obese Zucker rat (fa/fa). Role of hyperphagia and hyperinsulinemia', Diabetologia 14, 191-197.

GODBOLE, V., YORK, D. A. and BLOXHAM, D. P. (1978): 'Developmental changes in the fatty (fa/fa) rat: evidence for defective thermogenesis preceding the hyperlipogenesis and hyperinsulinemia', Diabetologia 15, 41-44.

GOODBODY, A. E. and TRAYHURN, P. (1981): 'GDP binding to brown-adipose-tissue mitochondria of diabetic-obese (db/db) mice', Biochem. J. 194, 1019-1022.

GOUBERN, M. and PORTET, R. (1981): 'Circadian rhythm and hormonal sensitivity of lipoprotein lipase activity in cold acclimated rats', Horm. Metab. Res. 13, 73-77.

GRANDISON, L. and GUIDOTTI, A. (1977): 'Stimulation of food intake by Muscimol and beta-endorphin', Neuropharmacology 16, 533-536.

GREEN, C. J. (1975): 'Neuroleptanalgesia drug combination in the anaesthetic management of small laboratory animals', Laboratory Animals 9, 161-178.

GREEN, I. C., PERRIN, D., PEDLEY, K. C., LESLIE, R. D. G. and PYKE, D. A. (1980): 'Effect of enkephalins and morphine on insulin secretion from isolated rat islets', Diabetologia 19, 158-161.

GRUBE, D., VOIGHT, K. H. and WEBER, E. (1978): 'Pancreatic glucagon cells contain endorphin-like immunoreactivity', Histochemistry 59, 75-79.

GRUEN, R. E., HIETANEN, E. and GREENWOOD, M. R. C. (1978): 'Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa)', Metabolism 27, 1955-1966.

HABEREY, P., BACH, B., SCHAEFER, A. and PIQUARD, F. (1980): 'Spontaneous activity and food requirements for maintenance and for growth in the genetically obese Zucker rat', Nutr. Metab. 24, 218-227.

HAHN, P., DRAHOTA, Z., SKALA, J., KAZDA, S. and TOWELL, M. E. (1969): 'The effect of cortisone on brown adipose tissue of young rats', Can. J. Physiol. Pharmacol. 47, 975-980.

HALES, C. N. and RANDLE, P. J. (1963): 'Immunoassay of insulin with insulin antibody precipitate', Bioch. J. 88, 137-146.

HALLFRISCH, J., COHEN, L., and REISER, S. (1981): 'Effect of feeding rats sucrose in a high-fat diet', J. Nutr. 111, 531-536.

HALLFRISCH, J., LAZAR, F. L. and REISER, S. (1978): 'Epididymal fat metabolism in rats fed sucrose or starch', Nutr. Rep. Int. 18, 359-367.

HAN, P. W. (1967): 'Hypothalamic obesity in rats without hyperphagia', Trans. N.Y. Acad. Sci. 30, 229-243.

HAN, P. W. (1968): 'Energy metabolism of tube-fed hypophysectomized rats bearing hypothalamic lesions', Am. J. Physiol. 215, 1343-1350.

HARDEVELD, C. van, ZUIDWIJK, M. J. and KASSENAAR, A. A. H. (1979a): 'Studies on the origin of altered thyroid hormone levels in the blood of rats during cold exposure', Acta Endocr. 91, 473-483.

HARDEVELD, C. van, ZUIDWIJK, M. J. and KASSENAAR, A. A. H. (1979b): 'Studies on the origin of altered thyroid hormone levels in the blood of rats during cold exposure', Acta Endocr. 91, 484-492.

HARRIS, A., FANG, S. L., VAGENAKIS, A. G. and BRAVERMAN, L. E. (1978): 'Effect of starvation, nutriment replacement and hypothyrodisim on *in vitro* hepatic T_4 to T_3 conversion in the rat', Metabolism 27, 1680-1690.

HASHIM, S. (1981): 'Human eating behaviour', In: *Obesity: Pathogenesis and Treatment*, Enzi, G., Crapaldi, G., Pozza, G. and Renold, A. E. (Eds), Academic Press, London, 225-236.

HAUSBERGER, F. X. (1958): 'Action of insulin and corticosterone on adipose tissue', Diabetes 7, 211-220.

HAUSBERGER, F. X. and WIDELITZ, M. M. (1963): 'Distribution of labelled erythrocytes in adipose tissue and muscle in the rat', Am. J. Physiol. 204, 649-652.

HEATON, G. M. and NICHOLLS, D. G. (1976): 'Hamster brown-adipose-tissue mitochondria', Eur. J. Biochem. 67, 511-517.

HEATON, G. M., WAGENVOORD, R. J., KEMP, A. and NICHOLLS, D. G. (1978): 'Brown adipose tissue mitochondria: Photoaffinity labelling of the regulatory site of energy dissipation', Eur. J. Biochem. 82, 515-521.

HEATON, J. M. (1972): 'The distribution of brown adipose tissue in the human', J. Anat. 112, 35-39.

HEICK, H. M. C., VACHON, C., KALLAI, M. A., BEGIN-HEICK, N. and LE BLANC, J. (1973): 'The effects of thyroxine and isopropyl-noradrenaline on cytochrome oxidase activity in brown adipose tissue', Can. J. Physiol. Pharmacol. 51, 751-758.

HEMINGWAY, A. (1963): 'Shivering', Physiol. Rev. 43, 397-422.

HERBERG, L. and COLEMAN, D. L. (1977): 'Laboratory animals exhibiting obesity and diabetes syndromes', Metabolism 26, 59-99.

HETHERINGTON, A. W. and RANSON, S. W. (1940): 'Hypothalamic lesions and adiposity in the rat', Anat. Rec. 78, 149-172.

HIMMS-HAGEN, J. (1967): 'Sympathetic regulation of metabolism', Pharmacol. Rev. 19, 361-461.

HIMMS-HAGEN, J. (1975): 'Role of the adrenal medulla in adaptation to cold', In: Handbook of Physiology VI, 637-665.

HIMMS-HAGEN, J. (1976): 'Cellular thermogenesis', Ann. Rev. Physiol. 38, 315-351.

HIMMS-HAGEN, J. (1983a): 'Brown adipose tissue thermogenesis in obese animals', Nutr. Rev. 41, 261-267.

HIMMS-HAGEN, J. (1983b): 'Role of thermogenesis in brown adipose tissue in the regulation of energy balance', In: The Adipocyte and Obesity: Cellular and Molecular Mechanisms , Angel, A., Hollenberg, C. H., and Roncari, D. (Eds), Raven Press, New York.

HIMMS-HAGEN, J. (1983c): 'Thyroid hormones and thermogenesis', In: Mammalian Thermogenesis, Girardier & Stock (Eds), Chapman and Hall, London, 141-177.

HIMMS-HAGEN, J. and DESAUTELS, M. (1978): 'A mitochondrial defect in brown adipose tissue of the obese (ob/ob) mouse: Reduced binding of purine nucleotides, and a failure to respond to cold by an increase in binding', Biochem. Biophys. Res. Commun. 83, 628-634.

HIMMS-HAGEN, J., DITTMAR, E. and ZAROR-BEHRENS, G. (1980): 'Polypeptide turnover in brown adipose tissue mitochondria during acclimation of rats to cold', Can. J. Biochem. 58, 336-344.

HIMMS-HAGEN, J., TRIANDAFILLOU, J. and GWILLIAM, C. (1981): 'Brown adipose tissue of cafeteria fed rats', Am. J. Physiol. 241, E116-120.

HOEBEL, B. G. and TEITELBAUM, P. (1966): 'Weight regulation in normal and hypothalamic hyperphagic rats', J. Comp. Physiol. Psychol. 61, 180-193.

HOGAN, S., COSCINA, D. V. and HIMMS-HAGEN, J. (1982): 'Brown adipose tissue of rats with obesity-inducing ventromedial hypothalamic lesions', Am. J. Physiol. 243, E338-344.

HOGAN, S. and HIMMS-HAGEN, J. (1980): 'Abnormal brown adipose tissue in obese mice (ob/ob), response to acclimation to cold', Am. J. Physiol. 239, E301-309.

HOHORST, H. J. and RAFAEL, J. (1968): 'Oxydative phosphorylierung durch mitochondrion aus braunem fettgewebe', Hoppe-Seyler's Z Physiol. Chem. 349, 268-270.

HOOK, W. E. and BARRON, E. S. G. (1941): 'The respiration of brown adipose tissue and kidney of the hibernating and non-hibernating ground squirrel', Am. J. Physiol. 133, 56-63.

HORWITZ, B. A. (1973): 'Oubain sensitive component of brown fat thermogenesis', Am. J. Physiol. 224, 352-355.

HSIEH, A. C. L. and CARLSON, L. D. (1957): 'Role of adrenaline and noradrenaline in chemical regulation of heat production', Am. J. Physiol. 190, 243-246.

HSIEH, A. C. L., CARLSON, L. D. and GRAY, G. (1957): 'Role of the sympathetic nervous system in the control of chemical regulation of heat production', Am. J. Physiol. 190, 247-251.

HSIEH, A. C. L., PUN, C. W., LI, K. M. and TI, K. W. (1966): 'Circulatory and metabolic effects of noradrenaline in cold-adapted rats', Fed. Proc. 25, 1205-1209.

IKEDA, H., NISHIKAWA, K. and MATSUO, T. (1980): 'Feeding responses of Zucker fatty rats to 2-deoxy-D-glucose, norephineprine and insulin', Am. J. Physiol. 239, E379-384.

INGBAR, D. H. and GALTON, V. A. (1975): 'The effect of food deprivation on the peripheral metabolism of thyroxine in rats', Endocrinology 96, 1525-1531.

ISMAIL-BEIGI, F. and EDELMAN, I. S. (1970): 'Mechanism of thyroid calorigenesis, role of active sodium transport', Proc. Nat. Acad. Sci. 67, 1071-1078.

IPP, E., DOBBS, R. and UTIGER, R. H. (1978): 'Morphine and β-endorphin influence on the secretion of the endocrine pancreas', Nature 276, 190-191.

JAMES, W. P. T. and TRAYHURN, P. (1976): 'An integrated view of the metabolic and genetic basis for obesity', Lancet 2, 770-772.

JAMES, W. P. T. and TRAYHURN, P. (1981): 'Obesity in mice and men', In: Nutritional Factors: Modulating Effects on Metabolic Processes, Raven Press, New York.

JANSKY, L. (1973): 'Non shivering thermogenesis and its thermoregulatory significance', Biol. Rev. 48, 85-132.

JEANRENAUD, B. (1978): 'An overview of experimental models of obesity', In: Recent Advances in Obesity Research 2, Bray, G. A. (Ed), Newman Publishing Co., London, 111-122.

JOEL, C. D. and BALL, E. G. (1962): 'The electron transmitter system of brown adipose tissue', Biochemistry 1, 281-287.

JOHNSON, P. R., STERN, J. S., GREENWOOD, M. R. C., ZUCKER, L. M. and HIRSCH, J. (1973): 'Effect of early nutrition on adipose cellularity and pancreatic insulin release in the Zucker rat', J. Nutr. 103, 738-743.

JOHNSON, P. R., ZUCKER, L. M., CRUCE, J. A. and HIRSCH, J. (1971): 'Cellularity of adipose depots in the genetically obese Zucker rat', J. Lipid Res. 12, 704-714.

JUNG, R. T., SHETTY, P. S., JAMES, W. P. T., BARRAND, M. and CALLINGHAM, B.A. (1979): 'Reduced thermogenesis in obesity', Nature 279, 322-323.

KANAREK, R. and HIRSCH, E. (1977): 'Dietary-induced overeating in experimental animals', Fed. Proc. 36, 154-158.

KANAREK, R. B. and MARKS-KAUFMANN, R. (1979): 'Development aspects of sucrose induced obesity in rats', Physiol. Behav. 23, 881-885.

KANAREK, R. B. and ORTHEN-GAMBILL, N. (1982): 'Differential effects of sucrose, fructose and glucose on carbohydrate induced obesity in rats', J. Nutr. 112, 1546-1554.

KANTER, R. A., ENSINCK, J. W. and FUJIMOTO, W. Y. (1980): 'Disparate effects of enkephalin and morphine upon insulin glucagon secretion by islet cell cultures', Diabetes 29, 84-86.

KAPLAN, M. L. (1977): 'Identification of fa/fa genotype during preobese phase of development', Fed. Proc. 36, 1149.

KAPLAN, M. L. (1979b): 'Consumption of oxygen and early detection of fa/fa genotype in rats', Metabolism 28, 1147-1151.

KAPLAN, M. L. (1981): 'Oxygen consumption by Zucker obese rats, obese yellow mice, and obese hyperglycemic mice with body protein used for metabolic mass', Int. J. Obesity 5, 51-56.

KAPLAN, M. M. (1979a): 'Subcellular alterations causing reduced hepatic thyroxine-5'-monodeiodinase activity in fasted rats', Endocrinology 104, 58-64.

KENNEDY, D. R., HAMMOND, R. P. and HAMOLSKY, M. W. (1977): 'Thyroid cold acclimation influences on norepinephrine metabolism in brown fat', Am. J. Physiol. 232, E565-569.

KING, B. M., BANTA, A. R., THAREL, G. N., BRUCE, B. K. and FROHMAN, L. A. (1983): 'Hypothalamic hyperinsulinemia and obesity: role of adrenal glucocorticoids', Am. J. Physiol. 245, E194-199.

KING, B. M., STAMOUTSOS, B. A. and GROSSMAN, S. P. (1978): 'Delayed response to 2-deoxy-D-glucose in hypothalamic obese rats', Pharmacol. Biochem. Behav. 8, 259-262.

KUROSHIMA, A., KONNO, N., DOI, K. and ITOH, S. (1968): 'Effect of corticotrophin and adrenocortical hormone on the blood flow through brown adipose tissue in the rat', Jap. J. Physiol. 18, 446-452.

LABRIE, F., RAYNAUD, J. P., DUNCOMMUN, P. and FORTIER, C. (1964): 'Influence of thyroid activity or corticosterone binding by plasma proteins', Proc. Can. Fed. Biol. Soc. 7, 44.

LACHANCE, J. P. and PAGE, E. (1953): 'Hormonal factors influencing fat deposition in the interscapular brown adipose tissue of the white rat', Endocrinology 52, 57-64.

LANDSBERG, L. and YOUNG, J. B. (1978): 'Fasting, feeding and regulation of the sympathetic nervous system', New Eng. J. Med. 298 1295-1301.

LANDSBERG, L. and YOUNG, J. B. (1981): 'Diet-induced changes in sympathoadrenal activity: Implications for thermogenesis', Life Sci. 28, 1801-1807.

LANDSBERG, L. and YOUNG, J. B. (1982): 'Effect of nutritional status on autonomic nervous system function', Am. J. Clin. Nutr. 35, 1234-1240.

LANDSBERG, L. and YOUNG, J. B. (1983): 'Autonomic regulation of thermogenesis', In: Mammalian Thermogenesis, Girardier, L. and Stock, M. J. (Eds), Chapman & Hall, London, 99-140.

LARSSON, L. I., BODER, G. B. and SHAW, W. W. (1977): 'Changes in the islet of Langerhans in the obese Zucker rat', Lab. Invest. 36, 593-598.

LAUBIE, M., SCHMITT, H., VINCENT, M. and REMOND, G. (1977): 'Central cardiovascular effects of morphinomimetic peptides in dogs', Eur. J. Pharmacol. 46, 67-71.

LAURY, M. C. and PORTET, R. (1977): 'Corticotrophin and nonshivering thermogenesis', Experimentia 33, 1474-1475.

LAVAU, M. and BAZIN, R. (1982): 'Inguinal fat pad weight plotted versus body weight as a method of genotype identification in 16 day old Zucker rats', J. Lipid Res. 23, 941-943.

LAVAU, M., BAZIN, R., KARAOGH-LANIAN, Z and GUICHARD, C. (1982): 'Evidence for a high fatty acid synthesis activity in interscapular brown adipose tissue of genetically obese Zucker rats', Biochem. J. 204, 503-507.

LEAN, M. E. J., BRACH, W. J., JAMES, W. P. T., JENNINGS, G. and ASHWELL, M. (1983): 'Measurement of rat brown adipose tissue mitochondrial uncoupling protein by radioimmunoassay: Increased concentration after cold acclimation', Biosci. Rep. 3, 67-71.

LEBLANC, J. and VILLEMAIRE, A. (1970): 'Thyroxine and noradrenaline on noradrenaline sensitivity cold resistance and brown fat', Am. J. Physiol. 218, 1742-1745.

LEDUC, J. (1961): 'Catecholamine production and release in acclimation to cold', Acta Physiol. Scan. 53, 5-101.

LEMONNIER, D. (1972): 'Effect of age, sex and site on the cellularity of adipose tissue in mice and rats rendered obese by a high fat diet', J. Clin. Inv. 51, 2907-2915.

LEONARD, J., MELLEN, S. A. and LARSEN, P. (1983): 'Thyroxine 5' deiodinase activity in brown adipose tissue', Endocrinology 112, 1153-1155.

LEVIN, B. E., TRISCARI, J. and SULLIVAN, A. C. (1980): 'Abnormal sympathoadrenal function and plasma catecholamine in obese Zucker rats', Pharmacol. Biochem. Behav. 13, 107-113.

LEVIN, B. E., TRISCARI, J. and SULLIVAN, A. C. (1981a): 'Defective catecholamine metabolism in peripheral organs of genetically obese Zucker rats', Brain Res. 224, 353-356.

LEVIN, B. E., TRISCARI, J. and SULLIVAN, A. C. (1981b): 'Metabolic and sympathoadrenal abnormalities in the obese Zucker rat: Effect of chronic phenoxybenzamine treatment', Pharmacol. Biochem. Behav. 14, 517-525.

LEVIN, B. E., TRISCARI, J. and SULLIVAN, A. C. (1982): 'Sympathetic activity in thyroid treated Zucker rats', Am. J. Physiol. 243, R170-178.

LIN, C. S., HACKENBERG, H. and KLINGENBERG, E. M. (1980): 'The uncoupling protein from brown adipose tissue mitochondria is a dimer. A hydrodynamic study', FEBS. Lett. 113, 304-306.

LIN, C. S. and KLINGENBERG, M. (1982): 'Characteristics of the isolated purine nucleotide binding protein from brown fat mitochondria', Biochemistry 21, 2950-2956.

LINDBERG, O. (1970): In: Brown Adipose Tissue, Elsevier, Holland.

LINDBERG, O., BEIBER, L. L. and HOUSTEK, J. (1976): 'Brown adipose tissue metabolism: an attempt to apply results from in vitro experiments on tissue in vivo', In: Regulation of Depressed Metabolism and Thermogenesis, Jansky, L. and Musacchia, X. J., (Eds), Thomas, Springfield, U.S.A., 117-136.

LINDBERG, O., DE PIERRE, J., RYLANDER, E. and AFZELIUS, B. A. (1967): 'Studies of the mitochondrial energy-transfer system of brown adipose tissue', J. Cell. Biol. 34, 293-310.

LOCKE, R. M. and NICHOLLS, D. G. (1981): 'A re-evaluation of the role of fatty acids in the physiological regulation of the proton conductance of brown adipose tissue mitochondria', FEBS. Lett. 135, 249-252.

LOCKE, R. M., RIAL, E. and NICHOLLS, D. G. (1982b): 'The acute regulation of mitochondrial proton conductance in cells and mitochondria from the brown fat of cold adapted and warm adapted guinea-pigs', Eur. J. Biochem. 129, 381-387.

LOCKE, M. RIAL, E., SCOTT, I. D. and NICHOLLS, D. G. (1982a): 'Fatty acids as acute regulators of the proton conductance of hamster brown fat mitochondria', Eur. J. Biochem. 129, 373-380.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951): 'Protein measurement with the Folin phenol reagent', J. Biol. Chem. 193, 267-275.

McCORMACK, J. G. (1982): 'The regulation of fatty acid synthesis in brown adipose tissue by insulin', Prog. Lipid Res. 21, 195-223.

McCRACKEN, K. J. and GRAY, R. (1976): 'A futile energy cycle in adult rats given a low protein diet at high levels of energy intake', Proc. Nutr. Soc. 35, 59A-60A.

MACDONALD, I. A., ROTHWELL, N. J. and STOCK, M. J. (1976): 'Lipolytic and lipogenic activities of adipose tissue during spontaneous fat depletion and repletion', Proc. Nutr. Soc. 35, 129A.

McEWEN, B. S. (1976): 'Interactions between hormones and nerve tissue', Sci. Am. 235 , 49-58.

MAINS, R. E., EIPPER, B. A. and LING, N. (1977): 'Common precursor to corticotrophins and endorphins', Proc. Natl. Acad. Sci. 74, 3014-3018.

MARCHINGTON, D., ROTHWELL, N. J., STOCK, M. J. and YORK, D. A. (1983): 'Energy balance, diet induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy', J. Nutr. 113, 1395-1402.

MARGULES, D. L., LEWIS, M. J., SHIBUYA, H. and PERT, C. B. (1978): 'β endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa)', Science 202, 988-991.

MARKS-KAUFMANN, R. and KANAREK, R. B. (1981): 'Modifications of nutrient selection induced by nalaxone in rats', Psychopharmacology 74, 321-324.

MARTIN, R. J. (1974): '<u>In vivo</u> lipogenesis and enzyme levels in adipose tissue and liver from pair-fed genetically obese and lean rats', Life Sci. 14, 1447-1453.

MARTIN, R. J. and LAMPREY, P. M. (1975): 'Early development of adipose cell lipogenesis and glycerol utilization in Zucker obese rats', Proc. Soc. Exp. Biol. Med. 149, 35-39.

MARTIN, R. J., WANGSNESS, P. J. and GAHAGAN, J. H. (1978): 'Diurnal changes in serum metabolites and hormones in lean and obese Zucker rats', Horm. Metabol. Res. 10, 187-192.

MAZZUCCHELLI, M. V., CONFALONIERI, C. and SCHLECHTER, P. (1961): 'The nervous system and lipid metabolism of adipose tissue', Metabolism 10, 330-334.

MEJSNAR, J. and JIRÁK, E. (1981): 'Thermogenic response as the function of extravascular influx of infused noradrenaline', Experientia 37, 482-483.

MILLER, D. S. (1979): 'Non genetic models of obesity', In: Animal Models of Obesity, Festing, M. F. W. (Ed), MacMillan Press Ltd., London, 131-140.

MILLER, D. S. and PARSONAGE, S. (1975): 'Resistance to slimming. Adaptation or illusion?', Lancet 1, 773-775.

MILLER, D. S. and PAYNE, P. R. (1962): 'Weight maintenance and food intake', J. Nutr. 78, 255-262.

MITCHELL, P. and MOYLE, J. (1969): 'Estimation of membrane potential and pH differences across the cristae membrane of rat liver mitochondria', Eur. J. Biochem. 7, 471-484.

MORLEY, J. E. (1980): 'The neuroendocrine control of appetite: The role of the endogenous opiates, cholecystokinin, TRH, gamma-amino-butyric-acid and the diazepam receptor', Life Sci. 27, 355-368.

MORY, G.,RICQUIER, D., NECHAD, M. and HEMON, P. (1982): 'Impairment of trophic response of brown fat to cold in guanethidine-treated rats', Am. J. Physiol. 242, C159-165.

MORY, G., RICQUIER, D., PESQUIES, P. and HEMON, P. (1981): 'Effects of hypothyroidism on the brown adipose tissue of adult rats: comparison with the effects of adaptation to cold', J. Endocrinol. 91, 515-524.

MOWERY, R. A. and HERSHBERGER, T. V. (1982): 'Effect of age and body weight on the maintenance requirement of lean and obese Zucker rats', J. Nutr. 112, 2116-2121.

MUSTEN, B., PEARCE, D. and ANDERSON, G. H. (1974): 'Food intake regulation in the weanling rat: Self selection of protein and energy', J. Nutr. 104, 563-572.

NAKANISHI, S., INOUE, A., KITA, T., NAKAMURA, M., CHANG, A., COHEN, S. and NUMA, S. (1979): 'Nucleotide sequence of cloned DNA for bovine corticotrophin-beta-endorphin prescursor', Nature 278, 423-427.

NEDERGAARD, J. and LINDBERG, O. (1982): 'The brown fat cell', Int. Rev. Cytol. 74, 188-286.

NICHOLLS, D. G. (1974): 'Hamster brown adipose tissue mitochondria. The chloride permeability of the inner membrane under respiring conditions. The influence of purine nucleotides', Eur. J. Biochem. 49, 583-593.

NICHOLLS, D. G. (1976a): 'The bioenergetics of brown adipose tissue mitochondria', FEBS Lett. 61, 103-110.

NICHOLLS, D. G. (1976): 'Hamster brown adipose tissue mitochondria. Purine nucleotide control of the ion conductance of the inner membrane. The nature of the nucleotide binding site', Eur. J. Biochem. 62, 223-228.

NICHOLLS, D. G. (1979): 'Brown adipose tissue mitochondria', Biochim. Biophys. Acta. 549, 1-29.

NICHOLLS, D. G., HANS, J. G. and LINDBERG, O. (1972): 'Mitochondria from hamster brown adipose tissue', Eur. J. Biochem. 31, 526-533.

NICHOLLS, D. G. and LINDBERG, O. (1973): 'Brown adipose tissue mitochondria: The influence of albumen and nucleotide on passive ion permeabilities', Eur. J. Biochem. 37, 523-530.

OOMURA, Y., OOYAMA, H., NAKA, F., YAMAMOTO, T., ONO, T. and KOBAYASHI, N. (1969): 'Some stochastical patterns of single unit discharges in the cat hypothalamus under chronic conditions', Ann. N. Y. Acad. Sci. 157, 666-689.

ORWOLL, E. S. and KENDALL, J. W. (1980): 'β-endorphin and adreno-corticotrophin in extrapituitary sites: gastrointestinal tract', Endocrinology 107: 438-442.

PAMENTER, R. W. and HEDGE, G. A. (1980): 'Inhibition of thyrotropin secretion by physiological levels of corticosterone', Endocrinology 106, 162-166.

PEDERSEN, J. I. (1970): 'Coupled endogenous respiration in brown adipose tissue mitochondria', Eur. J. Biochem. 16, 12-18.

PEDERSEN, J. I. and GRAV, H. J. (1972): 'Physiologically-induced loose coupling of brown-adipose-tissue mitochondria correlated to endogenous fatty acids and adenosine phosphates,' Eur. J. Biochem. 25, 75-83.

PEDERSEN, J. I., SLINDE, E., GRYNNE, B., and AAS, B. (1975): 'The intracellular localization of long-chain acyl-CoA synthetase in brown adipose tissue', Biochim. Biophys. Acta 398, 191-203.

PERKINS, M. N., ROTHWELL, N. J., STOCK, M. J. and STONE, T. W. (1981): 'Activation of brown adipose tissue thermogenesis by the ventro-medial hypothalamus', Nature 289, 401-402.

PETERSON, A. D. and BAUMGARDT, B. R. (1971): 'Food and energy intake of rats fed diets varying in energy concentration and density', J. Nutr. 101, 1057-1068.

PETTERSSON, B. (1977): 'CO_2-mediated control of fatty acid metabolism in isolated hamster brown-fat cells during norepinephrine stimulation', Eur. J. Biochem. 72, 235-240.

PETTERSSON, B. and VALLIN, I. (1976): 'Norephinephrine shift in levels of adenosine 3'-5' monophosphate and ATP parallel to increased respiratory rates and lypolysis in isolated hamster brown fat cells', Eur. J. Biochem. 62, 383-390.

PITTMAN, J. A., BROWN, R. W. and REGISTER, H. B. Jr. (1962): 'Biological activity of 3, 3'5'-triiodo-DL-thyronine', Endocrinology 70, 79-83.

PLANCHE, E., JOLIFF, M., DE GASQUET, P. and LELIEPVRE, X. (1983): 'Evidence of a defect in energy expenditure in 7 day old Zucker rats (fa/fa)', Am. J. Physiol. 245, E107-113.

PORTET, R. (1981): 'Lipid biochemistry in the cold-acclimated rat', Comp. Biochem. Physiol. 70B, 679-688.

PORTET, R., LAURY, M. C., BERTIN, R., SENAULT, C., HLUSZKO, M. T. and CHEVILLARD, L. (1974): 'Hormonal stimulation of substrate utilization in brown adipose tissue of cold acclimated rats', Proc. Soc. Exp. Biol. Med. 147, 807-812.

POSPISILOVA, D. & JANSKY , L. (1976): 'Effect of various adaptational temperatures on oxidative capacity of the brown adipose tissue', Physiol. Bohemoslov. 25, 519-527.

POWLEY, T. and MORTON, S. (1976): 'Hypophysectomy and regulation of body weight in genetically obese Zucker rats', Am. J. Physiol. 230, 982-987.

POWLEY, T. L. and OPSAHL, C. A. (1974): 'Ventromedial hypothalamic obesity abolished by subdiaphragmatic vagotomy', Am. J. Physiol. 226, 23-33.

PRESSMAN, B. C. and LARDY, H. A. (1956): 'Effect of surface active agents on the latent ATPase of mitochondria', Biochim. Biophys. Acta 21, 458-466.

PRUSINER, S. B., CANNON, B. and LINDBERG, O. (1968b): 'Oxidative metabolism in cells isolated from brown adipose tissue. Catecholamine and fatty acid stimulation of respiration', Eur. J. Biochem. 6, 15-22.

PRUSINER, S. B., EISENHARDT, R. H., RYLANDER, E. and LINDBERG, O. (1968a): 'The regulation of oxidative metabolism of isolated brown fat cells', Biochem. Biophys. Res. Commun. 30, 508-515

PRUSINER, S., WILLIAMSON, J. R., CHANCE, B. and PADDLE, R. M. (1968c): 'Pyridine nucleotide changes during thermogenesis in brown fat tissue in vivo', Arch. Biochem. Biophys. 123, 368-377.

PULLAR, J. D. and WEBSTER, A. J. F. (1974): 'Heat loss and energy retention during growth in congenitally obese and lean rats', Br. J. Nutr. 31, 377-392.

PULLAR, J. D. and WEBSTER, A. J. (1977): 'The energy cost of fat and protein deposition in the rat', Br. J. Nutr. 37, 355-363.

RADCLIFFE, J. D. and WEBSTER, A. J. F. (1976): 'Regulation of food intake during growth in 'fatty' and lean female rats given diets of different protein content', Br. J. Nutr. 36, 457-469.

RADOMSKI, M. W. and ORME, T. (1971): 'Response of lipoprotein lipase in various tissues to cold exposure', Am. J. Physiol. 220, 1852-1856.

RAFAEL, J. and HELDT, H. W. (1976): 'Binding of guanine nucleotides to the outer surface of the inner membrane of guinea-pig brown fat mitochondria in correlation with the thermogenic capacity of the tissue', FEBS Lett. 63, 304-308.

RAKIETEN, N., RAKIETEN, M. L. and NADKARNI, M. V. (1963): 'Studies on the diabetogenic action of Streptozotocin (NSC-3791M)', Cancer Chemother. Rep. 29, 91-98.

RECANT, L., VOYLES, N., WADE, A., AWOKE, S. and BHATHENA, S. (1983b): 'Studies on the role of opiate peptides in two forms of genetic obesity ob/ob mouse and fa/fa rat', Horm. Metabol. Res. 15, 589-593.

RECANT, L., VOYLES, N., WADE, A., AWOKE, S., SMITH, S., BHATHENA, S. J. and PERT, C. B. (1983a): 'Gastrointestinal and pancreatic opiod activity: alteration with fasting and diabetes in rats', Endocrinology (in press).

REEDS, P. J., HAGGARTY, P., WAHLE, W. J. and FLETCHER, J. M. (1982): Tissue and whole-body protein synthesis in immature Zucker rats and their relationship to protein deposition', Biochem. J. 204, 393-398.

RENOLD, A. E. (1981): 'Epidemiologic considerations of overweight and of obesity', In: Obesity: Pathogenesis and Treatment, Enzi, G., Crepaldi, G., Pozza, G. and Renold, A. E. (Eds) Academic Press, 1-6.

RICQUIER, D., GERVAIS, C., KADER, J. C. and HEMON, Ph. (1979a): 'Partial purification by guanosine-5'-diphosphate-agarose affinity chromatography of the 32,000 molecular weight polypeptide from mitochondria of brown adipose tissue', FEBS Lett. 101, 35-38.

RICQUIER, D. and KADER, J. C. (1976): 'Mitochondrial protein alteration in active brown fat and SDS polyacrylamide gel electrophoretic study', Biochem. Biophys. Res. Commun. 73, 577-583.

RICQUIER, D., MORY, G. and HEMON, P. (1979b): 'Changes induced by cold-adaptation in the brown adipose tissue from several species of rodents with special reference to the mitochondrial component', Can. J. Biochem. 57, 1262-1266.

RICQUIER, D., MORY, G., NECHAD, M. and HEMON, P. (1978): 'Effects of cold adaptation and re-adaptation upon the mitochondrial phospholipids of brown adipose tissue', J. Physiol. (Paris) 74, 695-702.

ROHNER-JEANRENAUD, F., HOCHOTRASSER, A. C. and JEANRENAUD, B. (1983): 'Hyperinsulinemia of preobese and obese fa/fa rats is partly vagus nerve mediated', Am. J. Phys. 244, E317-322.

ROTHWELL, N. J., SAVILLE, M. E. and STOCK, M. J. (1981a): 'Acute effects of food, 2-deoxy-D-glucose and noradrenaline on metabolic rate in normal and atropinised lean and obese (fa/fa) Zucker rats', Pflugers Arch. 392, 172-177.

ROTHWELL, N. J., SAVILLE, M. E. and STOCK, M. J. (1982a): 'Factors influencing the acute effect of food on oxygen consumption in the rat', Int. J. Obesity 6, 53-59.

ROTHWELL, N. J., SAVILLE, M. E. and STOCK, M. J. (1982e): 'Effects of feeding a cafeteria diet on energy balance and diet-induced thermogenesis in four strains of rat', J. Nutr. 112, 1515-1524.

ROTHWELL, N. J., SAVILLE, M. E. and STOCK, M. J. (1982f): 'Sympathetic and thyroid influence on metabolic rate in fed, fasted and refed rats', Am. J. Physiol. 243, R339-346.

ROTHWELL, N. J., SAVILLE, M. E. and STOCK, M. J. (1983b): 'Metabolic responses to fasting and refeeding in lean and genetically obese rats', Am. J. Physiol. 244, R615-620.

ROTHWELL, N. J., SAVILLE, M. E. and STOCK, M. J. (1983c): 'Role of insulin in thermogenic responses to refeeding in 3-day-fasted rats', Am. J. Physiol. 245, E160-165.

ROTHWELL, N. J., SAVILLE, M. E., STOCK, M. J. and WYLLIE, M. G. (1982d): 'Catecholamine and thyroid hormone influence on brown fat $Na^{+}K^{+}$-ATPase activity and thermogenesis in the rat', Horm. Metab. Res. 14, 261-265.

ROTHWELL, N. J., SAVILLE, M. E., STOCK, M. J. and WYLLIE, M. G. (1983a): 'Influences of thyroid hormone on diet-induced thermogenesis in the rat', Horm. Metab. Res. 15, 394-399.

ROTHWELL, N. J. and STOCK, M. J. (1979a): 'A role for brown adipose tissue in diet-induced thermogenesis', Nature 281, 31-35.

ROTHWELL, N. J. and STOCK, M. J. (1979b): 'Regulation of energy balance in two models of reversable obesity in the rat', J. Comp. Physiol. Psychol. 93, 1024-1034.

ROTHWELL, N. J. and STOCK, M. J. (1980a): 'Similarities between cold and diet-induced thermogenesis in the rat', Can. J. Physiol. Pharmacol. 58, 842-848.

ROTHWELL, N. J. and STOCK, M. J. (1980b): 'Intra-strain difference in the response to overfeeding in the rat', Proc. Nutr. Soc. 39, 20A.

ROTHWELL, N. J. and STOCK, M. J. (1981a): 'A role for insulin in the diet-induced thermogenesis of cafeteria fed rats', Metabolism 30, 673-678.

ROTHWELL, N. J. and STOCK, M. J. (1981b): 'Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis', Pflugers Arch 389, 237-242.

ROTHWELL, N. J. and STOCK, M. J. (1981c): 'Regulation of energy balance', Ann. Rev. Nutr. 1, 235-256.

ROTHWELL, N. J. and STOCK, M. J. (1982a): 'Effects of feeding a palatable 'cafeteria' diet on energy balance in young and adult lean (+ / ?) Zucker rats', Br. J. Nutr. 47, 461-471.

ROTHWELL, N. J. and STOCK, M. J. (1982b): 'Energy expenditure derived from measurements of oxygen consumption and energy balance in hyperphagic 'cafeteria'-fed rats', J. Physiol. 324, 59-60P.

ROTHWELL, N. J. and STOCK, M. J. (1982c): 'Neural regulation of thermogenesis', T.I.N.S. 5, 124-126.

ROTHWELL, N. J. and STOCK, M. J. (1983a): 'Acute effects of fat and carbohydrate on metabolic rate in normal, cold-acclimated and lean and obese (fa/fa) Zucker rats', Metabolism 32, 371-376.

ROTHWELL, N. J. and STOCK, M. J. (1983b): 'Diet-induced thermogenesis', In: Mammalian Thermogenesis , Girardier L. & Stock, M. J. (Eds), University Press, Cambridge, 208-233.

ROTHWELL, N. J., STOCK, M. J. and STRIBLING, D. (1982b): 'Diet-induced thermogenesis', Pharmacol. Ther. 17, 251-268.

ROTHWELL, N. J., STOCK, M. J. and TYZBIR, R. S. (1982c): 'Energy balance and mitochondrial function in liver and brown adipose tissue of rats fed cafeteria diets of varying protein content', J. Nutr. 112, 1663-1672.

ROTHWELL, N. J., STOCK, M. J. and WYLLIE, M. G. (1981b): 'Na^+ K^+-ATPase activity and noradrenaline turnover in brown adipose tissue of rats exhibiting diet-induced thermogenesis', Biochem. Pharmacol. 30, 1709-1712.

ROTHWELL, N. J., STOCK, M. J. and YORK, D. A. (1984): 'Effect of adrenalectomy on energy balance, diet induced thermogenesis and brown adipose tissue in adult cafeteria fed rats', Comp. Biochem. Physiol. (in press).

SALAMAN, M. R. and ROBINSON, D. S. (1966): 'Clearing factor lipase in adipose tissue. A medium in which the enzyme activity of tissue from starved rats increases in vitro', Biochem. J. 99, 640-647.

SCAMMEL, J. G., BARNEY, C. E. and FREGLY, M. J. (1981): 'Proposed mechanism for increased thyroxine deiodination in cold-acclimated rats', J. Appl. Physiol. 51, 1157-1161.

SCHACHTER, S. and RODIN, J. (1974): 'Obese humans and rats', In: Obese Humans and Rats, Festinger, L. and Schachter, S. (Eds), Erlbaum Halsted, Washington.

SCHEMMEL, R. and MICKELSON, O. (1973): 'Influence of diet, strain, age and sex on fat depot mass and body composition of the nutritionally obese rat', In: Regulation of Adipose Tissue Mass, Vague, J. and Boyer, J. (Eds), Excerpta. Medica. Amsterdam, 238-253.

SCHEMMEL, R. A., TEAGUE, R. J. and BRAY, G. A. (1982): 'Obesity in Osborne-Mendel and S5B/Pl rats: effects of sucrose solutions, castration, and treatment with estradiol or insulin', Am. J. Physiol. 243, R347-353.

SCLAFANI, A. (1981): 'The role of hyperinsulinemia and the vagus nerve in hypothalamic hyperphagia re-examined', Diabetologia 20, 402-410.

SCLAFANI, A. and GORMAN, A. N. (1977): 'Effects of age, sex and prior body weight on the development of dietary obesity in adult rats', Physiol. Behav. 18, 1021-1026.

SCLAFANI, A. and SPRINGER, D. (1976): 'Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes', Physiol. Behav. 17, 461-471.

SELLERS, E. A., FLATTERY, K. V. and STEINER, G. (1974): 'Cold acclimation of hypoththyroid rats', Am. J. Physiol. 226, 290-294.

SELYE, H. (1946): 'The general adaptation syndrome and the diseases of adaptation', J. Clin. Endocrinol. Metab. 6, 117-230.

SEYDOUX, J., CHINET, A., SCHNEIDER-PICARD, G., BAS, S., IMESCH, E., ASSIMACOPOULOS-JEANNET, F., GIACOBINO, J. P. and GIRARDIER, L. (1983): 'Brown adipose tissue metabolism in Streptozotocin-diabetic rats', Endocrinology 113, 604-610.

SEYDOUX, J. and GIRARDIER, L. (1977): 'Control of brown fat thermogenesis by the sympathetic nervous system', Experientia 33, 1128-1129.

SEYDOUX, J., ROHNER-JEANRENAUD, F., ASSIMACOPOULOS-JEANNET, F., JEANRENAUD, B. and GIRARDIER, L. (1981): 'Functional disconnection of brown adipose tissue in hypothalamic obesity in rats', Pflugers Arch. 390, 1-4.

SEYDOUX, J., RICQUIER, D., ROHNER-JEANRENAUD, F., ASSINACOPOULOS-JEANNET, F., GIACOBINO, J. P., JEANRENAUD, B. and GIRARDIER, L. (1982): 'Decreased guanine-nucleotide binding and reduced equivalent production by brown adipose tissue in hypothalamic obesity', FEBS Lett. 146, 161-164.

SHARGILL, N. S., YORK, D. A. and MARCHINGTON, D. R. (1983): 'Regulation of hepatic tyrosine aminotransferase in genetically obese rats', Biochim. Biophys. Acta 756, 297-307.

SHIMAZU, T. (1981): 'Central nervous system regulation of liver and adipose tissue metabolism', Diabetologia 20, 343-356.

SCHIMMEL, M. and UTIGER, R. D. (1977): 'Thyroidal and peripheral production of thyroid hormones. Review of recent findings and their clinical implications', Ann. Intern. Med. 87, 760-768.

SHINO, A., MATSUO, T. and SULUOKI, Z. (1973): 'Structural changes of pancreatic islets in genetically obese rats', Diabetologia 9, 413-421.

SHIRAISHI, T. and MAGER, M. (1980a): 'Hypothermia following injection of 2-deoxy-D-glucose into selected hypothalamic sites', Am. J. Phys. 239, R265-269.

SHIRAISHI, T. and MAGER, M. (1980b): '2-Deoxy-D-glucose-induced hypothermia: thermoregulatory pathways in the rat', Am. J. Physiol. 239, R270-276.

SILVA, J. E. and LARSEN, P. R. (1983): 'Adrenergic activation of triiodothyronine production in brown adipose tissue', Nature 305, 712-713.

SIMS, E. A. H., DANFORTH, E. Jr., HORTON, E. S., BRAY, G. A., GLENNON, J. A. and SALANS, L. B. (1973): 'Endocrine and metabolic effects of experimental obesity in man', Rec. Progr. Horm. Res. 29, 457-496.

SKALA, J. and HAHN, P. (1971): 'Effects of single cortisone injections on brown adipose tissue of developing rats', Can. J. Physiol. Pharmacol. 49, 501-507.

SLAVIN, B. G. and BERNICK, S. (1974): 'Morphological studies on denervated brown adipose tissue', Anat. Rec. 179, 497-506.

SMALLEY, R. L. (1970): 'Changes in composition and metabolism during adipose tissue development', In: Brown Adipose Tissue, Lindberg, O. (Ed), Elsevier, Holland, 77-95.

SMALLEY, R. L. and DRYER, R. L. (1967): 'Brown fat in hibernation', In: Mammalian Hibernation III, Fisher, K. C., Dawe, A. R., Lyman, C. P., Schonbaum, E. and South, Jr. F. E. (Eds), Elsevier, New York, 325-345.

SMITH, O. L. K. (1978): 'Some effects of acute cold stress on carbohydrate metabolism in the rat', Experientia Suppl. 32, 281-285.

SMITH, P. E. (1927): 'The disabilities caused by hypophysectomy and their repair', J. Am. Med. Assoc. 88, 159.

SMITH, R. E. and HORWITZ, B. A. (1969): 'Brown fat and thermogenesis, Physiol. Rev. 49, 330-425.

SMITH, P. A. and KAPLAN, M. L. (1980): 'Development of hepatic and adipose tissue lipogenesis in the fa/fa rat', Int. J. Biochem. 11, 217-228.

SMITH, R. E. and ROBERTS, J. C. (1964): 'Thermogenesis of brown adipose tissue in cold acclimated rats', Am. J. Physiol. 206, 143-148.

SMOCK, T. and FIELDS, H. L. (1981): '$ACTH_{1-24}$ blocks opiate-induced analgesia in the rat', Brain Res. 212, 202-206.

SOLOMON, J., BRADWIN, G., COCCHIA, M., COFFEY, D., CONDON, T., GARRITY, W. and GRIECO, W. (1977): 'Effect of adrenalectomy on body weight and hyperglycemia in five months old ob/ob mice', Horm. Metab. Res. 9, 152-156.

STEINER, G., LOVELAND, M. and SCHONBAUM, E. (1970): 'Effect of denervation on brown adipose tissue metabolism', Am. J. Physiol. 218, 566-570.

STERN, J. S. and HIRSCH, J. (1971): 'Obesity and pancreatic function', In: Handbook of Physiology. Endocrinology. The Endocrine Pancreas 1, Blascho, H., Sayers, G. and Smith, A. D. (Eds), Am. Physiol. Soc., Washington D.C., 641-651.

STERN, J. S. and JOHNSON, P. (1977): 'Spontaneous activity and adipose tissue cellularity in genetically obese Zucker rats (fa/fa)', Metabolism 26, 371-380.

STERN, J. S., JOHNSON, P. R., BATCHELOR, B. R., ZUCKER, L. M. and HIRSCH, J. (1975): 'Pancreatic insulin release and peripheral tissue resistance in Zucker obese rats fed high and low carbohydrate diets', Am. J. Physiol. 228, 543-548.

STERN, J., JOHNSON, P., GREENWOOD, M., ZUCKER, L. and HIRSCH, J. (1972): 'Insulin resistance and pancreatic insulin release in the genetically obese Zucker rat', Proc. Soc. Exp. Biol. Med. 139, 66-69.

STIRLING, J. L. and STOCK, M. J. (1968): 'Metabolic origins of thermogenesis induced by diet', Nature 220, 801-802.

STOCK, M. J. and ROTHWELL, N. J. (1979): In: Animal Models of Obesity, Festing, M. F. W. (Ed), Macmillan Press, London 141-151.

STOCK, M. J. and ROTHWELL, N. J. (1981): 'Sympathetic control of brown adipose tissue in the regulation of body weight', Biol. Soc. Trans. 9, 525-527.

SUNDIN, U. (1981): 'GDP binding to rat brown fat mitochondria: effects of thyroxine at different ambient temperatures', Am. J. Physiol. 241, C134-139.

SUNDIN, U. and CANNON, B. (1980): 'GDP binding to the brown fat mitochondria of developing and cold adapted rats', Comp. Biochem. Physiol. 65B, 463-471.

SVOBODA, P., SVARTENGREN, J., SNOCHOWSKI, M., HOUSTEK, J. and CANNON, B. (1979): 'High number of high-affinity sites for (-) [^{3}H] dihydroalprenolol on isolated hamster brown-fat cells', Eur. J. Biochem. 102, 203-210.

TARTTELIN, M. F. and GORSKI, R. A. (1973): 'The effects of ovarian steroids on food and water intake and body weight in the female rat', Acta. Endocrinol. 72, 551-568.

TEDESCO, J. L., FLATTERY, K. V. and SELLERS, E. A. (1977): 'Effects of thyroid hormones and cold exposure on turnover of norephinephrine in cardiac and skeletal muscle', Can. J. Physiol. Pharmacol. 55, 515-522.

TERENIUS, L. (1976): 'Somatostatin and ACTH are peptides with partial-antagonist-like selectivity for opiate receptors', Eur. J. Pharmacol. 38, 211-213.

THURLBY, P. L. and TRAYHURN, P. (1980): 'Regional blood flow in genetically obese (ob/ob) mice: The importance of brown adipose tissue to the reduced energy expenditure on non-shivering thermogenesis', Pflugers Arch. 385, 193-201.

TISDALE, H. D. (1967): 'Preparation and properties of succinic cytochrome c reductase. Complex II-III', Methods in Enzymology 10, 213-215.

TRAYHURN, P., JONES, P. M., MCGUCKIN, M. M. and GOODBODY, A. E. (1982): 'Effects of overfeeding on energy balance and brown fat thermogenesis in obese (ob/ob) mice', Nature 295, 323-325.

TRAYHURN, P. and FULLER, L. (1980): 'The development of obesity in genetically diabetic-obese (db/db) mice pair-fed with lean siblings: The importance of thermoregulatory thermogenesis', Diabetologia 19, 148-153.

TRAYHURN, P. and JAMES, W. P. T. (1978): 'Thermoregulation and nonshivering thermogenesis in the genetically obese (ob/ob) mouse', Pflugers Arch. 373, 189-193.

TRAYHURN, P., THURLBY, P. L. and JAMES, W. P. T. (1976): 'A defective response to cold in the obese (ob/ob) mouse and the obese Zucker (fa/fa) rat', Proc. Nutr. Soc. 35, 133A.

TRIANDAFILLOU, J., GWILLIAM, C and HIMMS-HAGEN, J. (1982): 'Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria', Can. J. Biochem. 60, 530-537.

TRIANDAFILLOU, J. and HIMMS-HAGEN, J. (1983): 'Brown adipose tissue in genetically obese (fa/fa) rats: response to cold and diet', Am. J. Physiol. 244, E145-150.

TULP, O. L., FRINK, R. and DANFORTH, E. Jr. (1982): 'Effect of cafeteria feeding on brown and white adipose tissue cellularity, thermogenesis and body composition in rats', J. Nutr. 112, 2250-2260.

TULP, O., HORTON, E. S., TYZBIR, E. D., DANFORTH, E. Jr., KRUPP, P. P., and BOLLINGER, J. (1977): 'Thyroid function in experimental protein malnutrition', Am. J. Clin. Nutr. 30, 621A.

TURKENKOPF, I. J., JOHNSON, P. R. and GREENWOOD, M. R. C. (1982): 'Development of pancreatic and plasma insulin in prenatal and suckling Zucker rats', Am. J. Physiol. 242, E220-225.

TYZBIR, R. S., KUNIN, A. S., SIMS, N. M. and DANFORTH, E. Jr. (1981): 'Influence of diet composition on serum triiodothyronine (T_3) concentration, hepatic mitochondrial metabolism and shuttle system activity in rats', J. Nutr. 111, 252-259.

VANDERTUIG, J. G., KNEHANS, A. W. and ROMSOS, D. R. (1982): 'Reduced sympathetic nervous system activity in rats with ventromedial hypothalamic lesions', Life Sci. 30, 913-920.

VAN NOORDEN, C. J. F., WEIRSINGA, W. M. and TOUBER, J. L. (1979): 'Propranalol inhibits the in vitro conversion of thyroxine into triiodothyronine by isolated rat liver parenchymal cells', Horm. Metab. Res. 11, 366-370.

VON DER PORTEN, K. and DAVIS, J. R. (1979): 'Weight loss following lateral hypothalamic lesions independent of changes in motor activity or metabolic rate', Physiol. Behav. 23, 813-819.

WALBERG, J. L., MOLE, P. A. and STERN, J. S. (1982): 'Effect of swim training on development of obesity in the genetically obese rat', Am. J. Physiol. 242, R204-211.

WANGSNESS, P. J., DILLETUSO, B. A. and MARTIN, R. J. (1978): 'Dietary effects on body weight, food intake and diurnal feeding behaviour of genetically obese rats', J. Nutr. 108, 356-364.

WICKLER, S. J., HORWITZ, B. A. and STERN, J. S. (1982): 'Regional blood flow in genetically-obese rats during non-shivering thermogenesis', Int. J. Obesity 6, 481-490.

WILBER, J. F. and UTIGER, R. D. (1969): 'The effect of glucocorticoids on thyrotrophin secretion', J. Clin. Invest. 48, 2096-2103.

WILLIAMS, L. T., LEFKOWITZ, R. J., WATANABE, A. M., HATHAWAY, D. R., and BESCH, H. R. (1977): 'Thyroid hormone regulation of β-adrenergic receptor number', J. Biol. Chem. 252, 2787-2789.

WILLIAMSON, J. R. (1970): 'Control of energy metabolism in hamster brown adipose tissue', J. Biol. Chem. 245, 2043-2050.

YEN, T. T., SHAW, W. N. and PAO-LO, Y. (1977): 'Genetics of obesity in Zucker rats and Koletsky rats', Heredity 38, 373-378.

YORK, D. A. (1979): 'The characteristics of genetically obese mutants', In: Animal Models of Obesity, Festing, M. F. W. (Ed), Macmillan Press Ltd., London, 39-64.

YORK, D. A. and BRAY, G. A. (1973a): 'Genetic obesity in rats II. The effect of food restriction on metabolism of adipose tissue', Metabolism 22, 443-454.

YORK, D. A. and BRAY, G. A. (1973b): 'Adipose tissue metabolism in six-week-old fatty rats', Horm. Metab. Res. 5, 355-360.

YORK, D. A. and GODBOLE, V. (1979): 'Effect of adrenalectomy on obese 'fatty'rats', Horm. Metab Res. 11, 646.

YORK, D. A., HERSHMAN, J. M., UTIGER, R. D. and BRAY, G. A. (1972): 'Thyrotropin secretion in genetically obese rats', Endocrinology, 90, 67-72.

YORK, D., HOLT, S., ROTHWELL, N. and STOCK, M. (1984): 'The effect of age and gene-dosage on brown adipose tissue of Zucker obese fa/fa rats', Am. J. Physiol. (in press).

YORK, D. A. and MARCHINGTON, D. R. (1984): 'Regulation of brown adipose tissue by corticosterone in obese (fa/fa) rats', Int. J. Obesity (in press).

YORK, D. A., SHARGILL, N. S. and GODBOLE, V. (1981): 'Serum insulin and lipogenesis in the suckling 'Fatty' fa/fa rat', Diabetologia 21, 143-148.

YOSHIDA, T., KEMNITZ, J. W. and BRAY, G. (1983): 'Lateral hypothalamic lesions and norephinephrine turnover in rats', J. Clin. Invest. 72, 919-927.

YOUNG, J. B. and LANDSBERG, L. (1977a): 'Suppression of sympathetic nervous system during fasting', Science 196, 1473-1475.

YOUNG, J. B. and LANDSBERG, L. (1977b): 'Stimulation of the sympathetic nervous system during sucrose feeding', Nature 269, 615-617.

YOUNG, J. B. and LANDSBERG, L. (1979): 'Effect of diet and cold exposure on norephinephrine turnover in pancreas and liver', Am. J. Physiol. 236, E524-533.

YOUNG, J. B. and LANDSBERG, L. (1983): 'Diminished sympathetic nervous system activity in the genetically obese (ob/ob) mouse', Am. J. Physiol. 245, E148-154.

YOUNG, J. B., SAVILLE, E., ROTHWELL, N. J., STOCK, M. J. and LANDSBERG, L. (1982): 'Effect of diet and cold exposure on norephinephrine turnover in brown adipose tissue in the rat', J. Clin. Invest. 69, 1061-1071.

YOUNG, R. A., TULP, O. L. and HORTON, E. S. (1980): 'Thyroid and growth responses of young Zucker obese and lean rats to a low protein, high carbohydrate diet', J. Nutr. 110, 1421-1431.

YUKIMURA, Y. and BRAY, G. A. (1978): 'Effects of adrenalectomy on body weight and the size and number of fat cells in the Zucker (fatty) rat', Endocr. Res. Commun. 5, 189-198.

YUKIMURA, Y., BRAY, G. A. and WOLFSEN, A. R. (1978): 'Some effects of adrenalectomy in the fatty rat', Endocrinology 103, 1924-1928.

YUKIMURA, Y., PARLOW, A. and BRAY, G. A. (1977): 'Effect of adrenalectomy in the fatty rat,' 59th Annual Meeting of the Endocrine Society, Chicago, 340.

ZAROR-BAHRENS, G. and HIMMS-HAGEN, J. (1983): 'Cold stimulated sympathetic activity in brown adipose tissue of obese (ob/ob) mice', Am. J. Physiol. 244, E361-366.

ZELIS, R., MANSOUR, E. J., CAPONE, R. J. and MASON, D. T. (1974): 'The cardiovascular effects of morphine: The peripheral capacitance and resistance vessels in human subjects', J. Clin. Invest. 54, 1247-1258.

ZUCKER, L. M. (1975): 'Efficiency of energy utilization by the Zucker hereditarily obese rat 'fatty',' Proc. Soc. Exp. Biol. Med. 148, 498-500.

ZUCKER, L. M. and ANTONIADES, H. N. (1972): 'Insulin and obesity in the Zucker genetically obese rat 'fatty', Endocrinology 90, 1320-1330.

ZUCKER, L. M. and ZUCKER, T. F. (1961): 'Fatty, a new mutation in the rat', J. Heredity 52, 275-278.

ZUCKER, T. F. and ZUCKER, L. M. (1963): 'Fat accretion and growth in the rat,' J. Nutr. 80, 6-19.