



National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

**THE ROLE OF CORTICOSTERONE IN THE
DEFECTIVE CONTROL OF BROWN ADIPOSE
TISSUE THERMOGENESIS IN MICE WITH
GOLD THIOGLUCOSE-INDUCED OBESITY**

by

Christiane Villemure

**A thesis submitted to the School of Graduate
Studies of the University of Ottawa
in partial fulfilment of the requirements
for the degree of Master of Science**

**Department of Biochemistry
Faculty of Medicine
University of Ottawa
Ottawa, Canada**



Christiane Villemure, Ottawa, Canada, 1990.



of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

ISBN 0-315-60584-7



UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ABSTRACT

Gold thioglucose (GTG) causes hypothalamic lesions in mice that lead to the development of obesity associated with both hyperphagia and reduced energy expenditure. GTG-mice have a reduced brown adipose tissue (BAT) thermogenic activity. Adrenalectomy normalizes many abnormalities in GTG-mice as in other models of obesity such as the genetically obese ob/ob mouse. The ob/ob mouse is known to be hypersensitive to an action of corticosterone on development of obesity and on BAT thermogenesis. The purpose of this study was to find out if hypersensitivity to corticosterone is also present in mice with hypothalamic obesity as opposed to mice with genetic obesity.

Mice were injected with either saline or GTG (700mg/kg body weight) at 8-9 weeks of age and sham-adrenalectomized or adrenalectomized (adx) 2 weeks later. At that time the adx animals received cholesterol implants containing 0, 1, 2, 5, or 20mg corticosterone. They were killed 2 weeks later. One group was killed at the time of surgery as a control group.

It was found that certain abnormalities of GTG-mice, namely the hyperphagia, the increased white adipose tissue and BAT weights and hyperinsulinemia are normalized by adrenalectomy and returned in virtually full force at low levels of corticosterone. Other abnormalities like the high body weight and reduced BAT mitochondrial GDP binding are not affected by adrenalectomy or corticosterone replacement. BAT protein was not affected by adrenalectomy in either lean or obese animals and was reduced by corticosterone replacement in both.

It is concluded that GTG-mice are hypersensitive to an action of corticosterone which plays an important role in the development of their obesity. However, some abnormalities, like the suppressed BAT thermogenesis, persist in the adx state and seem to be due to metabolic defects primarily caused by the GTG lesions that are not dependent on corticosterone.

DEDICATION

This thesis is dedicated to my parents who taught me the satisfaction of work well done and to my husband Robert who was always there to support me by his encouragement and patience.

ACKNOWLEDGEMENTS

I would like first to thank my supervisor, Dr. J. Himms-Hagen, for giving me the opportunity of working in her laboratory and for her excellent guidance and patience. I am grateful to Dr. Anna-Lisa Kates and Dr. Gloria Zaror-Behrens for their time and patience while teaching me various methods that were used in this study. I am also grateful to Dr. Anna-Lisa Kates for helping me with the statistical analysis. I thank Gisèle Larose for excellent care of the animals and for help with the surgery and many other things! I also thank M. Robert Latreille and M. Daniel Lacroix for help with the use of the Macintosh computer. Finally, I would like to thank everybody that worked in this laboratory while I was there Jingying Cui, Anna-Lisa Kates, Gloria Zaror-Behrens, Lynn Sigurdson, Mahin Baqi and Erica Valentine for their advice and friendship.

TABLE OF CONTENTS

CHAPTER ONE:	INTRODUCTION.....	1
	Role of the hypothalamus in energy balance.....	2
	Role of brown adipose tissue (BAT) in energy balance.....	4
	Gold thioglucose-induced obesity.....	14
	STATEMENT OF THE PROBLEM.....	20
CHAPTER TWO:	MATERIALS AND METHODS.....	22
1)	Animals.....	22
2)	GTG lesions.....	23
3)	Food intake measurement.....	23
4)	Corticosterone pellets preparation.....	23
5)	Adrenalectomy and pellet implantation.....	24
6)	Temperature measurement.....	24
7)	Isolation of BAT mitochondria.....	24
8)	GDP binding procedure.....	25
9)	Protein determination.....	26
10)	Serum corticosterone determination.....	26
11)	Serum thyroid hormone determination.....	27
12)	Serum insulin radioimmunoassay.....	29
13)	Measurement of BAT T4 5'-deiodinase activity.....	29
14)	Cytochrome c oxidase assay.....	31
15)	BAT UCP determination.....	31
CHAPTER THREE:	RESULTS.....	35
PART ONE:	CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON MOUSE BAT FUNCTION.....	35
	Methods.....	35
	Statistical analysis.....	36
	Results.....	36
	Conclusion.....	41
PART TWO:	GTG-OBESITY EXPERIMENT.....	41
	Methods.....	42
	Statistical analysis.....	44
	Results.....	45
CHAPTER FOUR:	DISCUSSION.....	60
PART ONE:	CAGING EXPERIMENT.....	60
PART TWO:	GTG-OBESITY EXPERIMENT.....	62
	CONCLUSION.....	73

REFERENCES:	75
APPENDIX A:	LIST OF ABBREVIATIONS.....	92
APPENDIX B:	CURRICULUM VITAE.....	94

LIST OF FIGURES

FIGURE	TITLE	PAGE
1	Effect of the concentration of homogenate protein on the rate of cytochrome c oxidation by cytochrome c oxidase.....	32
2	Example of a Lineweaver-Burk plot for cytochrome c oxidase.....	32
3	Caging experiment: Effect of housing conditions on GDP binding to BAT mitochondria.....	38
4	Caging experiment: Effect of housing conditions on mouse BAT UCP content.....	38
5	Caging experiment: Effect of housing conditions on mouse BAT cytochrome c oxidase activity.....	40
6	Caging experiment: Effect of housing conditions on mouse BAT T4 5'-deiodinase activity.....	40
7	GTG-obesity experiment: Effect of corticosterone on body weight gain of adrenalectomized GTG-obese and lean mice.....	49
8	GTG-obesity experiment: Effect of corticosterone on gonadal white adipose tissue weight of adrenalectomized GTG-obese and lean mice.....	51
9	GTG-obesity experiment: Effect of corticosterone on brown adipose tissue weight of adrenalectomized GTG-obese and lean mice.....	53
10	GTG-obesity experiment: Effect of corticosterone on brown adipose tissue total protein of adrenalectomized GTG-obese and lean mice.....	55
11	GTG-obesity experiment: Effect of corticosterone on brown adipose tissue mitochondrial GDP binding of adrenalectomized GTG-obese and lean mice.....	57
12	GTG-obesity experiment: Effect of corticosterone on the concentration of insulin in serum of adrenalectomized GTG-obese and lean mice.....	58

LIST OF TABLES

TABLE	TITLE	PAGE
1	Caging experiment: Effect of housing conditions on mouse body weight and temperature, brown adipose tissue and serum levels of thyroid hormones.....	37
2	GTG-obesity experiment: Effect of adrenalectomy, sham-adrenalectomy and corticosterone replacement on daily food intake of GTG-obese and normal lean mice.....	46
3	GTG-obesity experiment: Effect of adrenalectomy, sham-adrenalectomy and corticosterone replacement on the development of the obesity of GTG-obese mice.....	48
4	GTG-obesity experiment: grouping of mice according to their serum corticosterone levels.....	96

INTRODUCTION

"When we try to pick out
anything by itself,
We find it hitched to
everything else in the universe."
John Muir

Most animals maintain a stable body weight during most of their lifetime, indicating that energy intake and energy expenditure, although variable from day to day, are adjusted to each other in the long run (LeMagnen, 1983). This is possible only if the expenditure is tightly coupled to the intake of energy.

Although energy intake is attained through feeding and drinking only, various mechanisms are responsible for "burning up" this energy: the metabolic reactions leading to digestion and processing of the food, exercise and movement, maintenance of an adequate body temperature and all metabolic reactions required for the maintenance of living cells. A large portion of the energy is lost as heat due to the inefficiency of metabolic reactions. This is referred to as obligatory thermogenesis because heat production is a by-product of metabolic activity.

Interestingly, thermogenesis can also be facultative, that is, heat production can be turned on when needed. Skeletal muscle and brown adipose tissue (BAT) are the principal sites of facultative thermogenesis. In skeletal muscle heat production is associated with voluntary activity or exercise or with thermoregulatory shivering for maintaining body temperature in cold environments. In BAT, it is also associated with thermoregulation, a process called nonshivering thermogenesis or cold-induced thermogenesis (CIT) as

well as with diet-induced thermogenesis (DIT) which is turned on in response to changes in food quantity or quality.

Role of the hypothalamus in energy balance

The first description of hypothalamic injury leading to obesity was published by Mohr in 1840 (Bray and York, 1979). At the turn of the century it was found that brain tumors were associated with obesity (Babinski, 1901; Frohlich, 1900 in Bray and York, 1979). However, it was not until 1927 that the hypothalamus was found to be directly involved in hyperphagia and obesity (Smith, 1927). In 1939, the ventromedial hypothalamus (VMH) was identified as a more specific site for control of these functions (Hetherington and Ranson, 1939). The finding that lesions of the lateral hypothalamus (LH) could lead to reduced food intake (Anand and Brobeck, 1951) led to the promotion of the "dual-center" hypothesis (Stellar, 1954). This model proposed that the VMH and the LH were acting as satiety and feeding centers. Although this model received a lot of support at first (for example Oomura et al., 1974), it was later found that the hyperphagia could be produced by damage to other sites (mainly the medial hypothalamus (MH) and the paraventricular nucleus (PVN)) (Bray and York, 1979) and that VMH-lesioned rats could become obese without overeating or gaining weight excessively (Bernardis and Goldman, 1976; Han and Liu, 1966). It was then proposed that damage to the VMH and LH was due to the destruction of a feeding inhibitory pathway descending from the PVN and passing just lateral to the VMH in the direction of the brainstem (Sclafani and Kirchgessner, 1985). Consistent with this view was the fact that lesions of the PVN produce hyperphagia and obesity (Leibowitz et al., 1981) and that PVN

and VMH lesions induce similar changes in feeding behavior (Aravich and Sclafani, 1983). Meanwhile, Bray and York (1979) proposed that hypothalamically induced hyperphagia and obesity were secondary to autonomically-mediated changes in metabolism, mainly hyperinsulinemia and effects secondary to it. Various facts supported this hypothesis as reported by Sclafani and Kirchgessner (1985):

- lesion of the VMH produces hyperinsulinemia before the onset of hyperphagia.
- insulin release and fat deposition are elevated even when hyperphagia is prevented.
- administration of insulin induces overeating and obesity and this effect is dose-correlated.
- vagotomy blocks the hyperphagia, obesity and insulin release produced by VMH lesions.
- reduced sympathetic nervous system activity in these animals is thought to contribute to the hyperinsulinemia as well.

However, it was found that not all VMH lesioned animals were hyperinsulinemic, that the insulin-induced hyperphagia rarely approached the magnitude of that produced by VMH lesions and that vagotomy did not always eliminate hyperphagia in these animals (Powley and Opsahl, 1974; Sclafani and Kirchgessner, 1985; King and Frohman, 1982).

Fortunately, this concept (Bray and York, 1979) abolished the idea of a strict hypothalamic control of food intake. Moreover, it suggested that the hypothalamus still influences food intake, but neuroendocrine changes also contribute to the maximal expression of hyperphagia and obesity.

Consistent with that way of thinking, it is known that other humoral or hypothalamic factors, besides insulin, act as satiety or feeding signals. Glucagon (Penick and Hinkie, 1961), glucocorticoids (Leibowitz et al., 1974), neuropeptide Y (Morley et al., 1987; Leibowitz et al., 1988), corticotropin releasing factor (CRF) (Arase et al., 1988), cholecystinin (Silver et al., 1989) and various opioids (Yim and Lowy, 1984), to name just a few, are known to be involved in the control of feeding behavior.

Glucocorticoids are particularly interesting because their removal by adrenalectomy improves obesity due to hypothalamic damage. For example, adrenalectomy of rats with hypothalamic knife cuts (Romsos et al., 1987), of mice with hypothalamic lesions induced by gold thioglucose (GTG) (Debons et al., 1982, 1983) or monosodium glutamate (Tokuyama and Himms-Hagen, 1989) reverses the elevated body weight and the hyperphagia to normal levels. The obesity-promoting action of glucocorticoids appears to be on the hypothalamus (Debons et al., 1986; Leibowitz et al., 1984).

Role of BAT in energy balance

1) History

Research in the 1960s and 1970s contributed mainly to our understanding of BAT anatomy and physiology and of its role in cold-adapted small mammals (Nicholls and Locke, 1984). It was not until a decade ago that the importance of BAT as a site of energy expenditure was recognized (Nicholls and Locke, 1984; Himms-Hagen, 1984). Not only was BAT involved in cold-induced nonshivering thermogenesis (Foster and Frydman, 1979) but also in the response to diet (DIT) (Rothwell and Stock, 1979). BAT will grow in

response to prolonged stimulation by cold or high fat diets and will atrophy in absence of stimulation. Trophic responses vary both quantitatively and qualitatively with the nature of the external stimulus, and with the age, diet, species and genetic background of the subjects studied (Himms-Hagen, 1985).

2) Anatomy and histology

In rodents, BAT is located in relatively large depots in the interscapular, subscapular, cervical and axillary regions and in small depots around major organs and blood vessels (Smith and Horwitz, 1969; Né Chad, 1986). Because they are easily accessed the most studied depots are the first four mentioned. BAT cells are morphologically distinct from white adipose tissue (WAT) cells in many aspects, the most important being that they contain many lipid droplets (multilocular) and are very rich in mitochondria, which confer on the tissue its brownish color.

BAT is characterized by a rich sympathetic innervation (Né Chad, 1986; Foster et al., 1982) that mediates its growth and activation (Girardier and Seydoux, 1986).

The mature BAT cells account for only 40% of the cells in the tissue; the remaining portion being made up by endothelial cells, perivascular cells, preadipocytes, mast cells, Schwann cells and fibroblasts (Né Chad, 1986). Nevertheless, these other cells are small and are thought to contribute only little material other than DNA to homogenates of BAT (Himms-Hagen, 1989a). BAT is also remarkable for its rich vasculature organized to transfer heat rapidly to the vital organs of the body (Smith and Roberts, 1964).

3) Mechanisms of thermogenesis

Thermogenesis in BAT is possible due to the presence of a unique protein present only in BAT (Ricquier and Bouillaud, 1986 and references therein), referred to as the uncoupling protein (UCP). When BAT cells are stimulated the UCP, located in the inner membrane of mitochondria, acts as a proton carrier allowing the dissipation of the proton gradient generated by the action of the electron transport chain therefore causing an uncoupling of the respiration from the synthesis of ATP. As a result, the process is speeded up since the electron transport chain no longer has to work against a proton gradient, resulting in more substrate oxidation therefore more heat produced.

The activity of the UCP is thought to be controlled by the intracellular concentration of free fatty acids which act in such a way as to lower the membrane potential at which proton translocation occurs (Rial et al., 1983; Klingenberg and Winkler, 1985; Jezek et al., 1988). In addition, the binding to the UCP of purine nucleotides such as ATP and ADP inhibits proton translocation (Nicholls, 1976; Jezek et al., 1988; Klingenberg and Winkler, 1985) leading to a "recoupling" of the mitochondria. The binding of purine nucleotides is pH dependent and "unbinding" is thought to play a role in the activation of the UCP (Klingenberg, 1988; French et al., 1988). These processes are referred to as masking and unmasking.

Stimulation of BAT is achieved primarily by noradrenaline released at sympathetic nerve terminals, acting on β -adrenergic receptors. This causes an increase in cAMP and subsequent activation of various protein kinases (Nicholls and Locke, 1984), including protein kinase C (Barge et al., 1988), resulting in the activation of the hormone-sensitive lipase (HSL) (Skala, 1984;

Holm et al., 1987) and increased synthesis of lipoprotein lipase (LPL) (Carneheim, 1984). The HSL hydrolyses triacylglycerols to free fatty acids and glycerol and the LPL permits increased uptake of free fatty acids from the blood triacylglycerol in lipoproteins. Fatty acids, besides their role in activating the UCP, are the major fuel for thermogenesis (Himms-Hagen, 1989b). Cooney et al. (1985) reported that glucose uptake can also be increased by β -adrenergic stimulation but appears to be only a minor substrate for thermogenesis (Isler et al., 1987). It is believed that glycolysis provides the ATP necessary for other metabolic processes in the stimulated BAT cells.

There is also a fairly important effect of noradrenaline on α_1 -adrenergic receptors. It consists in the propagation of the stimulatory signals via gap junctions by modulating the activity of various ion pumps. This action of noradrenaline is mediated by the phosphatidylinositol bisphosphate pathway. The induction in synthesis of the T4 5'-deiodinase is also mediated by α_1 -adrenergic receptors. The function of this enzyme will be discussed later on. It was reported that the α_1 -adrenergic stimulation potentiates the action of noradrenaline at β -adrenergic receptors (Foster, 1984; Ma and Foster, 1984).

Another effect of noradrenaline is mediated by α_2 -adrenergic receptors and involves a small increase in pH that is caused by the activity of the Na^+/H^+ ion exchange mechanism (Giovannini et al., 1988). Although the increase in pH is small (0.2 pH unit), it is thought to be sufficient to cause unmasking (Giovannini et al., 1988).

The increased thermogenesis that occurs after acute treatment with noradrenaline is mainly due to stimulated function of the UCP already present (Trayhurn et al., 1987). However, prolonged sympathetic stimulation of BAT by

stimuli like cold exposure or diet leads to growth of the tissue. This is brought about by hyperplasia of the tissue through maturation of preadipocytes and hypertrophy through increases in proteins and mitochondrial content (Bukowiecki, 1984; Bukowiecki and Collet, 1983). A selective increase in UCP content also occurs (Trayhurn et al., 1987). For example the UCP increases 100-fold in rat BAT after 4°C exposure and this increase is preceded by an increase in UCP mRNA (Ricquier et al., 1984).

4) Measurement of BAT activity

The only quantitative method to assess BAT contribution to overall energy expenditure is by measuring the O₂ consumption of the various BAT depots (Foster and Frydman, 1978; Foster, 1984; Himms-Hagen, 1985, 1989a, 1989b, Trayhurn and Milner, 1989). This is done by measuring blood flow through BAT using radioactive microspheres and arterial-venous difference in O₂ tension (Foster and Frydman, 1978; Foster, 1984). Because this technique is difficult and requires a lot of expertise, various other methods have been used as qualitative indices of BAT thermogenic activity. The most commonly used of these methods is the measurement of purine nucleotide binding (usually GDP) to isolated mitochondria as described by Nicholls (1976). GDP binding is low in quiescent BAT but is increased upon stimulation of the tissue. The increase in binding occurs rapidly, is reversible (Swick and Swick, 1986), does not require protein synthesis (Desautels and Himms-Hagen, 1979) and shows a diurnal variation (Rothwell et al., 1983). It is associated with unmasking of the UCP. GDP binding is not only a function of the UCP concentration but also of the number of exposed and accessible binding sites (Himms-Hagen, 1985, 1989a,

1989b; Trayhurn et al, 1987; Swick and Swick, 1986, Trayhurn and Milner, 1989).

The measurement of GDP binding, combined with other measurements like protein content, mitochondrial content as measured from cytochrome c oxidase or succinate dehydrogenase activity, and UCP concentration can give a good reliable index of BAT thermogenic capacity and activity. However, it should be noted that BAT weight is not included in this list; it gives only a rough idea of lipid content of the tissue (Himms-Hagen, 1985, 1989a, 1989b).

5) Control of BAT function

It is now well established that BAT function and growth are controlled by the noradrenaline released at sympathetic nerves that, in turn, are controlled by the hypothalamus. Different studies with physical and chemical lesions of specific areas of the hypothalamus, hypothalamic injection of various substances and electrical stimulation at various sites have been used to assess which specific area(s) was (were) responsible for the control of thermogenesis. Unfortunately, it is very difficult to get a precise idea because these manipulations are rather non-specific, have wide spread effects or disrupt other endocrinological functions of the hypothalamus.

It is the VMH that appears to be the primary central regulator of BAT thermogenesis. When it is electrically stimulated, BAT thermogenesis (Sakaguchi and Bray, 1987; Holt et al., 1987), BAT blood flow (Iwai et al., 1987), BAT noradrenaline turnover rate (Saito et al., 1989) and BAT temperature (Perkins et al., 1981; Holt et al., 1988) are elevated. These effects are mediated by sympathetic nerves as shown by denervation studies (Minokoshi et al.,

1986). Neither the PVN nor the LH are likely to be involved in BAT regulation (Holt et al., 1987, 1988; Sakaguchi et al., 1988; Saito et al., 1989) but more experiments are required to fully demonstrate this fact.

DIT is absent in VMH-lesioned animals while CIT is normal (Hogan et al, 1982; Preston et al., 1989). Thus, signals derived from diet and from environmental temperature appear to be processed separately in the hypothalamus. Glucose injected either in the hypothalamus or intravenously causes BAT noradrenaline turnover rate to be increased (Nijjima, 1986). 2-Deoxyglucose, a glucoprivic agent, causes the reverse effect (Arase et al., 1987; Allars and York, 1986) and hypothalamic injections of insulin suppress BAT thermogenesis (Sakaguchi and Bray, 1987). It is evident that the hypothalamus recognizes signals generated by the ingestion of food.

6) Hormonal responses

a) Thyroid hormones

A normal thermogenic response of BAT to noradrenaline requires the presence of local conversion of thyroxine (T4) to triiodothyronine (T3) by the enzyme T4-5'-deiodinase (type II) (Bianco and Silva, 1987a, 1987b; Leonard et al., 1983), which is found exclusively in BAT, the brain and the pituitary (Leonard et al., 1983). The activity of this enzyme is essential since noradrenaline-stimulation of thermogenesis is decreased in hypothyroidism (Obregon, et al., 1987; Silva, 1988) or requires supraphysiological doses of circulating T3 (Bianco and Silva, 1987). Increased gene transcription and synthesis of the enzyme is brought about by noradrenaline via an action on α_1 -adrenergic receptors (Silva and Larsen, 1983; Jones et al., 1986). The

mechanism of action of T3 is unclear. It appears that binding of T3 to nuclear receptors amplifies the noradrenaline-induced stimulation of UCP gene transcription (Bianco et al., 1988). Thus, cold exposure is accompanied by a stimulation of T3 production and an increase in T4-5'-deiodinase activity up to 100-fold (Kopecky et al., 1986; Silva and Larsen, 1983). The response of BAT to a high fat diet is not as great as for cold. No increase in the T4-5'-deiodinase activity was recorded after feeding rats and hamsters with a palatable diet for 10 days (Kopecky et al., 1986).

b) Insulin

The action of insulin on BAT is two-fold. First, it acts directly on the tissue, modulating glucose metabolism and secondly, it acts centrally, modulating the sympathetic nervous system activity.

Injection of insulin causes increased glucose uptake by brown adipocytes (Rothwell and Stock, 1981; Cooney et al., 1985; Ferré et al., 1986; Smith et al., 1986; Vallerand et al., 1987) through an action on glucose transporters. In fact, insulin causes translocation of glucose transporters from a pool associated with the microsomal fraction to the plasma membrane and an increase in their intrinsic activity in BAT (Greco-Perroto, 1987a). Cold exposure brings about similar changes in glucose transporters and also increases the total number of glucose transporters (Greco-Perroto, 1987b). In the cold, BAT gradually becomes hyperresponsive to insulin (Smith et al., 1986; Howland and Bond, 1987; Vallerand et al., 1987). Streptozotocin-treated diabetic rats show decreases in BAT proteins, mitochondria, UCP (Seydoux et al., 1984; Bartness et al., 1986), GDP binding (Jamal and Saggerson, 1988), T4-5'-deiodinase

(Silva and Larsen, 1986) and noradrenaline turnover rate (Gualberto and Saggerson, 1989; Yoshida et al., 1983).

Thermogenesis is suppressed in animals that are insulin resistant, a symptom often encountered in the syndrome of obesity (Mercer and Trayhurn, 1984; Ferré et al., 1986). Insulin promotes lipogenesis in BAT (Saggerson et al., 1988; Ebner et al., 1987) as in other tissues and appears to be required for DIT (Rothwell and Stock, 1981). Insulin is likely to potentiate the action of noradrenaline to increase thermogenesis and promote growth of the tissue (Bartness et al., 1986; Ebner et al., 1987; Saggerson et al., 1988). In vitro studies by Ebner et al. (1987) have demonstrated that when BAT cells are stimulated by noradrenaline and insulin simultaneously glycolysis is augmented, but when insulin is present alone lipogenesis is predominant.

c) Glucagon

The exact mechanism of action of glucagon on BAT is still uncertain. First, plasma glucagon concentration is increased in animals upon cold exposure (Seitz et al., 1981). Chronic treatment with glucagon leads to growth of BAT in a similar way as with cold exposure (Billington et al., 1987). Growth of the tissue is characterized by increased mitochondrial mass, protein, DNA, cytochrome c oxidase activity, GDP binding (Billington et al., 1987). Injection of glucagon also increases T4-5'-deiodinase activity (Silva and Larsen, 1986). However, glucagon administration leads to liberation of catecholamines, therefore it is not sure which of these hormones exerts the effect (Himms-Hagen, 1985). BAT response to diet does not appear to be mediated by glucagon (Himms-Hagen, 1989a, 1989b).

d) Glucocorticoids

Glucocorticoids receptors are present in BAT (Feldman, 1978) but their role is unknown. Himms-Hagen (1985) suggests that they could control some enzyme activity or have unrelated function like their antiinflammatory action. The well known glucocorticoid potentiation of noradrenaline action on peripheral tissues appears to be absent in BAT (Wickler et al., 1986) and adrenalectomy in normal rats and mice does not affect BAT function (Tokuyama and Himms-Hagen, 1987; Allars et al., 1987; York et al., 1985). However, injection of large doses to normal animals will result in a suppression of BAT thermogenesis (Galpin et al., 1983). It is well established that glucocorticoids exert their suppressive effect on BAT through a central action on the hypothalamus, possibly due to modulation of CRF levels (Rothwell and Stock, 1984; Arase et al., 1987; Saphier and Feldman, 1988; Lefeuvre et al., 1987; Morley, 1986; King, 1988; Himms-Hagen, 1985, 1989a, 1989b).

7) Effects of housing conditions on BAT function.

Cold temperatures are often considered by experimenters to be those that are below 10-15°C. However, mice housed at a normal room temperature of 20-25°C, or at any temperature below their thermoneutral temperature of 28-32°C are already cold-stressed with respect to BAT function (Himms-Hagen, 1989a; Trayhurn and Milner, 1989). In fact their BAT UCP content is higher at 22°C compared to 32°C (Ashwell et al., 1983). The same temperature dependence is seen in rats and hamsters (Himms-Hagen, 1986).

Housing mice in groups can reduce the cold stress imposed on them and can suppress BAT thermogenesis (Jennings et al., 1986; Heldmaier, 1975).

This effect appears to vary with ambient temperature (Jennings et al., 1986). This is due to social thermoregulation, a concept introduced by Heldmaier (1975), which refers to the fact that mice tend to huddle together, thermoregulating as one entity with a smaller surface to volume ratio. In fact, ignoring this concept has led to conflicting results in the past (for example compare Desautels, 1985, and Trayhurn and Jennings, 1986). The kind of cage (plastic or wire mesh) can also affect BAT metabolism. Therefore in analysing data, it is important to take into account the number of animals per cage, the housing temperature as well as the kind of cage used and these must be specified in reports.

Gold thioglucose-induced obesity

Injection of gold thioglucose (GTG) was shown by Brecher and Waxler (1949) to cause obesity and hyperphagia in mice. Five years later, it was found that these symptoms were due to damage to the hypothalamus (Marshall et al., 1955). Since then, the "GTG-mouse" has become a widely used model of hypothalamically-induced obesity. It appears, as will be seen later, that this kind of obesity is characterized by alterations in the regulation of both energy intake and energy expenditure.

The effect of GTG to induce obesity is dose-dependent (Djazayery et al., 1979). The dose of GTG injected is also proportional to the mortality rate (Brecher and Waxler, 1949; Liebelt et al., 1960) which is due to the acute toxic effects of the drug. Autopsy of mice that succumbed to GTG showed gastric ulcers, extensive hypothalamic damage, severe tubular necrosis of the kidney and parenchymal destruction of the liver (Liebelt, 1960). The time of day at

which GTG is injected also influences the mortality rate suggesting that the metabolic state of the animals is also important (Wiepkema, 1966).

Lesions induced by GTG were located mainly in the VMH, the supraoptic nuclei, the ventral part of the anterior hypothalamus and in the LH, the arcuate nucleus and the median eminence (Perry and Liebelt, 1961). Lesions have also been identified outside the hypothalamus, in the area above the optic chiasm, the hippocampal commissure, the hindbrain and the area postrema, especially in the dorsomedial nucleus of the vagus (Perry and Liebelt, 1961; Powley and Prechtl, 1986). It is generally accepted that the syndrome of obesity of the GTG mouse is due principally to the lesions to the VMH, however, participation of other hypothalamic or brain lesions must not be neglected.

Mayer (1955) proposed that GTG was concentrated at lesion sites by binding of the glucose moiety to specific neuronal glucoreceptors that, in turn, brings about a focal accumulation of sufficient gold to produce necrosis of these sites (Debons et al., 1962). Evidence supporting this point can be summarized as follows:

- the firing rate of neurons appears to be influenced by glucose,
- the glucose moiety is needed for the necrotic effect. Damage by GTG is blocked by glucose and glucose analogues like 2-deoxyglucose (Likuski et al., 1967). Compounds containing gold but not glucose (goldthiogalactose, gold thioglycerol) are ineffective (Marshall et al., 1955; Meyer and Marshall, 1956; Debons et al., 1962).
- inhibitors of glucose uptake injected in the hypothalamus protect neurons from necrosis due to GTG (Debons et al., 1974; Brown and Viles, 1982).

-the effect of GTG is blocked in diabetic mice (Debons et al., 1968) and is restored by insulin replacement (Debons et al., 1969) which suggests the presence of an insulin dependent mechanism for uptake of glucose in the hypothalamus.

It is thought that GTG damage to neurons causes a massive release of serotonin that, in turn, causes damage to the surrounding capillaries producing local ischemia responsible for necrosis of the area (Debons et al., 1979a, 1979b).

It was suggested that the obesity syndrome developed by GTG-mice could be due to destruction of the feeding-inhibitory pathway. However, it is then difficult to explain the dose-dependent effect. Possible explanations are that high doses produce a greater damage to an anatomically diffuse area or that more than one system is involved in the control of food intake.

The development of obesity in GTG-mice can be divided into 3 phases. The first few days following the GTG injection are characterized by anorexia and weight loss due to the acute toxic effects of the drug. When animals recover from these effects they enter the second phase, referred to as the dynamic phase, which lasts 4 to 6 weeks, and is characterized by hyperphagia and weight gain. Animals also show mild hyperinsulinemia and insulin resistance (LeMarchand et al., 1978) despite normal glycemia and glucose disposal rates (LeMarchand et al., 1978; Saito and Bray, 1983) but levels of corticosterone are normal at this time (Saito and Bray, 1983). Levels of liver insulin receptors were also reported to be lower than normal, insulin binding being reduced (LeMarchand et al., 1978). During the last phase, called the static phase, body weight stabilizes at an elevated level compared to lean mice and normophagia

is restored (Gray and Liebelt, 1961). However, animals show hyperinsulinemia and insulin resistance (LeMarchand et al., 1978), hyperglycemia (Djazayery, 1976; LeMarchand et al., 1978) and elevated corticosterone levels during morning hours (Saito and Bray, 1983). GTG-mice are also characterized by a greater naso-anal length and greater bone mass (DeLeeuw et al., 1981). The cause of their faster growth rate has remained unexplained. Growth hormone serum levels are generally decreased but GTG-mice might actually oversecrete this hormone in response to stimulation (Sinha et al., 1975). Activity of the thyroid gland obtained via measurements of I⁻ uptake is not different in GTG-mice with reference to lean mice (Schindler and Liebelt, 1967).

The increased food intake exhibited by GTG-mice does not entirely account for their elevated body weight. GTG-mice become obese even when pair-fed to control lean mice (Zaror-Behrens and Himms-Hagen, 1984) or fed a restricted diet (Eley and Himms-Hagen, 1989b). This suggests a decrease in energy expenditure. In fact, the metabolic efficiency was estimated to be four times higher than normal lean mice (Djazayery et al., 1979).

The duration and the magnitude of the hyperphagia is influenced by diet, environmental temperature and the age of the animals when lesioned (DeLaey et al., 1974, 1975). It was suggested that the lower energy expenditure of these mice could be due also to thermoregulation at a lower than normal temperature during the night and part of the day (Zaror-Behrens and Himms-Hagen, 1989). Sympathetic activity is normal in BAT (Zaror-Behrens and Himms-Hagen, 1989) and heart (Young and Landsberg, 1980; Zaror-Behrens and Himms-Hagen, 1989).

BAT is not damaged in GTG-obese animals as shown by examination of the ultrastructure of its mitochondria (Hogan and Himms-Hagen, 1983). GTG-mice have a greater amount of BAT (Hogan and Himms-Hagen, 1983) which may be due to the overall faster growth of these animals. GTG-mice are able to activate their BAT in the cold (Hogan and Himms-Hagen, 1983) but their response is delayed and they are somewhat cold-intolerant (Eley and Himms-Hagen, 1989a).

DIT in BAT is absent in the dynamic phase of obesity (Hogan and Himms-Hagen, 1983) except for a transient activation at the time meal is eaten (Eley and Himms-Hagen, 1989b). This suggests an altered circadian rhythm in BAT of GTG-mice. Sympathetic activity and UCP content of BAT are normal (Zaror-Behrens and Himms-Hagen, 1984, 1989). Therefore, Himms-Hagen (1989b) suggests that, in the dynamic phase of obesity, the cephalic phase of BAT activation is present and occurs normally but the postprandial phase is absent. This might be due to a suppression of DIT by the thermic effect of food which is higher than usual due to the larger amount of food eaten by these animals or because neural circuits responsible for DIT are damaged. The latter effect is not supported by the fact that DIT and normal responsiveness of BAT to noradrenaline are restored in the static phase of obesity (Hogan and Himms-Hagen, 1983). The early establishment of insulin resistance in BAT of GTG-mice (Cooney et al., 1985, 1987) may also play a role in the suppression of the functioning of BAT and the attenuated response to cold stimulation.

In the GTG-obese mouse adrenalectomy (Debons et al., 1982, 1983) or hypophysectomy (Powley and Plocher, 1980; Debons et al., 1982) in the dynamic phase of obesity blocked the hyperphagia, weight gain and obesity

(Debons et al., 1982). These mice also develop gradual hypoglycemia that causes death eventually (Debons et al., 1983). The effect of adrenalectomy is reversed by glucocorticoid administration but not by mineralocorticoid administration (Debons et al., 1982). Adrenalectomy has no effect on lean, unlesioned animals (Debons et al., 1982).

The effect of corticosterone appears to be exerted in the central nervous system since intracerebroventricular injection of corticosterone in small amounts reverses the effect of adrenalectomy whereas the same dose given intraperitoneally has no effect (Debons et al., 1986).

In other animal models of obesity in which BAT thermogenesis is suppressed, such as the genetically obese ob/ob mouse and the genetically obese fa/fa rat, adrenalectomy improves BAT thermogenic function (Holt and York, 1984; Tokuyama and Himms-Hagen, 1987; Freedman et al., 1985, 1986). The effect of adrenalectomy on the suppressed BAT thermogenesis of the GTG-mouse is unknown.

STATEMENT OF THE PROBLEM

GTG-mice in the dynamic phase of obesity have a normal concentration of corticosterone in their blood and a normal circadian rhythm in this hormone as opposed to the genetically obese (*ob/ob*) mouse which shows higher levels at a young age at all times of day (Saito and Bray, 1983; Smith and Romsos, 1985). Yet, in both cases, adrenalectomy reverses the obesity of these animals. In addition, it was shown before that the genetically obese mouse is hypersensitive to an action of corticosterone that leads to obesity and altered BAT thermogenesis (Tokuyama and Himms-Hagen, 1987). The objectives of these experiments were to find out whether a mouse with obesity induced by hypothalamic lesions, the GTG-mouse, would show improved BAT thermogenesis after adrenalectomy and a similar hypersensitivity to a suppressive effect of corticosterone on BAT function as seen in the genetically obese mouse. The experimental approach consisted in studying dose-response relationships between corticosterone levels in the blood and various indices of BAT thermogenesis and obesity in adrenalectomized lean and GTG-mice with various levels of corticosterone replacement.

In order to design the study of GTG-mice it was necessary to know whether the housing conditions, i.e. single versus group housing, would influence BAT thermogenic activity. This experiment was undertaken in response to the finding of Jennings et al. (1986) that mice housed at 23°C singly or in groups showed different thermogenic activation of their BAT. I was

interested in knowing how BAT would be affected by housing mice at 28°C, the temperature at which mice are usually studied in this laboratory.

CHAPTER TWO

MATERIALS AND METHODS

1) Animals

Female mice of the C57Bl/6 strain obtained from Charles River Inc., St-Constant, Québec were used for the caging experiment. The experiment on GTG obesity was performed on female mice of the C57Bl/6J strain from Jackson Laboratories, Bar Harbor, ME. Because of the unavailability of Jackson Laboratories mice toward the end of this research project, mice obtained from Charles River Laboratories were used instead. Moreover, since only 3-week old mice were available from this supplier at that time, the mice were kept in the animal house at the Health Sciences building, University of Ottawa, until they were 8-weeks old. They were housed four per cage at 28°C, and fed Purina Rat chow. After this treatment the Charles River mice had attained body weights comparable to those from Jackson Laboratories (at the same age).

Experimental procedures were started after the mice had been acclimated for at least one week to their new housing conditions. Animals were housed three to five per cage unless otherwise specified, in plastic cages with wood chip bedding, with free access to food (Purina rat chow) and water. For all experiments the room temperature was 28±1°C and mice were maintained on a controlled lighting schedule of 12:12 light:dark with lights on at 7:30 hours.

2) GTG lesions

8-9 week-old mice (C57Bl/6J strain) were injected with GTG (Sigma) after an overnight fast of approximately 15 hours. In the morning, mice were divided randomly into a control group receiving a saline injection (0.9% sodium chloride) and a "GTG" group receiving an injection of GTG 7% in saline. In both cases, the injections were made intraperitoneally. The dosage used was 700mg GTG/kg body weight (based on the fasted weight). This dosage was expected to give a survival rate of 90% and an incidence of obesity of 65% (DeLaey et al., 1975; Hogan and Himms-Hagen, 1983). After the injection, food intake and body weight gain were monitored daily. GTG-mice were considered to be obese when their rate of weight gain was at least three times higher than that of lean mice. GTG-mice that did not meet this criterion were not included in the study.

3) Food intake measurement

The amount of food given to the animals was weighed between 8:00 and 9:30 hours daily. Intake was calculated by difference and expressed in gram per mouse per day (g/mouse/day). This was necessary since the number of mice per cage varied from 3 to 5.

4) Corticosterone pellets preparation

Corticosterone (Sigma) and cholesterol (Sigma) were mixed in amounts required to produce 100mg pellets containing 1, 2, 5 or 20mg corticosterone. The mixture was then melted over a gas flame while gently shaking just enough to mix the 2 compounds. The liquid was poured into 1.5ml Eppendorf centrifuge tube caps to shape the pellets. When completely solidified pellets were weighed, adjusted to 100 ± 2 mg and kept in a dry dark place until needed. Such pellets are expected to give a stable level of serum corticosterone within 3 days that lasts for another 10 days (Meyer et al., 1979).

5) Adrenalectomy and pellet implantation

Mice were anesthetized with halothane and bilateral adrenalectomy was performed by the dorsal approach. Sham adrenalectomy consisted of exposing the adrenal glands without removing them. Cholesterol pellets containing various amounts of corticosterone were implanted under the skin of the lower back. Such pellets are known to induce a stable level of serum corticosterone within 3 days that lasts for another 10 days (Meyer et al., 1979). Wound clips were used to close the incision. After the surgery, all mice received an injection of corticosterone 1mg/ml in saline (100 μ l). The completeness of adrenalectomy was checked by visual inspection of the glands and serum corticosterone concentration at the time of killing was measured (see method below).

6) Temperature measurements

For all experiments, rectal temperature at the time of sacrifice was measured using a Bailey Instruments digital thermocouple thermometer, model BAT-8, with a flexible Teflon-sheathed microprobe.

7) Isolation of BAT mitochondria

Animals were sacrificed by decapitation and blood was collected into chilled Eppendorf microtubes. Interscapular and subscapular BAT depots were removed and placed into ice-cold isolation medium (0.25M sucrose (BDH), 0.2mM dipotassium EDTA (Sigma) and 1.0mM HEPES (Sigma) adjusted to pH 7.2 with 1N NaOH (Fisher)). BAT depots were cleaned and weighed, then minced with scissors and homogenized in 3.5ml isolation medium with glass/Teflon homogenizers (5-6 strokes) at 400rpm. Samples were taken for protein determination (100 μ l) and for measurement of 5'-deiodinase and cytochrome c oxidase activities (2x200 μ l). They were homogenized further in

glass/glass homogenizers (8 stokes), frozen quickly in liquid nitrogen and kept at -20°C . The remaining homogenates were transferred to clear plastic centrifuge tubes, diluted to 14ml and centrifuged for 10 minutes at 3000rpm in a Sorvall RC-5B refrigerated centrifuge using a HB-4 rotor. From this point the method followed was as described by Slinde et al. (1975) with some modifications. Briefly, the supernatant obtained after the first centrifugation was filtered through gauze and centrifuged again at 10000rpm for 14 minutes. This 10000rpm-pellet was kept aside (pellet #1). The sediment from the 3000rpm centrifugation was resuspended in 14ml isolation medium, centrifuged at 3000rpm for 10 minutes, and the supernatant was filtered through gauze. The supernatant was centrifuged at 10000rpm (pellet #2). Pellet #1 and #2 were combined, resuspended and centrifuged at 10000rpm for 14 minutes. The final pellet was resuspended in a small volume (200 μl) of isolation medium in order to give a protein concentration of about 5mg/ml. Samples were maintained at 4°C or on ice at all times.

8) GDP binding procedure

The method for measuring GDP binding to isolated mitochondria was as described by Nicholls (1976) and modified by Desautels et al. (1978). Mitochondria were incubated for 2 minutes with or without 1mM ADP (Sigma) for measurement of non-specific binding in a medium containing disodium EDTA 1mM (Sigma), choline chloride 10mM (Sigma), TES buffer 20mM (Sigma), sucrose 100mM (BDH), rotenone 5 μM (Sigma), atractyloside 100 μM (Sigma), [U- ^{14}C]-sucrose 0.1 $\mu\text{Ci/ml}$ (Amersham), [8- ^3H]-GDP 0.78 $\mu\text{Ci/ml}$ (Amersham) and GDP 10 μM (Sigma). After the incubation, samples were centrifuged at 12000rpm in an Eppendorf microcentrifuge. The supernatant was

aspirated and the mitochondrial pellets were allowed to dissolve in 1ml NCS tissue solubilizer (Amersham) for 12 hours at 55°C. Then, 50µl of ascorbic acid 10% (BDH) was added as well as 10ml toluene (BDH) containing 0.65% 2,5-diphenyloxazole (Sigma). Samples were counted in a Beckman LS 6800 liquid scintillation counter. [U-¹⁴C]-sucrose was added in the incubation medium to correct for the amount of water trapped in the pellet. Results are expressed as pmol GDP bound per mg mitochondrial protein.

9) Protein determination

Protein concentration was determined in BAT total homogenates and mitochondrial suspensions using Lowry's method (1951) as modified by Schacterle and Pollack (1973). Bovine serum albumin (Sigma) was used as standard. 10µl of BAT homogenate or 8µl of mitochondria or an appropriate amount of standard was dissolved in 0.5N NaOH to make 1ml. Absorbance readings were made with a Bausch and Lomb Spectronic 20.

10) Serum corticosterone determination

Blood was centrifuged at 12000rpm for 15 minutes in a refrigerated microcentrifuge. Serum was collected and stored at -20°C until needed.

The method used was set up by Deeks (Bégin-Heick and Deeks, 1987) based on an original direct radioimmunoassay by Al-Dujaili et al. (1981). 5µl of serum was incubated for 60 minutes at 37°C in a phosphate buffer 0.05M (Fisher or BDH) pH 3.0, containing 0.025M sodium citrate (Fisher), 0.1% bovine serum albumin (Sigma, RIA grade), a 1:1 dilution of the commercially available antiserum to corticosterone-21-thyroglobulin (ICN Immunobiologicals) and [1,2,6,7-³H]-corticosterone (~5000-7000cpm) (Sigma). Sample corticosterone was determined using a standard curve spanning 0-1000pg/tube of

corticosterone. The antibody-corticosterone complex was separated from the free corticosterone by adding 0.5% Norit A charcoal (Bio-rad) and 0.05% dextran T70 (Sigma) in 0.05M NaH₂PO₄ (BDH) pH 7.4. The samples were centrifuged at 3200 rpm at 4°C for 15 minutes in a Beckman J6B centrifuge. An aliquot of the supernatant was diluted in Aquasol and counted in a Beckman LS 6800 liquid scintillation counter. The results are expressed as µg corticosterone per dl serum. The sensitivity of the assay is 0.15µg/dl.

11) Serum thyroxine and triiodothyronine determinations

Serum thyroid hormones were determined from samples frozen at -20°C until the time of the assay. A basic radioimmunoassay was taken from Larsen (1976) and modified as in Kates (1989, Ph.D. thesis).

Standard curves were prepared by diluting a solution made of 40µg/ml thyroxine (T₄, Sigma) or 40µg/ml triiodothyronine (T₃, Sigma) in 0.05N NaOH (Fisher) + 0.1% bovine serum albumin (Sigma, RIA grade) in thyroid hormone free mouse serum. The concentration of the initial solutions was checked by measuring the absorbance at 325nm for T₄ and 320nm for T₃ in a Gilford 2400-2 spectrophotometer. The range of concentrations covered was 0.25-8.00µg/dl for T₄ and 10-300ng/dl for T₃. Standards were frozen at -80°C until needed.

Thyroid hormone free mouse serum was prepared by incubating the serum with 50000 cpm/ml ¹²⁵T₄ or ¹²⁵T₃ (Amersham) and 0.023g/ml Norit A charcoal (Bio-Rad). The mixture was constantly stirred overnight at 4°C and centrifuged at 100000g for 15 minutes in a Beckman L8-55M ultracentrifuge with a Ti55 rotor. Serum could be considered free of thyroid hormones when it contained less than 5% of the counts that were put in at the beginning (Larsen, 1976). Thyroid hormone free serum was stored at -80°C until needed.

A) T4 determination

The assay consisted in incubating 5 μ l of sample or T4 standard in 95 μ l of assay buffer (0.2M glycine (Sigma), 0.2M sodium acetate (Analar), 0.02g/ml sodium salicylate (Fisher), 0.2mg/ml bovine serum albumin (Sigma, RIA grade)). Assay buffer only was used for total counts. $^{125}\text{T4}$ (Amersham) in assay buffer (7-8000 cpm/tube) was added to each tube to have a total volume of 1 ml. Then, T4 antibody (gift from Dr. P.R. Larsen) was added to give a final dilution of 1:10000. This dilution was found to be optimal. All tubes were incubated overnight at 4°C. Antibody-bound T4 was separated from the free T4 by adding 1ml of assay buffer containing 40ng/ml Dextran T70 (Sigma) and 400ng/ml Norit A charcoal. Total counts received assay buffer only. All tubes were incubated 45 minutes at 4°C and centrifuged at 1500g for 20 minutes in a Beckman J6B centrifuge. Supernatant was decanted in new tubes and counted for 2 minutes each in a Beckman gamma 5500 counter. Results were calculated with a IBM computer using the Lotus 1-2-3 package.

B) T3 determination

15 μ l of T3 sample or standard was added to 885 μ l of assay buffer containing T3 antibody (Gift from Dr. P.R. Larsen). The final dilution of antibody was 1:25000. Total counts consisted of assay buffer only. All tubes were incubated at 4°C for 24 hours. Then, 100 μ l assay buffer containing 3-4000 cpm $^{125}\text{T3}$ was added to each tubes. Tubes were incubated again at 4°C for 24 hours. The separation of antibody-bound and free T3, the counting and the calculations were done as for the T4 assay.

12) Serum Insulin radioimmunoassay

Serum insulin was determined as described in Dalpé-Scott et al. (1982) except that the assay was adapted to use 50µl of serum instead of 100µl because of the small amount of blood that can be obtained from a mouse. The principle of this radioimmunoassay is that a fixed amount of radiolabelled insulin molecules are competing with a variable number of unlabelled (the sample) for a fixed amount of insulin antibody binding sites. Briefly, an antibody mix containing guinea pig serum 1:500 (Bio/Can Scientific), rabbit antibody to rat insulin 1.66µl/ml and rabbit antibody to guinea pig gamma-globulin 1:30 in borate buffer pH 8.0 (0.04M sodium borate 0.5% (J.T. Baker) and methiolate 0.1mg/ml (Sigma) was incubated overnight. Then 50µl of serum + 100µl borate buffer or 150µl standard rat insulin (Novo Biolabs) was added followed by a second incubation at 4°C for 5-6 hours. Then, ¹²⁵I-insulin (porcine, NEN specific activity between 80-120µCi/µg) was added (10000cpm/tube) and the mixture was incubated again overnight. Samples were then centrifuged at 1200rpm at 4°C for 30 minutes in a Beckman J6B centrifuge. Supernatant was discarded and pellet was counted for 2 minutes in a Beckman gamma 5500 counter. The antibody to rat insulin and the second antibody to guinea pig gamma-globulin were raised in rabbits and kindly provided by Dr. N. Bégin-Heick.

13) Measurement of BAT thyroxine 5'-deiodinase activity

This method measures the amount of ¹²⁵I released when T3 is produced from T4 by the action of the enzyme. The basic method (Visser et al., 1982;

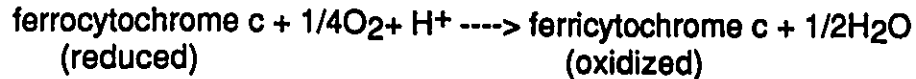
Leonard and Rosenberg, 1980; Leonard et al., 1983) was used as described by Kates and Himms-Hagen (1985).

Samples were frozen at -20°C until assay (no more than one month). A $30\mu\text{l}$ sample containing between $15\text{-}150\mu\text{g}$ protein was incubated under nitrogen for 30 minutes at 37°C in 0.01M potassium phosphate buffer pH 7.0 (BDH) containing 1mM EDTA (Sigma), 10mM dithiothreitol (Sigma), 1mM propylthiouracil (Sigma), 2.32 nM T₄ (Sigma) , 0.15nM ^{125}I -T₄ (Amersham) for a total concentration of T₄ of 2.47nM (about 50000 counts/sample). Reaction was stopped by placing samples on ice and by adding $50\mu\text{l}$ thyroid hormone free human serum containing T₃ and T₄ binding proteins and then $300\mu\text{l}$ TCA 12.5% (BDH). Samples were centrifuged at 14000 rpm for 2 minutes at room temperature in an Eppendorf microfuge. $500\mu\text{l}$ supernatant was passed through a column containing Dowex AG 50 W-X2 $100\text{-}200$ mesh cation exchange resin (Bio-Rad) in acetic acid:H₂O (1:10). Columns were washed 3 times with acetic acid:H₂O. The total eluate was counted for 2 minutes in a Beckman gamma 4000 counter.

Due to spontaneous autoradiolysis of ^{125}I -T₄ , this product was purified prior to use according to Larsen (personal communication to Dr. J. Himms-Hagen). ^{125}I -T₄ was diluted 1:5 in 0.01M potassium phosphate buffer pH 7.0 containing 1mM EDTA and passed through a column containing LH-20 Sephadex (Bio-rad) prewashed with buffer. The eluate, containing free ^{125}I was discarded. Columns were washed 2 times with 2ml H₂O and the pure ^{125}I -T₄ was obtained by adding 2ml ethanol:H₂O (70:30) to the column. The volume of the solution was reduced to about 1ml by gently blowing nitrogen gas into the tube.

14) Cytochrome c oxidase assay

This assay was adapted from Yonetani and Ray (1965). It consisted of the spectrophotometric determination of the oxidation of reduced cytochrome c by the enzyme.



BAT homogenate was prepared as described above and samples were frozen at -20°C until needed. Samples were treated with Lubrol WX 0.3% (1 μl Lubrol/mg protein) and diluted in 0.1M potassium phosphate buffer pH 7.0 (BDH) so as to have a final protein concentration of 0.65mg/ml. 10 μg protein was added in disposable spectrophotometric cuvetts (Sarstedt) containing reduced cytochrome c (Sigma, horse heart type III) and absorbance was measured immediately for 2 minutes at 550nm in a Beckman DU-50 spectrophotometer using the kinetic soft-pac module (program kindata). The plot of initial velocity against protein concentration was linear up to 15 μg protein (figure 1). Determinations were done in duplicate at 3 different concentrations of cytochrome c (26.4, 33.0 and 39.6 μM) so as to construct a Lineweaver-Burk plot and get the V_{max} for each sample. A Lineweaver-Burk plot that includes these values is shown in figure 2.

Cytochrome c was reduced prior to use by dissolving 100mg in buffer containing 1mg% ascorbic acid (BDH). This solution was used for the assay.

15) BAT UCP determination

UCP was determined in BAT homogenates using a solid phase RIA. Samples were diluted to 0.5mg/ml in PBS (phosphate buffer 6.5mM (BDH)

FIGURE ONE: PROTEIN CURVE: INITIAL RATES OF OXIDATION OF FERROCYTOCHROME C BY CYTOCHROME C OXIDASE AT INCREASING CONCENTRATIONS OF BAT HOMOGENATE PROTEINS.

BAT homogenates were prepared as described in the method section. 0-18 μ g of homogenate proteins were added to spectrophotometric cuvetts containing 26.4 μ M of ferrocytochrome c in 0.1M phosphate buffer pH 7.0 for a final volume of 3ml. Measurements were done at room temperature. Each point represents the mean of triplicates. S.E.M. bars are too small to be seen outside symbols.

FIGURE TWO: REPRESENTATIVE LINEWEAVER-BURK PLOT OF INITIAL RATES OF OXIDATION OF FERROCYTOCHROME C BY BAT CYTOCHROME C OXIDASE.

Assay was done as described in the method section. Concentrations of ferrocytochrome c tested were between 6 and 40 μ M. Each point is the mean of duplicates. This assay was done several times and the same results were obtained each time.

FIGURE 1

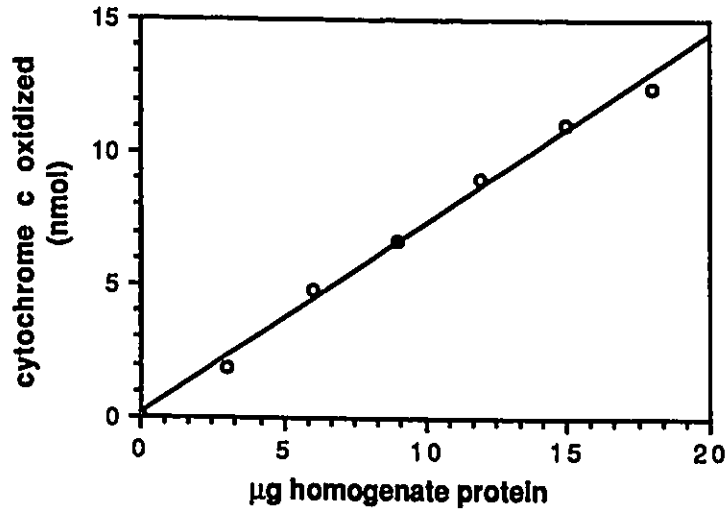
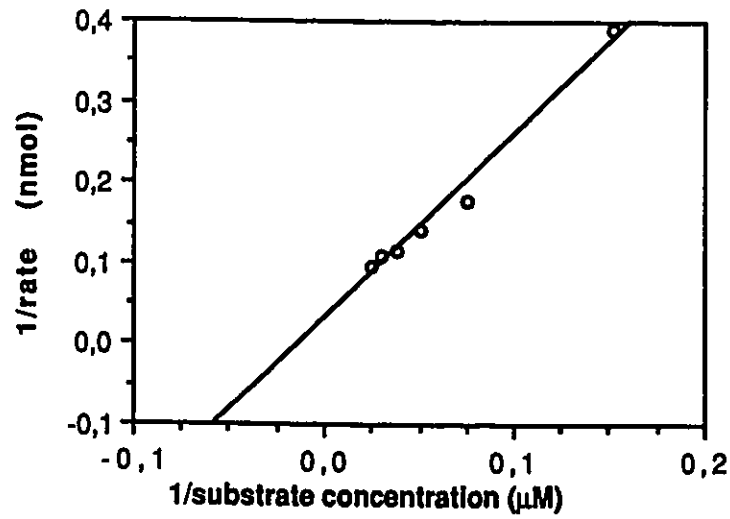


FIGURE 2



containing NaCl 0.14M (Fisher) and KCl 3mM (Fisher)) and extracted with 5% Triton X-100 (BDH) (Triton:homogenate 1:4) for 30 minutes at room temperature as described by York (personal communication). Samples were then diluted 5 times with PBS and centrifuged 2 minutes in an Eppendorf microfuge. The supernatant was removed and kept at -20°C for less than 3 days.

Flexible 96-well microtitre plates (Falcon 3912) were soaked in Pierce RBS 35 detergent and washed thoroughly with distilled H₂O to remove residues from the manufacturing process so as to increase protein binding to the plates and reduce variability in binding.

Assay plates were coated with mouse UCP at 10µg/ml (50µl per well) and incubated at 37°C for 2 hours. For non-specific binding, 50µl/well of PBS+Triton 0.1% was used. After the incubation period the liquid was removed by vigorously "slapping" the plates onto layers of Kleenex tissue. Plates were washed 5 times with PBS containing 1% bovine serum albumin (BSA, Sigma RIA grade) and 0.1% sodium azide (BDH), slapping between each wash. The first wash was incubated at 37°C for 10 minutes to block sites not bound with UCP. After the last wash plates were allowed to drain in the inverted position at least 5 minutes.

The samples were placed in wells to give a final protein concentration of 0.80µg/well. Standards used consisted of mouse UCP in the range 0.1-5.3ng UCP. Some wells were reserved for total and non-specific binding. They contained PBS+Triton 0.1%. Freshly diluted rabbit anti-hamster UCP antiserum (diluted 1:3000 in PBS, 10µl/well) was added to all wells. The plates were covered with parafilm and incubated at 4°C overnight.

The next day plates were washed 5 times with PBS+BSA1%+sodium azide 0.1% and 50 μ l of 125 I-protein A (60000-70000cpm/well, ICN) was added to all wells. Plates were incubated 1.5 hour at room temperature. The incubation was followed by another series of washes, plates were drained and wells were cut out into plastic tubes and counted in a Beckman 5500 gamma counter. Results were calculated from the linear regression line of logit-In UCP and expressed as μ g UCP/mg BAT protein or as μ g UCP per total tissue. For these calculations an IBM computer with the Lotus 1-2-3 package was used.

Mouse and hamster UCP were purified by chromatography on hydroxylapatite as described by Lin and Klingenberg (1980, 1982) except that the sucrose density gradient step was not performed. Hamster UCP antibodies were raised in rabbit as described by Fernandez et al. (1987). I am grateful to Dr. G. Zaror-Behrens for preparing mouse UCP and rabbit anti-hamster UCP antiserum.

CHAPTER THREE

RESULTS

PART ONE: CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON MOUSE BROWN ADIPOSE TISSUE FUNCTION.

Previous studies demonstrated that the extent of BAT thermogenesis is related to the way animals are housed. It is greater in singly-housed mice than in group-housed mice (Jennings et al., 1986). When mice are caged in groups they tend to huddle and regulate their body temperature as one organism, a phenomenon referred to as social thermoregulation, which limits the need for heat production. Jennings et al. (1986) studied the effect of caging mice in groups of different sizes at 23° and 4°C. However, the usual room temperature of 20-25°C is already cold-stressing the animals. The following experiment was undertaken to assess the effects of housing conditions on BAT thermogenic state when mice are kept at 28°C, a temperature that is closer to thermoneutrality. I was particularly interested in the effects of caging mice singly or in groups on BAT function at the temperature at which mice are normally studied in this laboratory. In addition, the study included a determination of the activity of T4 5'-deiodinase in BAT since it seems likely that the expected alterations in BAT thermogenic state might be accompanied by alterations in the activity of this enzyme.

Methods:

Female C57Bl/6 mice were allowed to acclimate to their new environment for one week after their arrival. Then, they were divided randomly in groups of 1, 2 or 6 mice per cage (6 cages of each group). They were

maintained at 28°C for 15 days and then killed 12 per day until all were sacrificed (killing more than 12 animals per day becomes difficult in terms of time and labor). All mice in one cage were killed the same day. All mice were killed by decapitation, blood was collected and put on ice immediately. Inter- and subscapular BAT was removed, weighed, homogenized and aliquots taken for protein determination which was done the same day and for later determination of T4 5'-deiodinase and cytochrome c oxidase activities as well as UCP content. Mitochondria were isolated from the remaining homogenate and used for the measurement of GDP binding. Blood was centrifuged and serum was stored at -20°C for later determination of T3 and T4 content.

Statistical analysis:

Data was analysed by completely randomized design one way ANOVA using a SAS computer statistic program. Scheffé's post hoc test (Bruning and Kintz, 1977) was used to assess the difference between all possible means. Means were considered significantly different when $P < 0.05$.

Results are presented in tabular form but BAT GDP binding, T4-5'-deiodinase and cytochrome c oxidase activities as well as UCP content are also presented graphically to allow an easier evaluation of the data.

Results:

1) Effects of housing conditions on BAT function.

BAT GDP binding was increased about two-fold in mice that were housed singly compared to mice housed in pairs (table 1, figure 3). The effect of caging singly was even more dramatic when compared to mice housed in groups of 6, with a six-fold increase in GDP binding. When mice housed in

TABLE 1: CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON MOUSE BODY WEIGHT AND TEMPERATURE, BROWN ADIPOSE TISSUE AND SERUM LEVELS OF THYROID HORMONES.

Animals were housed individually, in pairs and in groups of 6 for 15 days. The number of mice per cage is indicated at the top of each column. Values represent means \pm S.E.M.. The number of animals is indicated in parentheses. All values for BAT are for activities or amounts in homogenates except for mitochondrial GDP binding.

SYMBOLS:

- * significant difference ($p < 0.05$) compared to the first group (one mouse per cage)
- ◇ significant difference ($p < 0.05$) compared to the second group (two mice per cage)

TABLE 1

	NUMBER OF MICE PER CAGE		
	1	2	6
Body weight change (g)	3.2±0.4(6)	3.0±0.2(12)	3.4±0.1(36)
Rectal temperature (°C)	36.7±0.3(6)	37.2±0.2(11)	37.1±0.1(35)
Serum T3 (ng/dl)	64.8±10.7(5)	54.0±3.4(12)	64.6±2.2(36)
Serum T4 (µg/dl)	3.08±0.18(6)	3.24±0.14(12)	3.47±0.16(36)
BAT weight (mg)	112.0±8.2(6)	126.6±4.8(12)	136.2±5.0(36)
protein (mg)	9.9±0.65(6)	10.1±0.27(12)	8.8±0.29(36)
T4 5'-deiodinase specific activity (fmol/mg protein/hr)	83.5±11.2(6)	76.9±6.9(11)	59.0±6.0(27)
total (fmol/hr)	854±148(6)	774±75(11)	533±59(28)
UCP			
specific (µg/mg protein)	6.9±1.4(6)	4.3±0.5(12)*	2.0±0.2(32)* [∅]
total (µg)	67±12(6)	44±5(12)*	17±1(32)* [∅]
cytochrome c oxidase			
specific activity (nmol/min/µg protein)	1.77±0.45(5)	2.38±0.79(8)	2.38±0.50(16)
total (nmol/min)	16.4±3.2(5)	23.5±7.4(8)	21.7±5.0(16)
mitochondrial GDP binding (pmol/mg protein)	274±37(6)	156±20(12)*	47±7(36)* [∅]

FIGURE 3: CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON GDP BINDING TO BAT MITOCHONDRIA.

Data from Table 1 have been plotted to allow an easier assessment of the effect of housing conditions on BAT GDP binding. Animals were housed individually, in pairs and in groups of six for 15 days. Values are means \pm S.E.M. Refer to Table 1 for the number of animals for each determination.

SYMBOLS:

- * significant difference ($p < 0.05$) compared to the first group (one mouse per cage)
- ◇ significant difference ($p < 0.05$) compared to the second group (two mice per cage)

FIGURE 4: CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON MOUSE TOTAL BAT UNCOUPLING PROTEIN.

Data taken from Table 1. For legend, see above.

FIGURE 3

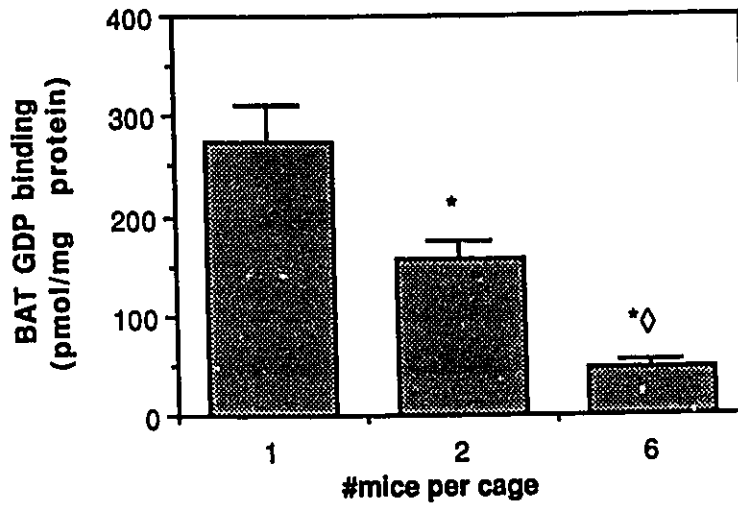
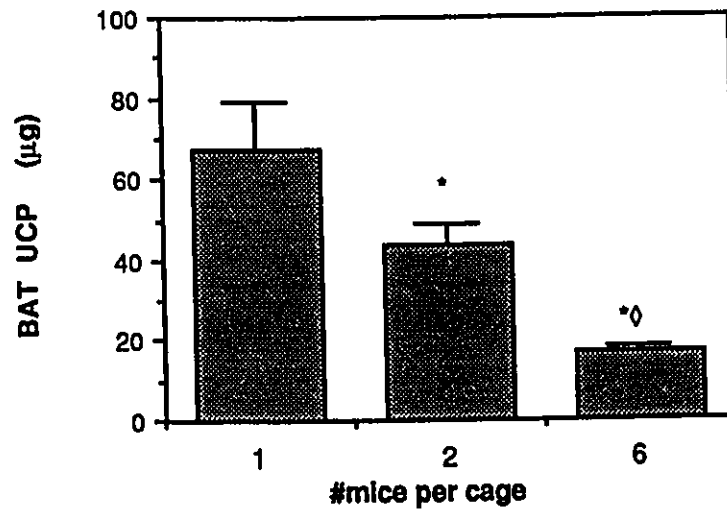


FIGURE 4



groups of 2 were compared to those housed in groups of 6, a three-fold increase in GDP binding was observed. In all cases, means were statistically different.

The same trend was observed in the case of the UCP content (table 1, figure 4). However, the effect was less dramatic. Housing mice singly or in groups of 2 caused a significant increase (4X and 1.5X respectively) in the level of UCP when compared to mice housed in groups of 6. When comparing mice grouped in pairs and in 6's, an almost three-fold significant increase in UCP content was recorded. These results apply to UCP expressed either specifically (content per mg protein) or as a total (total tissue content).

The total mitochondrial cytochrome c oxidase activity was not affected by housing conditions in this experiment (table 1, figure 5).

There were no significant differences in specific or total T4 5'-deiodinase activity between mice housed singly, in pairs or in groups of 6. However, there was a trend to an increase in singly-housed mice that did not reach statistical significance due to the large variation in the data as reflected by the S.E.M. (table 1, figure 6).

BAT level of protein (total) was not affected by housing mice in different groups (table one). There was a trend to a decrease in BAT wet weight as the number of mice per cage was decreased which did not reach statistical significance.

FIGURE 5: CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON MOUSE BAT CYTOCHROME C OXIDASE ACTIVITY.

Data taken from Table 1.

SYMBOLS:

- * significant difference ($p < 0.05$) compared to the first group (one mouse per cage)
- ◇ significant difference ($p < 0.05$) compared to the second group (two mice per cage)

FIGURE 6: CAGING EXPERIMENT: EFFECT OF HOUSING CONDITIONS ON MOUSE BAT T4 5'-DEIODINASE ACTIVITY.

Data taken from Table 1. For legend, see above.

FIGURE 5

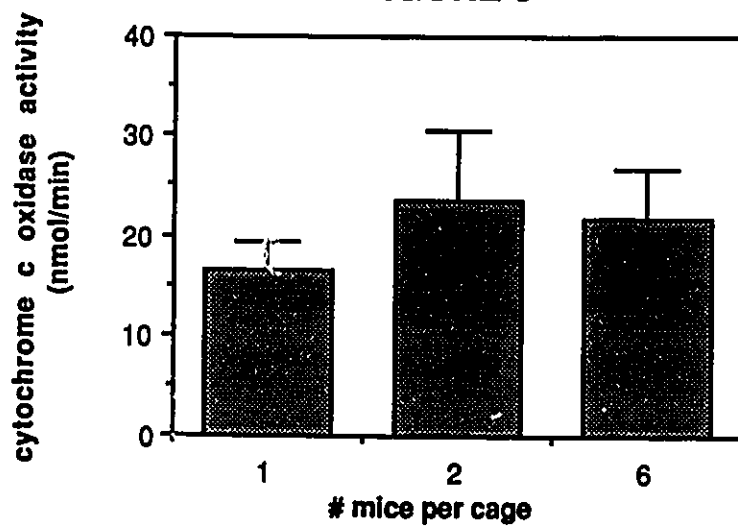
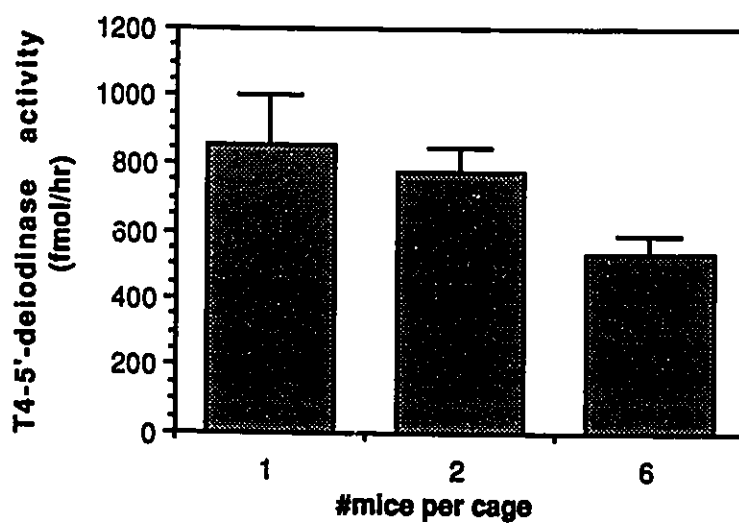


FIGURE 6



2) Effects of housing conditions on body weight, rectal temperature and serum T4 and T3 levels.

Caging mice singly or in groups of 2 or 6 did not affect the change in body weight in 15 days nor the rectal temperature at the time of killing (table one). No difference was obtained in T4 and T3 serum concentrations.

Conclusion

Results indicate that housing mice singly at 28°C increases BAT thermogenic activity (higher mitochondrial GDP binding) and thermogenic capacity (higher total content of UCP) without altering total mitochondrial content (unchanged cytochrome c oxidase activity) or total metabolic mass (unchanged total protein).

The findings of this experiment were taken into account when planning the next one that is presented below. The number of mice per cage was always higher than two, four most of the time. Due to the high mortality rate of GTG-mice especially after adrenalectomy some cages contained 3 or 5 mice in order to keep together those that received the same treatment and to prevent some mice from being caged alone or in pairs.

PART TWO: GTG-OBESITY EXPERIMENT

Studies on various types of obese animals have shown that their suppressed BAT function could be partly responsible for the development of obesity. Removal of corticosterone by adrenalectomy reverses the obesity of the genetically obese ob/ob mouse (Saito and Bray, 1984; Grogan et al., 1987; Shimomura et al., 1987), the GTG-mouse (Debons et al., 1982, 1983, 1986a,

1986b), the diabetic db/db mouse (Saito and Bray, 1984), the monosodium glutamate (MSG) treated mouse (Tokuyama and Himms-Hagen, 1989), the genetically obese yellow mouse (Shimizu et al., 1989), the parasagittal-knife cut-lesioned rat (Romsos et al., 1987), the PVN-lesioned rat (Tokunaga et al., 1989) and the fatty fa/fa rat (Freedman et al., 1985, 1986). Moreover, it was demonstrated that the ob/ob mouse appears to be hypersensitive to a suppressive effect of corticosterone on BAT (Tokuyama and Himms-Hagen, 1987).

The objective of these experiments was to find out whether a mouse with obesity induced by hypothalamic lesions, the GTG-mouse, would show a similar hypersensitivity to a suppressive effect of corticosterone on BAT as seen in the genetically obese mouse and rat. The following questions directed the research:

- what is the effect of adrenalectomy on BAT function and the development of obesity in the GTG-mouse?
- Is there a dose-response relationship between corticosterone level in the blood and BAT thermogenesis in the GTG-mouse?
- Are insulin levels affected by adrenalectomy and corticosterone replacement in GTG-mice?

Methods:

Jackson Lab or Charles River female C57Bl/6J mice were allowed to acclimate to their new environment for at least one week before the start of the experiment. They were housed initially 4 per cage and maintained at 28°C at all times with lights on at 7:30 and off at 19:30.

GTG was injected intraperitoneally at a dose of 700mg/kg body weight after an overnight fast. Control mice received saline. Food was returned to the cages one hour after the injections.

The effects of the drug were allowed to develop for 2 weeks. This was monitored by measuring food intake and body weight daily. GTG-mice were considered to be developing obesity when their weight gain was at least three times faster than controls. After this period a group of GTG- and saline-injected mice was killed to determine the level of obesity and BAT thermogenesis at this time point. This group will be referred as the "2-week control group". The rest of the mice were adrenalectomized (adx) and implanted with cholesterol pellets containing 0, 1, 2, 5 or 20mg corticosterone. After the adrenalectomy some cages contained 3 or 5 mice instead of 4 in order to keep together mice that received the same treatment and to prevent some mice from being caged alone or in pairs. Adrenalectomy was considered successful when the body weight at killing did not exceed by more than 10% the weight at the time of adrenalectomy. This criterion was based on the fact that adrenalectomy reverses the weight gain of GTG-mice as reported previously by Debons et al. (1982, 1983, 1986a, 1986b, also Tse and Debons, 1986). A group was sham-adrenalectomized (sham-adx).

Body weight and food intake were recorded daily for 2 weeks for all groups (9 days for the adx group that did not receive a replacement dose of corticosterone; it was decided that this group would be killed before the others because of the low survival of the adx GTG-mice).

The rest of the mice were sacrificed by decapitation 2 weeks after surgery. At the time of killing body weight and rectal temperature were recorded.

Trunk blood was collected and chilled until serum could be prepared. Serum was kept at -20°C for later determination of insulin and corticosterone concentrations.

Inter- and subscapular BAT was removed, cleaned and weighed. BAT was homogenized and aliquots were taken for protein determination which was done the same day of the experiment. Mitochondria were isolated from the remaining homogenate and GDP binding measured.

Gonadal white adipose tissue (WAT) was also removed and weighed.

Statistical analysis:

Mice with similar serum corticosterone levels were grouped together and the mean corticosterone level calculated (see appendix c, p.96). Results are presented in 2 ways. First, body weight, WAT and BAT weights, BAT protein, BAT GDP binding, serum insulin, serum corticosterone and rectal temperature for 2-week controls, sham-adx and adx lean and obese mice are presented in tabular form (Table 3). t-Tests were used to assess the difference between sham-adx, 2-week control and adx GTG-injected mice and their respective saline-injected controls. t-Tests were also used to compare sham-adx and 2-week control mice to adx mice that received the same treatment (GTG or saline injection).

Secondly, body weight, WAT and BAT weights, BAT proteins, BAT GDP binding and serum insulin are expressed graphically as a function of serum corticosterone levels. The values obtained for sham-adx and 2-week control mice are also included in these graphs. Adx mice that were implanted with corticosterone pellets were compared to adx mice that did not receive a replacement dose of corticosterone by two-way ANOVA with the Tukey-Kramer's test (Bruning and Kintz, 1977) as post hoc test.

The effects of corticosterone replacement per se on the development of obesity was also tested. For that, all GTG or lean mice that received a corticosterone implant were grouped together and an overall mean and S.E.M. calculated. These results are found in table three, together with results for the 2-week control, sham-adx and adx groups. The values contained in this table were analyzed using multiple t-tests.

Results:

1) Food Intake

Since mice were housed 3-5 per cage it was not possible to measure the food intake of each mouse individually. Food intake was measured on a per cage basis and mice were caged in homogeneous groups according to the treatment and the amount of corticosterone they received. For statistical purposes the value of "n" was taken as the amount of cages used for measurement of food intake but the results were converted to the amount eaten per mouse per day because all cages did not contain the same number of mice. Unfortunately, this way of presenting the results will give only a rough estimate of the effect of corticosterone on food intake.

2 weeks after GTG injection the obese mice ate significantly more than lean mice (about twice as much) (table 2). Sham-adx GTG-mice (4-weeks after injection) did not eat significantly more than lean ones. Food intake of adx GTG-mice was significantly reduced compared to the 2-week control and the sham-adx GTG-mice. Adx GTG-mice were also hypophagic compared to adx lean mice. Corticosterone replacement of adx GTG-mice caused the food intake to be increased to values comparable to or slightly higher than their lean counterparts (table 2).

TABLE 2: GTG OBESITY EXPERIMENT: EFFECT OF ADRENALECTOMY, SHAM-ADRENALECTOMY AND CORTICOSTERONE REPLACEMENT ON DAILY FOOD INTAKE OF GTG-OBESSE MICE.

Mice were housed 3-5 per cage. Food intake for each cage was measured daily for the 4 days before the animals were killed and averaged. Values represent these means \pm S.E.M. Since it was not possible to express the data as a function of the serum corticosterone levels, food intake are expressed according to the amount of corticosterone that was implanted. Numbers in parentheses represent the number of cages for each group.

- 2WKS: GTG- or saline-injected mice that were sacrificed 2 weeks after the injection.
- SHAM: GTG- or saline-injected mice were sham-adx 2 weeks after the injection and sacrificed at 4 weeks.
- ADX: GTG- or saline-injected mice were adx 2 weeks after the injection and were implanted with pellets containing no corticosterone (cholesterol only).
- ADX+1, ADX+2, ADX+5, ADX+20: GTG- or saline-injected mice were adx 2 weeks after the injection and were implanted with pellets containing 1, 2, 5 and 20mg corticosterone respectively.

SYMBOLS:

- * significant difference compared to the corresponding control ($p < 0.05$)
- + significant difference compared to mice that received the same treatment (GTG or saline) and sacrificed 2 weeks after the injection ($p < 0.05$)
- significant difference compared to sham-adx mice that received the same treatment ($p < 0.05$)
- ◇ significant difference compared to adx mice of the same group that received no corticosterone ($p < 0.05$).

TABLE 2

	FOOD INTAKE	
	LEAN CONTROL (g/mouse/day)	GTG-OBESE (g/mouse/day)
2-WEEK CONTROL	2.3 ±0.3(2)	4.5 [◇] ±0.7(2)
SHAM-ADX	3.0 ±0.4(2)	3.5 ±0.6(3)
ADX	2.4 ±0.2(4)	1.8 [□] ±0.1(3)
ADX+1	2.3 ±0.2(2)	3.2 [◇] ±0.2(3)
ADX+2	2.2 [□] ±0.0(4)	2.8 [◇] ±0.1(3)
ADX+5	2.6 ±0.3(4)	2.9 [◇] ±0.0(2)
ADX+20	2.9 ±0.4(4)	3.4 [◇] ±0.4(5)

Neither adx nor corticosterone replacement affected the food intake of lean mice (table 2).

2) Serum corticosterone

Serum corticosterone levels were similar when comparing obese and lean mice treated in the same way (table 3). Results seem to show an age effect since sham-adx (4 weeks after the GTG injection) corticosterone levels were elevated compared to 2-week controls. This effect was observed in both obese and lean animals. It is also possible that the sham-adx mice were more stressed by the handling before sacrifice or that the killing was less rapid than usual. The fact that both lean and obese mice show higher levels of corticosterone supports this. It must be remembered that sham-adx mice, as well as the intact 2-week controls, have intact adrenals that respond quickly to stressful conditions.

Adrenalectomy was successful in obese and lean animals; serum corticosterone being undetectable in both groups (table 3). Corticosterone implantation caused an increase in serum levels of this hormone that was generally proportional to the amount present in the pellets. The mean increase was similar to 2-week controls for both obese and lean mice (table 3).

3) Body weight

2-week control GTG-mice and sham-adx GTG-mice were significantly heavier than lean controls at these same times (table 3, figure 7) as expected. Adx GTG-mice had body weight non significantly different from adx lean mice. Adrenalectomy of GTG-mice reversed the increase in body weight that was seen in sham-adx GTG-mice (table 3, figure 7).

TABLE 3:GTG OBESITY EXPERIMENT: EFFECT OF ADRENALECTOMY, SHAM-ADRENAL-ECTOMY AND CORTICOSTERONE REPLACEMENT ON THE DEVELOPMENT OF THE OBESITY OF GTG-OBESE MICE.

Data were pooled in order to assess if corticosterone replacement of adrenalectomized (adx) mice per se can affect the development of obesity of GTG-obese mice. All data on adx mice that were implanted with pellets containing various amounts of corticosterone were pooled, therefore producing two groups: the first being an adx implanted lean group, the second being an adx-implanted GTG-obese group. Data concerning the adx and the sham-adx mice as well as the group killed at the time of adx appears as in figures 7-12. Values represent means \pm S.E.M. The number of observations is indicated in parentheses if differing from what is reported at the top of each column.

2WKS: GTG- or saline-injected mice that were sacrificed 2 weeks after the injection.

SHAM: GTG- or saline-injected mice were sham-adx 2 weeks after the injection and sacrificed at 4 weeks.

ADX: GTG- or saline-injected mice were adx 2 weeks after the injection and

were implanted with pellets containing no corticosterone (cholesterol only).

ADX+CORTI: GTG- or saline-injected mice were adx 2 weeks after the injection and were implanted with pellets containing 1, 2, 5 or 20 mg corticosterone.

SYMBOLS:

- * significant difference compared to the corresponding group of lean mice ($p < 0.05$).
- + significant difference compared to mice that received the same treatment (GTG or saline) and sacrificed 2 weeks after the injection ($p < 0.05$).
- significant difference compared to sham-adx mice that received the same treatment ($p < 0.05$).
- ◇ significant difference compared to adx mice that received the same treatment but received no corticosterone ($p < 0.05$)

TABLE 3

	LEAN				GTG-OBESE									
	2WKS		ADX		ADX+CORTI		2WKS		SHAM		ADX		ADX+CORTI	
	n=6	n=7	n=13	n=45	n=6	n=7	n=5	n=5	n=7	n=5	n=5	n=49		
Body weight (g)	20.2 ±0.4	20.1 ±0.5	20.3 ±0.6	19.9 ±0.4	25.1* ±0.9	27.4* ±0.8	22.5□ ±1.0	26.2*0 ±0.4						
WAT weight (mg)	368 ±35	260* ±12	195* ±24	355 ±43	814* ±114	1024** ±84	532*□ ±94	1182*+0 ±55						
BAT weight (mg)	164.5 ±12.2	166.0 ±12.5	122.8+□ ±4.6(12)	153.7 ±8.1	287.8* ±14.6	306.1* ±12.4	143.2+□ ±13.9	263.5*0 ±9.9(48)						
BAT protein (mg)	10.4 ±0.84	9.1 ±0.71	14.7+□ ±1.32	8.30 ±0.53(42)	9.1 ±0.43	10.4 ±0.56	12.5+ ±0.86	8.10 ±0.52						
BAT GDP binding (pmol/mg mitochondrial protein)	48.7 ±11.5	121.9+ ±25.2	65.7□ ±7.7	131.2+0 ±12.4(42)	24.0 ±16.7	53.4* ±14.6	69.5+ ±5.86	76.6** ±8.4(44)						
Rectal temperature (°C)	37.4 ±0.20	37.8 ±0.20	37.0□ ±0.22	37.50 ±0.12(31)	36.9 ±0.13	37.1* ±0.22	37.0 ±0.26	37.2 ±0.10(30)						
Serum corticosterone (µg/dl)	3.23 ±1.11	7.58+ ±0.38	0.00+□	4.01□ ±0.61	4.58 ±0.58	9.44+ ±2.00	0.00+□	3.32□ ±0.44						
Serum insulin (ng/dl)	91.6 ±7.3	92.4 ±35.2	97.1 ±20.9(10)	176.0 ±43.5(37)	353.6* ±55.2	248.9* ±53.5	231.8 ±101.1	340.8* ±42.5(39)						

FIGURE 7: GTG OBESITY EXPERIMENT: EFFECT OF CORTICOSTERONE ON BODY WEIGHT GAIN OF ADRENALECTOMIZED GTG-OBESE AND LEAN MICE.

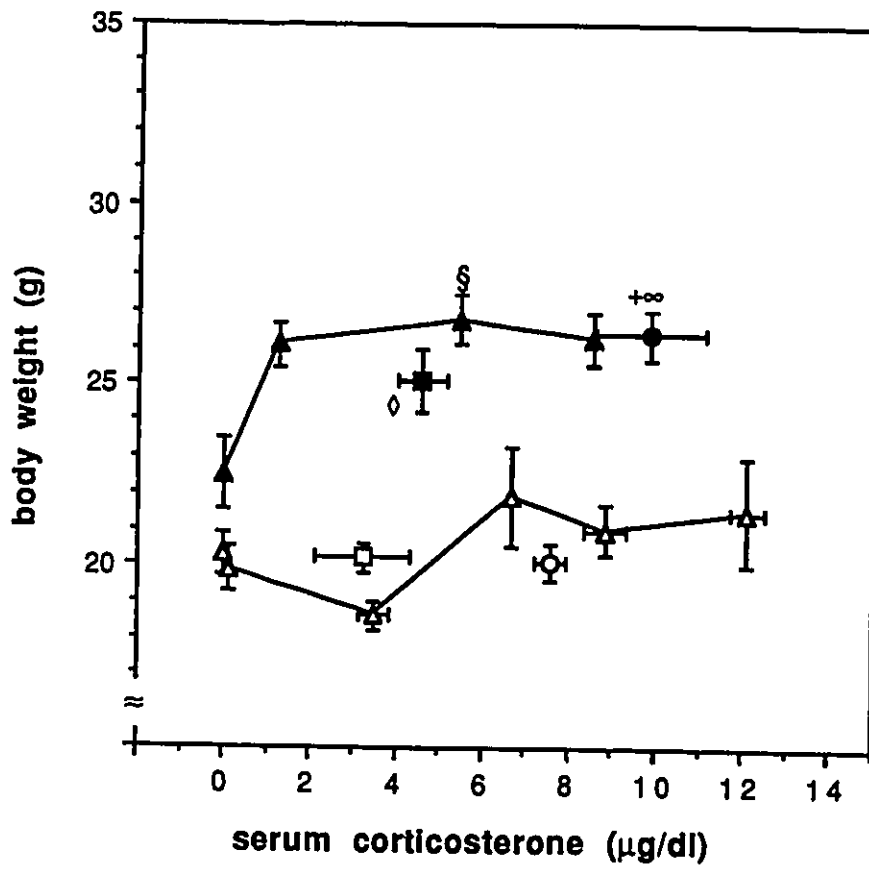
Mice were injected with saline or GTG at 7-8 weeks of age. Two weeks later, they were adrenalectomized and implanted with pellets containing various amounts of corticosterone. Final body weight is plotted against serum corticosterone levels at time of sacrifice. Values for sham-operated animals and those killed two weeks after injection of saline or GTG are included for comparison. Values are means \pm S.E.M. for 4-30 mice. Where no S.E.M. bar appears, it is too small to be seen outside symbol.

SYMBOLS:

- △- adrenalectomized lean mice
- ▲- adrenalectomized GTG-obese mice
- sham-adrenalectomized lean mice
- sham-adrenalectomized GTG-obese mice
- control lean group sacrificed two weeks after saline injection
- control obese group sacrificed two weeks after GTG injection

- * significant difference ($p < 0.05$) between adrenalectomized GTG-obese and adrenalectomized lean mice in absence of corticosterone
- § significant effect of corticosterone ($p < 0.05$) comparing adrenalectomized mice with mice of the same group that did not receive corticosterone
- + significant difference ($p < 0.05$) between sham-adrenalectomized GTG-obese and sham-adrenalectomized lean mice
- ◇ significant difference ($p < 0.05$) between GTG-obese and lean mice that were sacrificed two weeks after injection
- ∞ significant difference ($p < 0.05$) compared to adrenalectomized mice of the same group (obese or lean) that did not receive corticosterone.

FIGURE 7



Corticosterone replacement caused the body weight to be increased to levels similar to sham-adx GTG-mice (figure 7). All corticosterone-replaced GTG-mice had body weights higher than adx GTG-mice but no correlation with the serum levels of corticosterone was observed (figure 7); mice replaced with very low amounts of corticosterone being able to increase their body weight to a level similar to those that received a large dose of the hormone. When all the replaced GTG-mice were considered as a group (table 3), the mean body weight was significantly higher than the adx GTG group and the lean control mice.

Adrenalectomy, sham-adrenalectomy or corticosterone replacement had no effect on body weight of lean mice (table 3, figure 7).

4) WAT weight

GTG-mice were characterized by a gradual accumulation of WAT with time as seen with 2-week control GTG-mice and sham-adx GTG-mice at 4 weeks. WAT weight of sham-adx GTG-mice was significantly higher than 2-week control. WAT weight of GTG-mice was significantly higher than lean mice in all groups (table 3, figure 8).

Adrenalectomy caused the WAT weight of GTG-mice to decrease. The weight was significantly different from sham-adx GTG-mice (table 3, figure 8) but still significantly higher than in adx lean mice. Therefore, it is possible that the 2-weeks period after adrenalectomy was not long enough to allow a complete reversal of the WAT weight to normal lean levels.

WAT weight was related to serum corticosterone up to about 6 μ g/dl in corticosterone-replaced GTG-mice (figure 8). Higher levels of this hormone

FIGURE 8: GTG OBESITY EXPERIMENT: EFFECT OF CORTICOSTERONE ON GONADAL WHITE ADIPOSE TISSUE WEIGHT OF OF ADRENALECTOMIZED GTG-OBESE AND LEAN MICE.

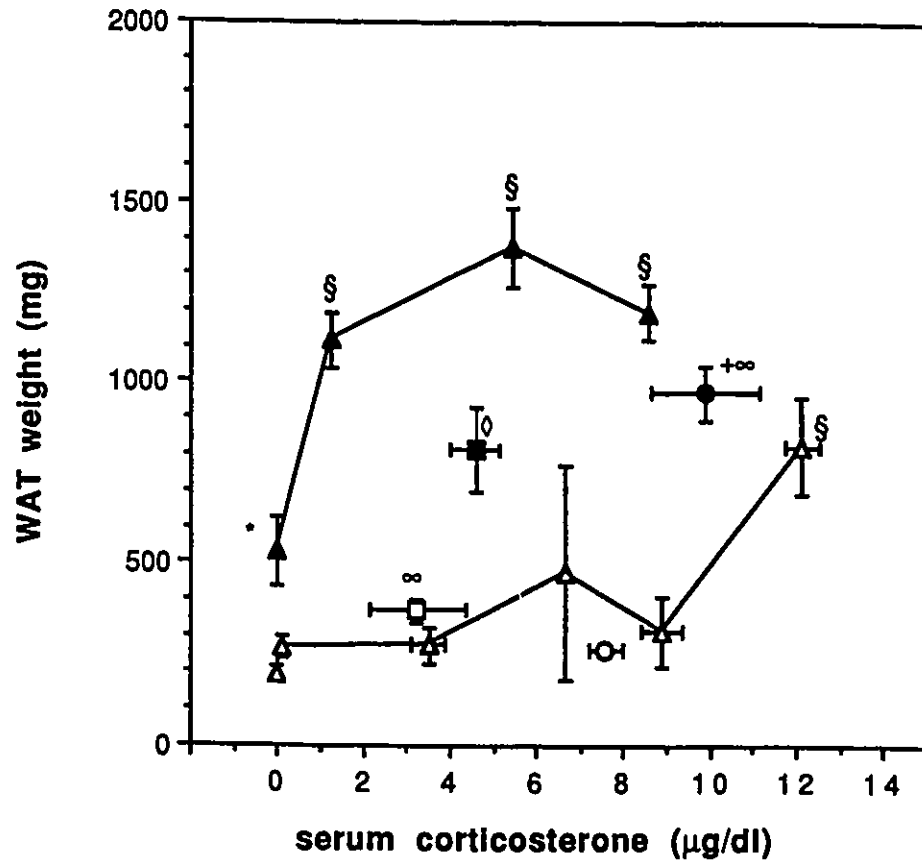
Gonadal white adipose tissue was removed at the time of sacrifice and weighed. For further information see legend to figure 7.

SYMBOLS:

- △- adrenalectomized lean mice
- ▲- adrenalectomized GTG-obese mice
- sham-adrenalectomized lean mice
- sham-adrenalectomized GTG-obese mice
- control lean group sacrificed two weeks after saline injection
- control obese group sacrificed two weeks after GTG injection

- * significant difference ($p < 0.05$) between adrenalectomized GTG-obese and adrenalectomized lean mice in absence of corticosterone
- § significant effect of corticosterone ($p < 0.05$) comparing adrenalectomized mice with mice of the same group that did not receive corticosterone
- + significant difference ($p < 0.05$) between sham-adrenalectomized GTG-obese and sham-adrenalectomized lean mice
- ◇ significant difference ($p < 0.05$) between GTG-obese and lean mice that were sacrificed two weeks after injection
- ∞ significant difference ($p < 0.05$) compared to adrenalectomized mice of the same group (obese or lean) that did not receive corticosterone.

FIGURE 8



tended to cause a slight reduction in WAT weight. When the corticosterone-replaced GTG-mice are considered as a group (table 3) the WAT weight is higher than in adx GTG-mice and in 2-week control GTG-mice, but not significantly different from that in sham-ADX GTG-mice.

Surprisingly, for an unknown reason, adx and sham-ADX lean mice had WAT weights that were lower than 2-week lean control. As a tentative explanation, it is possible that the surgery caused a mobilization of fat stores that could not be replenished to starting levels in 2 weeks. Also, the 2-week control mice were of different origin and had acclimated to the animal housing conditions for a longer period and thus might have been fatter. Such effects were obviously not observed with obese GTG-mice, perhaps because they were masked by the high rates of lipogenesis. High serum corticosterone levels caused an increase in WAT weight of lean mice that was significantly greater than the WAT weight of adx and sham-ADX lean mice.

5) BAT weight

BAT weight is used as a rough index for the accumulation of lipids in this tissue. GTG-mice showed increased BAT weights compared to their corresponding lean controls (table 3, figure 9). Sham-ADX and 2-week control GTG-mice had similar amount of BAT indicating that a greater proportion of lipid accumulated in the first 2 weeks after GTG injection (table 3, figure 9).

Adx caused a significant reduction in the weight of BAT in GTG-mice compared to sham-ADX and 2-week control obese mice and the weight was reduced to a level comparable to that in adx lean controls. Very low levels of corticosterone ($<2\mu\text{g}/\text{dl}$) are sufficient to sustain the elevated BAT weight seen in GTG-mice (figure 9). At about $6\mu\text{g}/\text{dl}$ corticosterone the accumulation of lipids

FIGURE 9: GTG OBESITY EXPERIMENT: EFFECT OF CORTICOSTERONE ON BROWN ADIPOSE TISSUE WEIGHT OF OF ADRENALECTOMIZED GTG-OBESE AND LEAN MICE.

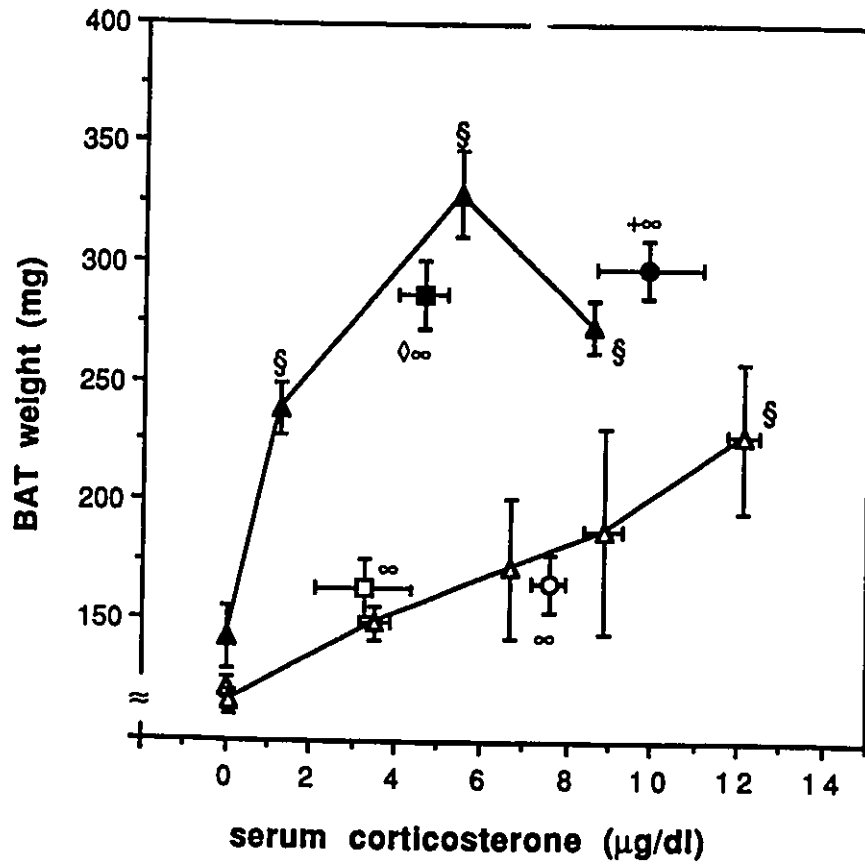
Brown adipose tissue was removed at the time of sacrifice and weighed. For further information see legend to figure 7.

SYMBOLS:

- △- adrenalectomized lean mice
- ▲- adrenalectomized GTG-obese mice
- sham-adrenalectomized lean mice
- sham-adrenalectomized GTG-obese mice
- control lean group sacrificed two weeks after saline injection
- control obese group sacrificed two weeks after GTG injection

- * significant difference ($p < 0.05$) between adrenalectomized GTG-obese and adrenalectomized lean mice in absence of corticosterone
- § significant effect of corticosterone ($p < 0.05$) comparing adrenalectomized mice with mice of the same group that did not receive corticosterone
- + significant difference ($p < 0.05$) between sham-adrenalectomized GTG-obese and sham-adrenalectomized lean mice
- ◇ significant difference ($p < 0.05$) between GTG-obese and lean mice that were sacrificed two weeks after injection
- ∞ significant difference ($p < 0.05$) compared to adrenalectomized mice of the same group (obese or lean) that did not receive corticosterone.

FIGURE 9



in BAT was maximal. There is then a strong positive correlation between BAT weight and serum corticosterone up to 6µg/dl. However, BAT weight was decreased by a higher level of serum corticosterone, as in the case of WAT weight. Generally speaking, the effects of corticosterone are similar on WAT and BAT weight but more pronounced in the latter.

BAT weight of lean mice was also very sensitive to corticosterone, increasing levels of serum corticosterone being associated with a progressive increase in BAT weight (figure 9). At the highest level tested (12µg/dl) the difference in BAT weight from that in adx lean mice was significant. Moreover, regression analysis of this relationship gives a coefficient of 0.995 ($p < 0.0004$). It seems, for an unknown reason, that the response to corticosterone in lean mice is subject to individual variations as seen by the large standard error bars. Adx lean mice showed a significantly reduced level of BAT weight compared to sham-adx and 2-week control lean mice (table 3, figure 9).

6) BAT proteins

The measurement of total BAT protein is an index of the trophic response of the tissue to stimulation. 2-week control, adx and sham-adx GTG-mice had BAT protein levels comparable to those found in their respective lean controls (table 3). Adx of GTG-mice caused a significant increase in BAT protein compared to 2-week control GTG-mice but not to sham-adx GTG-mice (table 3, figure 10). Interestingly, BAT protein was also significantly increased in lean control mice after adrenalectomy compared to 2-week control and sham-adx lean mice (table 3, figure 10).

BAT protein is inversely correlated with the serum level of corticosterone in both lean and obese mice (figure 10). The shape of the curves are similar in

FIGURE 10: GTG OBESITY EXPERIMENT: EFFECT OF CORTICOSTERONE ON BROWN ADIPOSE TISSUE TOTAL PROTEIN OF ADRENALECTOMIZED GTG-OBESE AND LEAN MICE.

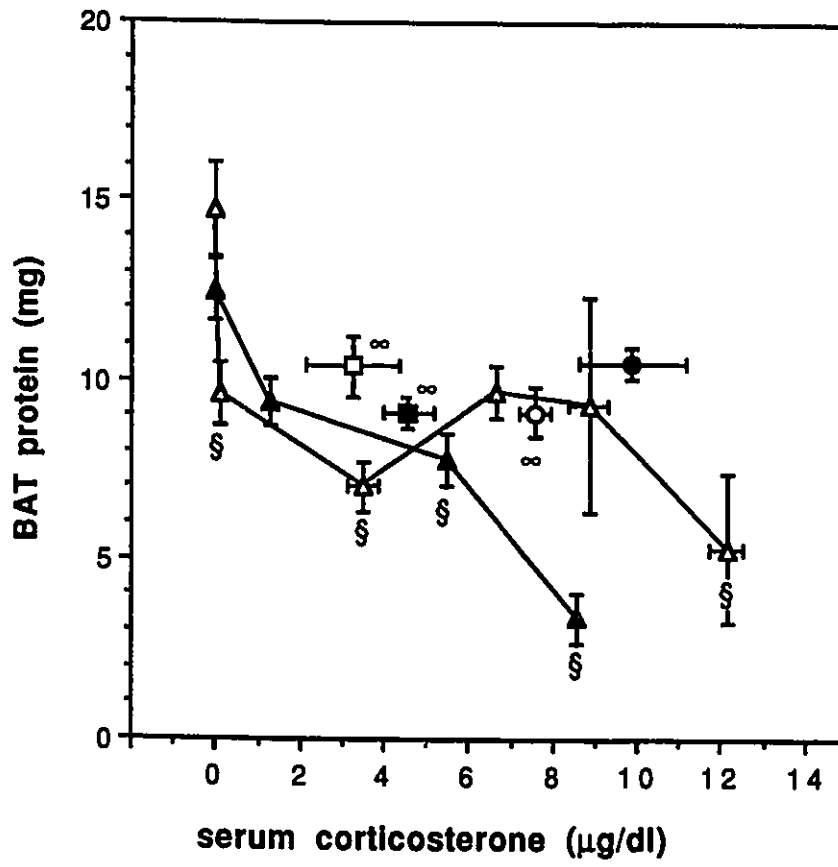
Brown adipose tissue was removed at the time of sacrifice and its total protein level determined. For further information see legend to figure 7.

SYMBOLS:

- △- adrenalectomized lean mice
- ▲- adrenalectomized GTG-obese mice
- sham-adrenalectomized lean mice
- sham-adrenalectomized GTG-obese mice
- control lean group sacrificed two weeks after saline injection
- control obese group sacrificed two weeks after GTG injection

- * significant difference ($p < 0.05$) between adrenalectomized GTG-obese and adrenalectomized lean mice in absence of corticosterone
- § significant effect of corticosterone ($p < 0.05$) comparing adrenalectomized mice with mice of the same group that did not receive corticosterone
- + significant difference ($p < 0.05$) between sham-adrenalectomized GTG-obese and sham-adrenalectomized lean mice
- ◇ significant difference ($p < 0.05$) between GTG-obese and lean mice that were sacrificed two weeks after injection
- ∞ significant difference ($p < 0.05$) compared to adrenalectomized mice of the same group (obese or lean) that did not receive corticosterone.

FIGURE 10



the two cases. These results suggest that BAT growth is gradually reduced as the corticosterone concentration is increased.

7) BAT GDP binding

The thermogenic activity as indicated by the measurement of GDP binding to isolated mitochondria was found to be significantly reduced in sham-*adx* GTG-mice compared to lean controls (table 3, figure 11). This was also observed with 2-week control GTG and lean mice but the difference was not significant largely because the values for the lean mice were unusually low at this time, for reasons that are not clear. Adrenalectomy of GTG-mice did not increase the low GDP binding. In lean mice, however, adrenalectomy caused a reduction in the binding to the same low level seen in *adx* GTG-mice (table 3, figure 11). This result was not expected and is possibly due to the fact that these lean mice were not completely awakened from their morning torpor as seen by their significantly lower body temperature (table 3). There was no relationship between the GDP binding and the serum corticosterone concentration (figure 11) in either GTG or lean mice. When *adx*-replaced GTG-mice were considered as one group GDP binding was not different from the *adx* GTG-mice but it was still lower than the corresponding lean control group (table 3).

8) Serum insulin determination (figure 12)

Although the shape of the curve suggests a positive relationship between serum insulin and corticosterone levels in GTG-mice, none of the values along the curve were significantly different from the *adx* GTG-mice (figure 12) due to the large variation in the data.

FIGURE 11: GTG OBESITY EXPERIMENT: EFFECT OF CORTICOSTERONE ON BROWN ADIPOSE TISSUE GDP BINDING OF ADRENALECTOMIZED GTG-OBESE AND LEAN MICE.

For further information see legend to figure 7.

SYMBOLS:

- △- adrenalectomized lean mice
- ▲- adrenalectomized GTG-obese mice
- sham-adrenalectomized lean mice
- sham-adrenalectomized GTG-obese mice
- control lean group sacrificed two weeks after saline injection
- control obese group sacrificed two weeks after GTG injection

- * significant difference ($p < 0.05$) between adrenalectomized GTG-obese and adrenalectomized lean mice in absence of corticosterone
- § significant effect of corticosterone ($p < 0.05$) comparing adrenalectomized mice with mice of the same group that did not receive corticosterone
- + significant difference ($p < 0.05$) between sham-adrenalectomized GTG-obese and sham-adrenalectomized lean mice
- ◇ significant difference ($p < 0.05$) between GTG-obese and lean mice that were sacrificed two weeks after injection
- ∞ significant difference ($p < 0.05$) compared to adrenalectomized mice of the same group (obese or lean) that did not receive corticosterone.

FIGURE 11

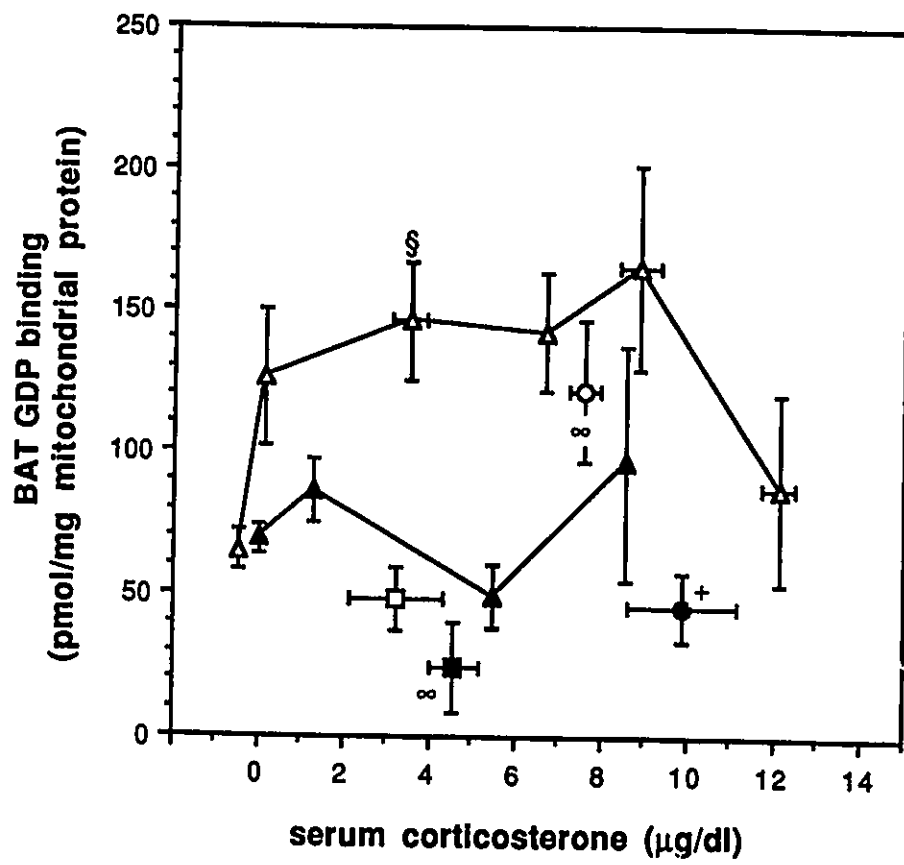


FIGURE 12: GTG OBESITY EXPERIMENT: EFFECT OF CORTICOSTERONE ON CONCENTRATION OF INSULIN IN SERUM OF ADRENALECTOMIZED GTG-OBESE AND LEAN MICE.

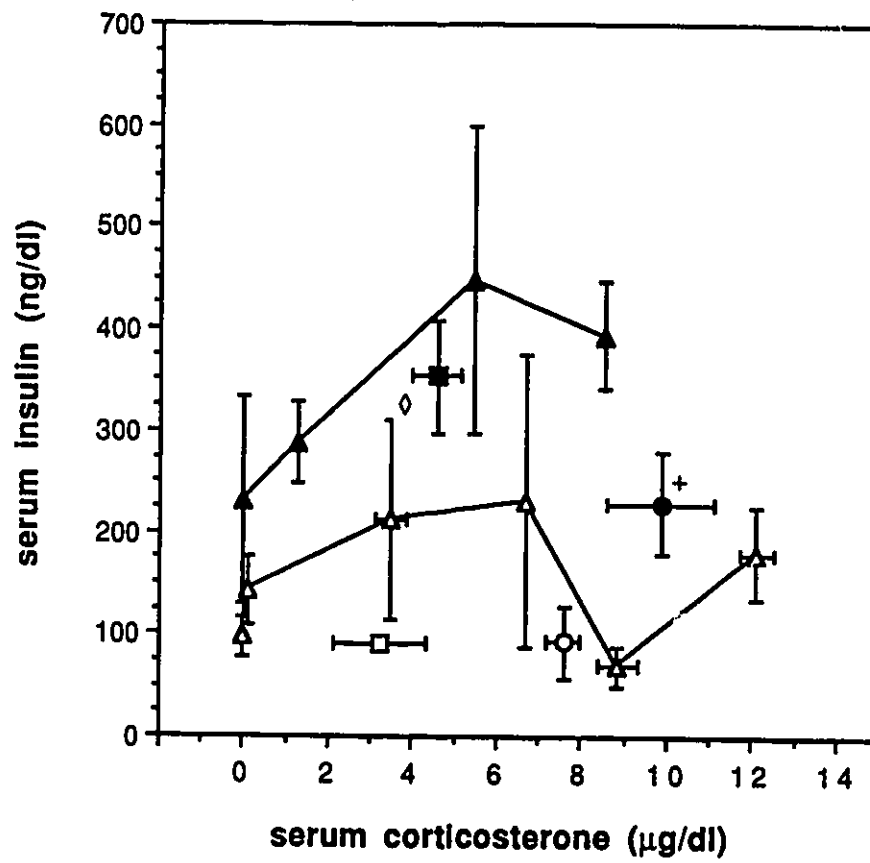
For further information see legend to figure 7.

SYMBOLS:

- △- adrenalectomized lean mice
- ▲- adrenalectomized GTG-obese mice
- sham-adrenalectomized lean mice
- sham-adrenalectomized GTG-obese mice
- control lean group sacrificed two weeks after saline injection
- control obese group sacrificed two weeks after GTG injection

- * significant difference ($p < 0.05$) between adrenalectomized GTG-obese and adrenalectomized lean mice in absence of corticosterone
- § significant effect of corticosterone ($p < 0.05$) comparing adrenalectomized mice with mice of the same group that did not receive corticosterone
- + significant difference ($p < 0.05$) between sham-adrenalectomized GTG-obese and sham-adrenalectomized lean mice
- ◇ significant difference ($p < 0.05$) between GTG-obese and lean mice that were sacrificed two weeks after injection
- ∞ significant difference ($p < 0.05$) compared to adrenalectomized mice of the same group (obese or lean) that did not receive corticosterone.

FIGURE 12



GTG-mice at 2 weeks, 4 weeks or replaced with corticosterone had higher insulin levels than similarly-treated lean mice (table 3, figure 12). The effect of adrenalectomy was inconclusive because of large variation in the data. However, insulin levels in adx GTG-mice was not significantly different from that in adx lean mice (table 3).

Serum insulin levels of lean mice were not affected by adrenalectomy, sham-adrenalectomy or corticosterone replacement (table 3, figure 12).

9) Rectal temperature

Rectal temperature of sham-adx GTG-mice was significantly lower than that of similarly treated lean mice. No difference was observed in 2-week control GTG and lean mice. Therefore, it seems that a defect in temperature takes more than two weeks to develop and is significant at 4 weeks. Adx lean mice showed a lower body temperature that was reversed by corticosterone replacement. However, it is not sure if this decrease in temperature is due to the adrenalectomy itself. As mentioned earlier, it could be due to the fact that these mice were not completely awakened from their morning torpor that is characterized by a lower body temperature. This effect was not observed in GTG-mice, possibly because of the already low body temperature found in these mice. No correlation was found between rectal temperature and serum corticosterone levels in these groups (data not shown).

CHAPTER FOUR

DISCUSSION

PART ONE: CAGING EXPERIMENT: EFFECTS OF HOUSING CONDITIONS ON BAT THERMOGENESIS

The main finding obtained with the caging experiment was that housing mice singly can markedly increase BAT thermogenesis and bring about an adaptive increase in UCP content. Mice housed singly also showed a marked activation of BAT function when compared to mice housed in pairs. These results are in accordance with the previous report by Jennings et al. (1986). However, in that report, mice were housed at 23°C whereas in the present experiment the animal room temperature was maintained at 28±1°C.

It is difficult to compare the level of GDP binding of these two studies because of slight difference in the GDP binding procedure between the two laboratories. However, it is seen that the effects on BAT thermogenesis of caging mice singly were more pronounced at 28°C than at 23°C or at 4°C. In other words, the increase in GDP binding when mice are housed singly, compared with mice grouped in pairs or in 6's is greater at 28°C than at 23°C. Therefore, a change of 5° is large in terms of maintaining a normal body temperature especially when mice are alone or in pairs. These results are perfectly in accordance with the statement of Heldmaier (1975) that the effects of social thermoregulation are temperature-dependent.

The GDP binding measurement reflects the thermogenic activation of BAT at the time of killing, as well as the specific concentration of UCP in mitochondria. The total UCP content reflects the total capacity of the tissue for thermogenesis. Since, in the present experiment, the total mitochondrial mass in the tissue was unchanged by housing conditions (unchanged cytochrome c oxidase activity) yet total UCP content was increased by single housing, it seems likely that the specific concentration of UCP in the mitochondria was also increased. Comparison of the ratio of GDP binding (mitochondrial) to UCP (total in homogenate) reveals a progressive increase from 2.8 in the mice housed in groups of 6 to 4.1 in the mice housed singly. It thus seems likely that both a selective increase in mitochondrial UCP concentration and an unmasking of binding sites had occurred in the singly-housed mice.

BAT protein and cytochrome c oxidase activity were not affected by housing mice in different sized groups indicating that the mitochondrial mass was maintained and that the increase in UCP content was due to a selective increase of UCP expression.

It is surprising that the increase in T4 5'-deiodinase activity was not significantly different despite the wide difference between the values obtained for GDP binding and for UCP content. It is recalled that the activity of this enzyme is increased dramatically in mice and rats upon cold-exposure (Silva and Larsen, 1983; Kates and Himms-Hagen, 1985; Jones et al., 1986). It is thus possible that the activity of the enzyme had returned to more basal levels after 15 days of housing treatment as it was shown by Eley and Himms-Hagen (1989a) that the T4 5'-deiodinase activity of BAT returned to normal low levels after 2 weeks of cold acclimation. Conversely, it is possible that housing mice

singly is not a strong enough stimulus to induce large changes in enzyme activity. However, the results show a consistent increase with decreasing number of mice per cage. It is then possible that the activities were not significantly different because of the large variation in the data, as shown by the large S.E.M.

Body weight, rectal temperature and serum T3 or T4 were not affected by the caging conditions as expected. Kates and Himms-Hagen (1985) reported no change in body weight, T3 and T4 levels in response to 14°C exposure in normal mice.

To conclude, it seems likely that both a selective increase in mitochondrial UCP concentration and an unmasking of binding sites had occurred in the singly-housed mice at 28°C.

Thus, as a general rule, in studying BAT thermogenesis, it is necessary to specify the conditions at which animals are housed (room temperature, number of animals/cage) and these conditions must be uniform throughout an experiment.

PART TWO: GTG OBESITY EXPERIMENT

The main objective of this study was to assess whether adrenalectomy of GTG-mice reverses their suppressed BAT thermogenesis and whether they show a hypersensitivity to corticosterone that could be responsible for the development of their obesity and the suppression of BAT function. Previous results have shown that adrenalectomy reverses the obesity and the suppressed BAT thermogenesis of the genetically obese ob/ob mouse

(Tokuyama and Himms-Hagen, 1987) and of the genetically obese fa/fa rat (Freedman et al., 1985, 1986). Moreover, the genetically obese mouse is hypersensitive to an action of corticosterone (Tokuyama and Himms-Hagen, 1987).

Contrarily to the genetically obese mice, which show higher levels of serum corticosterone at a young age (Saito and Bray, 1983; Smith and Romsos, 1985), GTG-mice in the dynamic phase of obesity are not hypercorticosteronemic (Saito and Bray, 1983; confirmed in the present experiment). Yet, in both cases, adrenalectomy reverses the obesity. It was thus of interest to know if the GTG-mice despite their normal corticosterone levels are hypersensitive to this hormone like the genetically obese mouse. The principal organ studied was BAT because it is generally accepted that obesity may result if the thermogenic mechanisms of BAT are impaired. The study of BAT was complemented with measurement of body weight, rectal temperature, WAT weight and serum insulin. Results were expressed as a function of serum corticosterone concentrations.

The present experiment showed that body weight, food intake, WAT and BAT weights and serum insulin are elevated in GTG-obesity while GDP binding is reduced. BAT protein and serum corticosterone were not changed. These results are consistent with previous reports (Hogan and Himms-Hagen, 1983, Eley and Himms-Hagen, 1989a, 1989b, Debons et al., 1982, 1983, 1986a, 1986b). It appears that the effects of GTG were more pronounced during the first two weeks following the injection. In fact, larger increases in body weight, WAT and BAT weights and food intake were found in 2-week control than in sham-adx (4 weeks) GTG-mice compared to lean mice. This is in accordance with

Debons et al. (1986b) and Hogan and Himms-Hagen (1983). These results are also consistent with the previous demonstration that the rate of body weight gain of GTG-mice is high at the beginning of the dynamic phase and is gradually decreased as mice are approaching the static phase, which starts at 4-6 weeks after the GTG injection and at which body weight is regulated at a constant elevated level (Djazayery et al., 1979). Moreover, the present experiment and results of Djazayery et al. show that food intake is rapidly reduced and maintained at normal levels, before the body weight gain starts to level off therefore indicating that the GTG-mice have a higher metabolic efficiency compared to lean mice.

Adrenalectomy incompletely reversed the elevated body weight and WAT weight of GTG-mice. Food intake was reduced below normal control levels. It is possible that the 9 day period between the time of adrenalectomy and the time of killing was not long enough to get a complete reversal of these parameters. However, it was not possible to extend this period because of the high mortality rate of adx GTG-mice. This effect of adrenalectomy was reported before (Debons et al., 1982, 1983). Adrenalectomized GTG-mice become anorexic, lose weight and finally die. The highly reduced food intake of the GTG-mice observed in this experiment obviously contributed to the mortality rate. The death of these mice did not appear to be due to surgical complications following adrenalectomy since the adrenalectomized lean group did not experience any mortality. In order to get a larger number of animals, the adx GTG- and lean mice were sacrificed 5 days before the rest of the animals. With respect to body weight, WAT weight and food intake the results obtained are in accordance with the work of Debons et al. (1982).

In both lean and obese mice, adrenalectomy caused a significant growth of BAT as reflected by increased total protein content of the tissue but did not have much influence on BAT GDP binding, which is a measure of the thermogenic activity of the tissue at the time of sacrifice. Therefore it seems that corticosterone is involved in restricting BAT growth even in normal lean animals. The reduced GDP binding of adx lean mice is not in accordance with the previous reports by Tokuyama and Himms-Hagen (1987). A possible explanation for this is that the mice were not completely awakened from their morning torpor as shown by their low body temperature. Although the effect of adrenalectomy on serum insulin concentrations were not very conclusive, the insulin levels of adx GTG-mice were not significantly different from those of adx lean mice.

The principal finding of this study is that there is a dose response relationship between serum levels of corticosterone and WAT and BAT weights, BAT total protein and possibly serum insulin levels in GTG obese mice up to a serum concentration of corticosterone of about $6\mu\text{g/dl}$ ($8.5\mu\text{g/dl}$ for protein). There is a positive correlation between WAT and BAT weights and serum insulin with respect to the serum corticosterone while BAT protein content is reduced by increasing levels of this hormone. Also, the dose-response of BAT protein is the same for lean and obese GTG-mice. Surprisingly, no dose response relationship was found between GDP binding and serum corticosterone levels in GTG-mice. Therefore, BAT is in an atrophied state in GTG-obesity (low GDP binding, low BAT protein) and this state is worsened by increasing corticosterone levels, an effect that is also seen in lean mice. GTG-mice are more sensitive and/or responsive to lower levels of corticosterone as

far as increased WAT and BAT weights and possibly serum insulin are concerned.

As BAT weight can be increased without an increase in the thermogenic function, the measurement of WAT weight aids in the interpretation of changes in the amount of BAT. Since the present results show an increase in BAT weight that is correlated with an increase in WAT weight ($r=0.993$, $p<0.007$), it is likely that the elevated BAT weight reflects an accumulation of lipids in the tissue. The effect of corticosterone on WAT and BAT weights were not large enough to be significantly reflected on the body weight of the obese GTG-mice but the results seemed to show this trend. The effects of corticosterone may vary between different WAT depots as they are responding differently to insulin (Hom and Goodner, 1984). Also, it is possible that the effect of the hormone on the body weight would have become significant if more than 2 weeks was allowed after the surgery and before the sacrifice. Then it can be concluded from these results that, although GTG-mice have normal serum levels of corticosterone, they are hypersensitive to the action of this hormone. Supporting this fact is that corticosterone will have the same effect in lean mice but at high physiological or supraphysiological levels (present results and Galpin et al., 1983).

In the genetically obese *ob/ob* mice the GDP binding is reduced as the concentration of corticosterone is increased in their blood (Tokuyama and Himms-Hagen, 1987) and a concentration of about 5 μ g/dl is sufficient to cause a significant reduction in the binding. In GTG-mice no reduction in binding was found even at the highest dose tested. However, contrary to findings with the *ob/ob* mouse, adrenalectomy did not increase the reduced BAT mitochondrial GDP binding seen in the GTG-obese mouse. Thus it can be concluded that the

suppressed thermogenic state of BAT mitochondria in the GTG-mouse is not due to any action of corticosterone. Dose-response curves for BAT protein were not reported for ob/ob mice.

GTG-mice maintained their body temperature at a normal level except for sham-adx GTG-mice. Eley and Himms-Hagen (1989b) showed that the usual circadian rhythm in rectal temperature was seen in GTG-mice. It thus appears that corticosterone is not involved in the control of body temperature in this model of obesity. Surprisingly, adrenalectomy of the lean mice caused a reduction in body temperature but, as suggested above, it is possible that these mice were still torpid at the time of killing.

In order to have a complete dose response curve including maximal response to corticosterone, higher levels of corticosterone should be tested. In this experiment, serum concentrations attained were always in the physiological range, below the highest level generally found in their blood. However, corticosterone in lean mice and GTG-mice, in the dynamic phase of obesity, show a circadian rhythm with the highest levels (about 12.5µg/dl) attained at night, a few hours before the animals start to eat and the lowest level (about 2.5µg/dl) attained in the early morning (Saito and Bray, 1983). It is important to point out that in the corticosterone-replaced mice no circadian rhythm would occur. Thus, for much of the day these mice would have been exposed to a higher than normal level of corticosterone but probably to lower than normal level for part of the night. Nevertheless, the results obtained are enough to conclude that GTG-mice show hypersensitivity to corticosterone since certain abnormalities of the GTG-mice are returned in virtually full force at low levels of this hormone.

Injection of MSG causes hypothalamic lesions mainly in the arcuate nucleus (as in the GTG-mice) and in the preoptic region, but not in the VMH. These mice become obese without a gain in body weight or an increase in food intake (Yoshida et al., 1984). In that case, it is the proportion of body fat that is increased at the expense of body proteins and carbohydrates (Tokuyama and Himms-Hagen, 1986). Accordingly, BAT weight is increased (Moss et al., 1985; Tokuyama and Himms-Hagen, 1986) but thermogenesis seems to occur normally or almost normally (Tokuyama and Himms-Hagen, 1986). The response to insulin is normal (Cameron et al., 1978) but these mice have high levels of corticosterone in their blood (Tokuyama and Himms-Hagen, 1986). It is interesting that the high serum corticosterone in these animals is not associated with a suppression of BAT. Therefore, it appears that it is the destruction of the VMH that is involved in the suppression of BAT thermogenesis in GTG-mice as MSG-mice have a normal BAT and an intact VMH. In addition, MSG-mice thermoregulate at a lower body temperature during the night (Tokuyama and Himms-Hagen, 1986) and adrenalectomy reverses the low body temperature to normal levels (Tokuyama and Himms-Hagen 1989). Thus, it is possible that the reduced body temperature of the MSG-mice is due to a lesion in the hypothalamus that is not present in the GTG-mouse.

It is important to note that if the elevated serum insulin is reduced by adrenalectomy, results suggest that it is still higher than that in adx lean mice despite the large variation in the data. It is possible that the lesions made by GTG in the VMH are responsible for this. In fact, electrical stimulation of the VMH has been shown to elicit a decrease in plasma insulin (Woods and Porte, 1974) while lesions cause an increase in insulin secretion that is blocked by

vagotomy (Berthoud and Jeanrenaud, 1979). Pace et al. (1989) also found enhance glucose-induced electrical activity from B-cells in the pancreas of GTG-mice. In addition, Cooney et al. (1989) reported an increase in insulin release in response to a meal (postprandial hyperinsulinemia) as soon as 2 weeks after the GTG lesions. Therefore it is possible that lesions of the VMH induce the basal defect in insulin secretion found in GTG-mice that is further modulated by an action of corticosterone on food intake. This hyperinsulinemia would lead eventually to a general insulin resistance that is found later on after GTG injection, toward the static phase of obesity (Cooney et al., 1989), and that is due possibly to down regulation of insulin receptors as suggested by Tanti et al.(1986) or glucose transporters as suggested by Garvey et al. (1987). Insulin resistance does not occur simultaneously in all sensitive organs of the GTG-mice. WAT even appears to be more sensitive to an action of this hormone soon after GTG injection (Cooney et al., 1987) but insulin resistance develops later on (Cooney et al., 1989). BAT becomes insulin resistant sooner than WAT after GTG (Cooney et al., 1987, 1989). Other fat depots may respond differently as shown by Hom and Goodner (1984).

I suggest that obesity in GTG-mice is primarily due to lesions of the VMH that cause hyperinsulinemia and reduced BAT thermogenesis. Destruction of the VMH or of another hypothalamic site might remove a suppressive action on the PVN leading to an increased food intake. Also, it is possible that the hyperphagia is brought about by the hyperinsulinemia as it was shown that chronic injection of high doses of insulin causes hyperphagia (Dulloo and Girardier, 1989). I suggest that the dose-response effect obtained in this study is due to an action of corticosterone on the alpha-noradrenergic feeding pathway

of the PVN as shown by Leibowitz et al.(1984). Corticosterone action on this pathway increases food intake in a dose dependent manner (Leibowitz et al., 1984) which in turn could cause an increased postprandial hyperinsulinemia. Therefore the dynamic phase of obesity would be characterized by a gradual development of hyperinsulinemia due to the increase in insulin secretion that is secondary to the increased food intake. Adrenalectomy partially reverses the hyperinsulinemia due to the decrease in food intake, that, in turn, leads to the mobilization of WAT and BAT fat stores. I suggest that this effect of corticosterone is superimposed on primary defects due to the VMH lesions, namely hyperinsulinemia and reduced BAT thermogenesis. WAT and BAT deposition as well as body weight gain are secondary to a defect in insulin secretion that is further modulated by an effect of corticosterone on food intake.

It is possible that corticosterone is also acting on peripheral organs. In vitro studies on isolated adipocytes showed that, contrary to the effect of insulin, glucocorticoids cause a decrease in the number of glucose transporters exposed at the cell surface (Garvey et al., 1989). Long term exposure to insulin could eventually cause insulin resistance through a down regulation of glucose transporters that can be potentiated by an increased sensitivity to corticosterone. However, the effect of glucocorticoids was demonstrated in vitro on adipocytes using large doses of the hormone and the relevance of this effect still has to be demonstrated in vivo.

The dose-response curves obtained for some of the measurements in the present experiment and by others (Tokuyama and Himms-Hagen, 1987) are bell shaped or show an effect at a low corticosterone concentration that is reversed at a high corticosterone concentration. Although the nonlinearity of

effect is often attributed to pharmacological effects, the corticosterone concentration was maintained in these cases in the normal range. Devenport et al. (1989) suggest that this is due to a dual action of corticosterone on two different receptor populations. In fact, it was shown previously that corticosterone, at low concentration, was binding to high affinity, low capacity receptors (type I) and, at high concentrations, to low affinity, high capacity (type II) receptors in the hypothalamus (Beaumont and Fanestil, 1983; DeKloet and Reul, 1987). Devenport et al. (1989) showed that with selective type I and type II agonists a bitonic dose-response curve for body weight gain was obtained that consisted of two monotonic functions representing successive binding to high and low affinity receptors. The anabolic effects of corticosterone are associated with binding to the type I receptors and eventually masked by a catabolic action at type II receptors. The results obtained in the present experiment for GTG but not normal lean mice are consistent with this hypothesis. It is possible that for lean mice corticosterone levels attained were not high enough to show this effect. In fact, Tokuyama and Himms-Hagen (1987) showed the bitonic response at levels of corticosterone higher than those attained in the present experiment. Therefore, it seems that GTG-mice are showing an altered sensitivity that involves both receptor populations as seen by the shift of the dose-response curve to the left. It could also be due to an imbalance between the two receptor subtype populations, possibly a greater proportion of type II receptors, or to the ablation of part of one population by the GTG-induced lesion. Consistent with this concept is the finding of Berdanier (1989) which showed that corticosterone was able to promote both anabolic (increase in enzymes for lipogenesis) and catabolic effects (stimulation of synthesis of

enzymes for glycogenolysis) in the liver and that such effects are blocked by adrenalectomy and reversed by corticosterone replacement. Both processes were observed together probably because the dose of corticosterone used was intermediate between type I and type II affinities. Other metabolic functions might be related to one or the other of the corticosterone receptors but more research is needed in this area.

To conclude, two different mechanisms for the development of obesity appear to be present in the GTG-mice. The first one is dependent on corticosterone which acts probably on the hypothalamus, is found in both GTG and normal lean mice and is responsible for the increased body weight, WAT and BAT weights and hyperphagia. The second one is independent of this hormone and is due to destruction of the VMH which causes hyperinsulinemia and reduced BAT thermogenesis. These two mechanisms also appear to be present in the genetically obese ob/ob mice but not in the MSG-mice. However, in the genetic model of obesity, adrenalectomy increases the suppressed BAT thermogenesis (as measured by GDP binding), an effect that is reversed by corticosterone replacement at low concentrations. In the GTG-mouse, adrenalectomy does not reverse the suppressed BAT thermogenesis that appears to be independent of corticosterone.

CONCLUSIONS

The caging experiment led to the finding that caging mice singly at 28°C greatly increases BAT thermogenesis compared to mice housed in pairs or in groups of 6. It seems likely that both a selective increase in mitochondrial UCP concentration and an unmasking of binding sites are responsible for the enhanced thermogenic activity. The UCP concentration and GDP binding in mice housed in pairs lie between those for mice housed singly and in groups of 6. The activity of the enzyme T4 5'-deiodinase is unaffected by the different housing conditions tested but there was a trend toward an increase in activity as the number of mice per cage decreased.

In the study of the role of glucocorticoids in GTG-induced obesity it was found that the development of obesity requires the presence of extremely small quantities of corticosterone. Mice with GTG-induced obesity are hypersensitive to a facilitory role of corticosterone in the development of obesity. In the adrenalectomized GTG-mice various abnormalities, such as increased WAT and BAT weights, food intake and elevated serum insulin levels, reappear in virtually full force at very low levels of corticosterone. Therefore, mice with GTG-induced hypothalamic lesions leading to obesity show an increased sensitivity to corticosterone that is in some respect like the genetic models of obesity.

However, it was also found that some abnormalities persist in the adrenalectomized state in GTG-mice and seem to be due to metabolic defects caused by GTG lesions that are not dependent on corticosterone. These include the suppressed BAT mitochondrial GDP binding (indicative of a suppressed thermogenic state). In this respect the GTG mouse differs from the genetically

obese ob/ob mouse. Thus, there would appear to be no role for corticosterone in the defective control of BAT thermogenesis in mice with GTG-induced obesity.

REFERENCES

- Al-Dujaili, E.A.S., Williams, B.C., Edwards, C.R.W. (1981) The development and application of a direct radioimmunoassay for corticosterone. *Steroids* 37:157-175.
- Allars, J., York, D.A. (1986) The effect of 2-deoxy-D-glucose on brown adipose tissue of lean and obese Zucker rats. *Int. J. Obesity* 10:147-158.
- Allars, J., Holt, S.J., York, D.A. (1987) Energetic efficiency and brown adipose tissue uncoupling protein of obese Zucker rats fed high-carbohydrate and high-fat diets: the effects of adrenalectomy. *Int. J. Obesity* 11:591-601.
- Amir, S., Lagiorgia, M., Pollock, R. (1989) Intra-ventromedial hypothalamic injection of insulin suppresses brown rat thermogenesis in the anaesthetized rat. *Brain Res.* 480:340-343.
- Anand, B.K., Brobeck, J.R. (1951) Hypothalamic control of food intake in rats in cats. *Yale J. Biol. Med.* 24:123-146.
- Anand, B.K., Chhina, G.S., Sharma, K.N., Dua, S., Singh, B. (1964) Activity of single neurons in the hypothalamic feeding centers: effect of glucose. *Am. J. Physiol.* 207:1146-1154.
- Arase, K., York, D.A., Bray, G.A. (1987) Corticosterone inhibition of the intracerebroventricular effect of 2-deoxy-D-glucose on brown adipose tissue thermogenesis. *Physiol. Behav.* 40:489-495.
- Arase, K., York, D.A., Shimizu, H., Shargill, N., Bray, G.A. (1988) Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats. *Am. J. Physiol.* 255:E255-E259.
- Aravich, P.R., Scalfani, A. (1983) Paraventricular hypothalamic lesions and medial hypothalamic knife cuts produce similar hyperphagia syndromes. *Behav. Neurosci.* 97:970-983.
- Ashwell, M., Jennings, G., Richard, D., Stirling, D.M., Trayhurn, P. (1983) Effect of acclimation temperature on the concentration of the mitochondrial "uncoupling" protein measured by radioimmunoassay in mouse brown adipose tissue. *FEBS Lett.* 161:108-112.
- Barge, R.M., Mills, I., Silva, J.E., Larsen, P.R. (1988) Phorbol esters, protein kinase C, and thyroxine 5'-deiodinase in brown adipocytes. *Am. J. Physiol.* 254:E323-E327.

- Bartness, T.J., Billington, C.J., Levine, A.S., Morley, J.E., Rowland, N.E., Brown, D.M. (1986) Insulin and metabolic efficiency in rats. II. Effects of norepinephrine and cold exposure. *Am. J. Physiol.* 251:R1118-R1125.
- Beaumont, K., Fanestil, D.D. (1983) Characterization of rat brain aldosterone receptors reveals high affinity for corticosterone. *Endocrinology* 113:2043-2051.
- Bégin-Heick, N., Deeks, J.R. (1987) Hypercorticism and manganese metabolism in brown adipose tissue of the obese mouse. *J. Nutr.* 117:1708-1714.
- Berdanier, C.D. (1989) Role of glucocorticoids in the regulation of lipogenesis. *FASEB J.* 3:2179-2183.
- Bernardis, L.L., Goldman, J.K. (1976) Origin of endocrine-metabolic changes in the weanling rat ventromedial syndrome. *J. Neurosci. Res.* 2:91-116.
- Berthoud, H.R., Jeanrenaud, B. (1979) Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology* 105:146-151.
- Bianco, A.C., Silva, J.E. (1987a) Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. *J. Clin. Invest.* 79:295-300.
- Bianco, A.C., Silva, J.E. (1987b) Optimal response of key enzymes and uncoupling protein to cold in BAT depends on local T₃ generation. *Am. J. Physiol.* 253:E255-E263.
- Bianco, A.C., Silva, J.E. (1988) Cold exposure rapidly induces virtual saturation of brown adipose tissue nuclear T₃ receptors. *Am. J. Physiol.* 255:E496-E503.
- Bianco, A.C., Sheng, X., Silva, J.E. (1988) Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *J. Biol. Chem.* 263:18168-18175.
- Billington, C.J., Bartness, T.J., Briggs, J., Levine, A.S., Morley, J.E. (1987) Glucagon stimulation of brown adipose tissue growth and thermogenesis. *Am. J. Physiol.* 252:R160-R165.
- Bray, G.A., York, D.A. (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol. Rev.* 59:719-807.
- Brecher, G., Waxler, S.H. (1949) Obesity in albino mice due to single injections of goldthioglucose. *Proc. Soc. Exp. Biol. Med.* 70:498-501.

Brown, D.F. (1986) Inhibition of gold thioglucose lesion formation in the ventromedial hypothalamus by a glucocorticoid. *Physiol. Behav.* 37:459-463.

Brown, D.F., Viles, J.M. (1982) Systemic phlorizin prevents gold thioglucose necrosis in the ventromedial hypothalamus. *Brain Res. Bull.* 8:347-351.

Bruning, J.L., Kintz, B.L. (1977) *Computational handbook of statistics.* (2nd edition). Scott, Foresman and Co., Gelview, Illinois, London.

Bukowiecki, L.J. (1984) Mechanisms of stimulus-calorigenesis coupling in brown adipose tissue. *Can J. Biochem. Cell Biol.* 62:623-630.

Bukowiecki, L.J., Collet, A.J. (1983) Regulation of brown adipose tissue metabolism. *J. Obesity Weight Regulation* 2:29-53.

Cameron, D.P., Cutbush, L., Opat, F (1978) Effects of monosodium glutamate-induced obesity in mice on carbohydrate metabolism in insulin secretion. *Clin. Exp. Pharmacol. Physiol.* 5:41-51.

Carneheim, C., Nedergaard, J., Cannon, B. (1984) β -adrenergic stimulation of lipoprotein lipase in rat brown adipose tissue during acclimation to cold. *Am. J. Physiol.* 246:E327-E333.

Cooney, G.J., Caterson, I.D., Newsholme, E.A. (1985) The effect of insulin and noradrenaline on the uptake of 2-[1- 14 C]deoxyglucose in vivo by brown adipose tissue and other glucose-utilizing tissues of the mouse. *FEBS Lett.* 188:257-261.

Cooney, G.J., Astbury, L.D., Williams, P.F., Caterson, I.D. (1987) Insulin response in individual tissues of control and gold thioglucose-obese mice in vivo with [1- 14 C]2-deoxyglucose. *Diabetes* 36:152-158.

Cooney, G.J., Vanner, M.A., Nicks, J.L., Williams, P.F., Caterson, I.D. (1989) Changes of the lipogenic response to feeding of liver, white adipose tissue and brown adipose tissue during the development of obesity in the gold-thioglucose-injected mouse. *Biochem. J.* 259:651-657.

Dalpé-Scott, M., Heick, H.M.C., Bégin-Heick, N. (1982) An improved double antibody radioimmunoassay for the determination of insulin in serum, plasma, and tissue incubation media. *Can. J. Biochem.* 60:962-966.

Debons, A.F., Silver, L., Cronkite, E.P., Johnson, H.A., Brecher, G., Tenzer, D., Schwartz, I.L. (1962) Localization of gold in mouse brain in relation to gold thioglucose obesity. *Am. J. Physiol.* 202:743-750.

- Debons, A.F., Krinsky, I., Likuski, H.J., From, A., Cloutier, R.J. (1968) Gold thioglucose damage to the satiety center: inhibition in diabetes. *Am. J. Physiol.* 214:652-658.
- Debons, A.F., Krinsky, I., From, A., Cloutier, R.J. (1969) Rapid effects of insulin on the hypothalamic satiety center. *Am. J. Physiol.* 217:1114-1118.
- Debons, A.F., Krinsky, I., From, A., Pattinian, H. (1974a) Phlorizin inhibition of hypothalamic necrosis induced by gold thioglucose. *Am. J. Physiol.* 226:574-578.
- Debons, A.F., Krinsky, I., From, A., Pattinian, H. (1974b) Diabetes-induced resistance of ventromedial hypothalamus to damage by gold thioglucose: reversal by adrenalectomy. *Endocrinology* 95:1636-1641.
- Debons, A.F., Krinsky, I., From, A., Siclari, E., Maayan, M.L., Fani, K., Jimenez, F.A. (1979a) Action of gold thioglucose on precapillary structures in the ventromedial hypothalamus. *J. Path.* 129:73-81.
- Debons, A.F., Krinsky, I., From, A., Siclari, E. (1979b) Mediation by serotonin of gold thioglucose-induced necrosis of the ventromedial hypothalamus. *J. Path.* 129:83-90.
- Debons, A.F., Das, K.C., Fuhr, B., Siclari, E. (1982) Inhibition by hypophysectomy of the hyperphagia and obesity following gold thioglucose. *Physiol. Behav.* 29:695-699.
- Debons, A.F., Siclari, E., Das, K.C., Fuhr, B. (1982) Gold thioglucose-induced hypothalamic damage, hyperphagia, and obesity: dependence on the adrenal gland. *Endocrinology* 110:2024-2029.
- Debons, A.F., Das, K.C., Fuhr, B., Siclari, E. (1983) Anorexia after adrenalectomy in gold thioglucose-treated obese mice. *Endocrinology* 112:1847-1851.
- Debons, A.F., Zurek, L.D., Tse, C.S., Abrahamsen, S. (1986a) Central nervous system control of hyperphagia in hypothalamic obesity: dependence on adrenal glucocorticoids. *Endocrinology* 118:1678-1681.
- Debons, A.F., Tse, C.S., Zurek, L.D., Abrahamsen, S., Maayan, L.A. (1986b) Adrenalectomy induced anorexia in gold thioglucose-treated obese mice: metabolic and hormonal changes. *Physiol. Behav.* 38:111-117.
- DeKloet, E.R., Reul, J.M.H.M. (1987) Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. *Psychoneuroendocrinology* 12:83-105.

DeLaey, P., Dent, C., Terry, A.C., Quinn, E.H. (1974) Gold thioglucose, its toxicity and efficacy in Charles River mice. *Arch. Int. Pharmacodyn.* 211:341-362.

DeLaey, P., Dent, C., Terry, A.C., Quinn, E.H. (1975) Food and water intake in gold thioglucose-induced obese Charles River mice. *Arch. Int. Pharmacodyn.* 213:145-162.

DeLœuw, I., van Rooy, P., D'Hollander, M. (1981) Bone mass in obese, goldthioglucose-treated, hyperglycaemic mice. *Diabetologia* 20:145-147.

Desautels, M. (1985) Mitochondrial thermogenin content is unchanged during atrophy of brown adipose tissue of fasting mice. *Am. J. Physiol.* 249:E99-E106.

Desautels, M., Zaror-Behrens, G., 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., 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.

Devenport, L., Knehans, A., Sundstrom, A., Thomas, T. (1989) Corticosterone's dual metabolic actions. *Life Sci.* 45:1389-1396.

Djazayery, A. (1976) Alterations in blood glucose concentrations in obese mice. *Proc. Nutr. Soc.* 36:15A.

Djazayery, A., Miller, D.S., Stock, M.J. (1979) Energy balances in obese mice. *Nutr. Metab.* 23:357-367.

Dulloo, A.G., Girardier, L. (1989) Energy expenditure and diet-induced thermogenesis in presence and absence of hyperphagia induced by insulin. *Am. J. Physiol.* 257:R717-R725.

Ebner, S., Burnol, A.-F., Ferré, P., Saintaurin, M.-A., Girard, J. (1987) Effects of insulin and norepinephrine on glucose transport and metabolism in rat brown adipocytes. Potentiation by insulin of norepinephrine-induced glucose oxidation. *Eur. J. Biochem.* 170:469-474.

Eley, J., Himms-Hagen, J. (1989a) Attenuated response to cold of brown adipose tissue of mice made obese with gold thioglucose. *Can. J. Physiol. Pharmacol.* 67:116-121.

Eley, J., Himms-Hagen, J. (1989b) Brown adipose tissue of mice with goldthioglucose-induced obesity: abnormal circadian control. *Am. J. Physiol.* 256:E773-E779.

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

Fernandez, M., Nicholls, D.G., Rial, E. (1987) The uncoupling protein from brown-adipose-tissue mitochondria: Chymotrypsin-induced structural and functional modifications. *Eur. J. Biochem.* 164:675-680.

Ferré, P., Burnol, A.-F., Leturque, A., Terretaz, J., Penicaud, L., Jeanrenaud, B., Girard, J. (1986) Glucose utilization in vivo and insulin-sensitivity of rat brown adipose tissue in various physiological and pathological conditions. *Biochem. J.* 233:249-252.

Foster, D.O. (1984) Quantitative contribution of brown adipose tissue thermogenesis to overall metabolism. *Can. J. Biochem. Cell Biol.* 62:618-622.

Foster, D.O., Depocas, F., Zaror-Behrens, G. (1982) Unilaterality of the sympathetic innervation of each pad of rat interscapular brown adipose tissue. *Can. J. Physiol. Pharmac.* 60:747-754.

Foster, D.O., Frydman, M.L. (1978) Nonshivering thermogenesis in the rat. II. Measurement of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorogenesis induced by noradrenaline. *Can. J. Physiol. Pharmacol.* 56:110-122.

Foster, D.O., Frydman, M.L. (1979) Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-adapted rats, the dominant role of brown adipose tissue. *Can. J. Physiol. Pharm.* 56:257-270.

Freedman, M.R., Castonguay, T.W., Stern, J.S. (1985) Effect of adrenalectomy and corticosterone replacement on meal patterns of Zucker rats. *Am. J. Physiol.* 249:R584-R594.

Freedman, M.R., Horwitz, B.A., Stern, J.S. (1986) Effect of adrenalectomy and glucocorticoids replacement on development of obesity in the genetically obese Zucker rats. *Am. J. Physiol.* 250:R595-R607.

French, R.R., Gore, M.G., York, D.A. (1988). A study of GDP binding to purified thermogenin protein from brown adipose tissue. *Biochem. J.* 251:385-389.

Galpin, K.S., Henderson, R.G., James, W.P.T., Trayhurn, P. (1983) GDP binding to brown-adipose-tissue mitochondria of mice treated chronically with corticosterone. *Biochem. J.* 214:265-268.

Garvey, T.W., Olefski, J.M., Matthaei, S., Marshall, S. (1987) Glucose and insulin co-regulate the glucose transport system in primary cultured adipocytes. *J. Biol. Chem.* 262:189-197.

Garvey, T.W., Huecksteadt, T.P., Monzon, R., Marshall, S. (1989) Dexamethasone regulates the glucose transport system in primary cultured adipocytes: different mechanisms of insulin resistance after acute and chronic exposure. *Endocrinology* 124:2063-2073.

Giovannini, P., Seydoux, J., Girardier, L. (1988). Evidence for a modulating effect of Na^+/H^+ exchange on the metabolic response of rat brown adipose tissue. *Pflügers Arch.* 411:273-277.

Girardier, L., Seydoux, J. (1986) Neural control of brown adipose tissue thermogenesis. In : *Brown Adipose Tissue* pp.122-151, Trayhurn, P. and Nicholls, D.G. (eds) Edward Arnold, London.

Gray, G.F., Liebelt, R.A. (1961) Food intake studies in goldthioglucose obese CBA mice. *Texas Rept. Biol. Med.* 19:80-88.

Greco-Perotto, R., Assimacopoulos-Jeannet, F., Jeanrenaud, B. (1987a) Insulin modifies the properties of glucose transporters in rat brown adipose tissue. *Biochem. J.* 247:63-68.

Greco-Perotto, R., Zaninetti, D., Assimacopoulos-Jeannet, F., Bobbioni, E., Jeanrenaud, B. (1987b) Stimulatory effect of cold adaptation on glucose utilization by brown adipose tissue. *J. Biol. Chem.* 262:7732-7736.

Grogan, C.K., Kim, H.-K., Romsos, D.R. (1987) Effects of adrenalectomy on energy balance in obese (*ob/ob*) mice fed high carbohydrate or high fat diets. *J. Nutr.* 117:1115-1120.

Gualberto, A., Saggerson, E.D. (1989) Differentiation of rapid and slower-acting effects of insulin on mitochondrial processes in brown adipose tissue from streptozotocin-diabetic rats. *Biochem. J.* 258:309-311.

Han, P.W., Liu, A.-C. (1966) Obesity and impaired growth of rats force fed 40 days after hypothalamic lesions. *Am. J. Physiol.* 209:627-631.

Heldmaier, G. (1975) The influence of the social thermoregulation on the cold-adaptive growth of BAT in hairless and furred mice. *Pflugers Archs* 355:261-266.

Hetherington, A.W., Ranson, S.W. (1939) Experimental hypothalamico-hypophysial obesity in the rat. *Proc. Soc. Exp. Biol. Med.* 41:465-466.

- Himms-Hagen, J. (1984). Nonshivering thermogenesis. *Brain Res. Bull.* 12:151-160.
- Himms-Hagen, J. (1985) Brown adipose tissue metabolism and thermogenesis. *Ann. Rev. Nutr.* 5:69-94.
- Himms-Hagen, J. (1986) Brown adipose tissue and cold-acclimation. In *Brown adipose tissue*. pp 214-268. Trayhurn, P. and Nicholls, D.G. (eds). Arnold, London.
- Himms-Hagen, J. (1989a) Brown adipose tissue thermogenesis: role in thermoregulation, energy regulation and obesity. *Pharmacol. Ther.* In press.
- Himms-Hagen, J. (1989b) Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.* 28:67-115.
- Hogan, S., Coscina, D.V., Himms-Hagen, J. (1982) Brown adipose tissue of rats with obesity-inducing ventromedial hypothalamic lesions. *Am. J. Physiol.* 243:E338-E344.
- Hogan, S., Himms-Hagen, J. (1983) Brown adipose tissue of mice with gold thioglucose-induced obesity: effect of cold and diet. *Am. J. Physiol.* 244:E581-E588.
- Holm, C., Fredrikson, G., Cannon, B., Belfrage, P. (1987) Hormone-sensitive lipase in brown adipose tissue: identification and effect of cold exposure. *Biosci. Rep.* 7:897-904.
- Holt, S.J., Wheal, H.V., York, D.A. (1987) Hypothalamic control of brown adipose tissue of obese Zucker lean and obese rats. Effects of electrical stimulation of the ventromedial nucleus and other hypothalamic centres. *Brain Res.* 405:227-233.
- Holt, S.J., Wheal, H.V., York, D.A. (1988) Response of brown adipose tissue to electrical stimulation of hypothalamic centers in intact and adrenalectomized Zucker rats. *Neurosci. Lett.* 84:63-67.
- Holt, S.J., York, D.A. (1989) The effects of adrenalectomy, corticotropin releasing factor and vasopressin on the sympathetic firing rate of nerves to interscapular brown adipose tissue in the Zucker rat. *Physiol. Behav.* 45:1123-1129.
- Hom, F.G., Goodner, C.J. (1984) Insulin dose-response characteristics among individual muscle and adipose tissues measured in the rat in vivo with $^3\text{(H)}$ 2-deoxyglucose. *Diabetes* 33:153-159.

Howland, R.J., Bond, K.D. (1987) Cold-acclimation increases insulin sensitivity of brown adipocytes. *Horm. Metabol. Res.* 19:503-504.

Isler, D., Hill H.-P., Meier, M.K., (1987) Glucose metabolism in isolated brown adipocytes under β -adrenergic stimulation. Quantitative contribution of glucose to total thermogenesis. *Biochem. J.* 245:789-793.

Iwai, M., Hell, N.S., Shimazu, T. (1987) Effect of ventromedial hypothalamic stimulation on blood flow of brown adipose tissue in rats. *Pflügers Arch.* 410:44-47.

Jamal, Z., Saggerson, E.D. (1988) Factors influencing the altered thermogenic response of rat brown adipose tissue in streptozotocin-diabetes. *Biochem. J.* 249:415-421.

Jennings, G., Richard, D., Trayhurn, P. (1986) Effect of caging singly or in groups of different sizes on the thermogenic activity of interscapular brown adipose tissue in mice. *Comp. Biochem. Physiol.* 85A:583-586.

Jezek, P., Houstek, J., Drahotka, Z. (1988). Alkaline pH, membrane potential, and magnesium cations are negative modulators of purine nucleotide inhibition of H^+ and Cl^- transport through the uncoupling protein of brown adipose tissue mitochondria. *J. Bioenerg. Biomembr.* 20:603-622.

Jones, R., Henschen, L., Mohell, N., Nedergaard, J. (1986) Requirement of gene transcription and protein synthesis for cold- and norepinephrine-induced stimulation of thyroxine deiodinase in rat brown adipose tissue. *Biochem. Biophys. Acta* 889:366-373.

Kates, A.-L. (1989) Ph. D. thesis. University of Ottawa.

Kates, A.-L., Himms-Hagen, J. (1985) Defective cold-induced stimulation of thyroxine 5'-deiodinase in brown adipose tissue of the genetically obese (ob/ob) mouse. *Biochem. Biophys. Res. Comm.* 130:188-193

Kates, A.-L., Himms-Hagen, J. (1990) Defective regulation of thyroxine 5'-deiodinase in brown adipose tissue of ob/ob mice. *Am. J. Physiol.* 258: in press.

King, B.M. (1988) Glucocorticoids and hypothalamic obesity. *Neurosci. Biobehav. Rev.* 12:29-37.

King, B.M., Frohman, L.A. (1982) The role of vagally-mediated hyperinsulinemia in hypothalamic obesity. *Neurosci. Biobehav. Rev.* 6:205-214.

Klingenberg, M., Winkler, E. (1985). The reconstituted isolated uncoupling protein is a membrane potential driven H^+ translocator. *EMBO J.* 4:3087-3092.

Klingenberg, M. (1988). Nucleotide binding to uncoupling protein. Mechanism of control by protonation. *Biochem.* 27:781-791.

Kopecky, J., Sigurdson, L., Park, I.R.A., Himms-Hagen, J. (1986) Thyroxine 5'-deiodinase in hamster and rat brown adipose tissue: effect of cold and diet. *Am. J. Physiol.* 251:E1-E7.

Larsen, P.R. (1976) Radioimmunoassay of thyroxine, triiodothyronine and thyrotropin and human serum. In: *Manual of clinical immunology*. Rose, N.R. and Friedman, H. eds. American Society for Microbiology, Washington D.C. pp. 222-230.

Lefevre, R.A., Rothwell, N.J., Stock, M.J. (1987) Activation of brown fat thermogenesis in response to central injection of corticotropin-releasing hormone in the rat. *Neuropharmacology* 26:1217-1221.

Leibowitz, S.F., Hammer, N.J., Chang, K. (1981) Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. *Physiol. Behav.* 27:1031-1040.

Leibowitz, S.F., Roland, C.R., Hor, L., Squillari, V. (1984) Noradrenergic feeding elicited via the paraventricular nucleus is dependent upon circulating corticosterone. *Physiol. Behav.* 32:857-864.

Leibowitz, S.F., Sladek, C., Spencer, L., Tempel, D. (1988) Neuropeptide Y, epinephrine and norepinephrine in the paraventricular nucleus: stimulation of feeding and the release of corticosterone, vasopressin and glucose. *Brain Res. Bull.* 21:905-912.

LeMagnen, J. (1983) Body energy balance and food intake: a neuroendocrine regulatory mechanism. *Physiol. Rev.* 63:314-386.

LeMarchand, Y., Freychet, P., Jeanrenaud, B. (1978) Longitudinal study of the establishment of insulin resistance in hypothalamic obese mice. *Endocrinology* 102:74-85.

Leonard, J.L., Rosenberg, I.N. (1980) Iodothyronine 5'-deiodinase from rat kidney: Substrate specificity and the 5'-deiodination of reverse triiodothyronine. *Endocrinology* 107:1376-1383.

Leonard, J.L., Mellen, S.A., Larsen, P.R. (1983) Thyroxine 5'-deiodinase activity in brown adipose tissue. *Endocrinology* 112:1153-1155.

Liebelt, R.A., Sekiba, K., Liebelt, A.G., Perry, J.H. (1960) Genetic susceptibility to gold-thioglucose-induced obesity in mice. *Proc. Soc. Exp. Biol. Med.* 104:689-693.

- Likuski, H.J., Debons, A.F., Cloutier, R.J. (1967) Inhibition of gold thioglucose-induced hypothalamic obesity by glucose analogues. *Am. J. Physiol.* 212:669-676.
- Lin, C.S., Klingenberg, M. (1980) Isolation of the uncoupling protein from brown adipose tissue mitochondria. *FEBS Lett.* 113:299-303.
- Lin, C.S., Klingenberg, M. (1982) Characteristics of the isolated purine nucleotide binding protein from brown fat mitochondria. *Biochemistry* 21:2950-2956.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Ma, S.W.Y., Foster, D.O. (1984) Potentiation of in vivo thermogenesis in rat brown adipose tissue by stimulation of alpha₁-adrenoreceptors is associated with increased release of cyclic AMP. *Can. J. Physiol. Pharmacol.* 62:943-948.
- Marshall, N.B., Barnett, R.J., Mayer, J. (1955) Hypothalamic lesions in goldthioglucose injected mice. *Proc. Soc. Exp. Biol. Med.* 90:240-244.
- Mayer, J., Marshall, N.B. (1956) Specificity of gold thioglucose for ventromedial hypothalamic lesions and hyperphagia. *Nature* 178:1399-1400.
- Mercer, S.W., Trayhurn, P. (1984) The development of insulin resistance in brown adipose tissue may impair the acute cold-induced activation of thermogenesis in genetically obese (ob/ob) mice. *Biosci. Rep.* 4:933-940.
- Meyer, J.S., Micco, D.J., Stephenson, B.S., Krey, L.C., McEwen, B.S. (1979) Subcutaneous implantation method for chronic glucocorticoid replacement therapy. *Physiol. Behav.* 22:867-870.
- Minokoshi, Y., Saito, M., Shimazu, T. (1986) Sympathetic denervation impairs responses of brown adipose tissue to VMH stimulation. *Am. J. Physiol.* 251:R1005-R1008.
- Morimoto, A., Murakami, N. (1985) [¹⁴C]-Deoxyglucose incorporation into rat brain regions during hypothalamic or peripheral thermal stimulation. *Am. J. Physiol.* 249:R84-R92.
- Morley, J.E. (1987) Neuropeptide regulation of appetite and weight. *Endocrinology Rev.* 8:256-287.
- Morley, J.E., Hernandez, E.N., Flood, J.F. (1987) Neuropeptide Y increases food intake in mice. *Am. J. Physiol.* 253:R516-R522.

- Moss, D., Ma, A., Cameron, D.P. (1985) Defective thermoregulatory thermogenesis in monosodium glutamate-induced obesity in mice. *Metabolism* 34:626-630.
- Né Chad M. (1986). Structure and development of brown adipose tissue. In *Brown adipose tissue*, pp.1-30, Trayhurn, P. and Nicholls, D.G. eds. Edward Arnold, London.
- 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., Locke, R.M. (1984) Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64:1-64.
- Nijima, A. (1986) Effect of glucose and other hexoses on efferent discharges of brown adipose tissue nerves. *Am. J. Physiol.* 251:R240-R242.
- Obregon, M.-J., Pitamber, R., Jacobsson, A., Nedergaard, J., Cannon, B. (1987) Euthyroid status is essential for the perinatal increase in thermogenin mRNA in brown adipose tissue of rat pups. *Biochem. Biophys. Res. Comm.* 148:9-14.
- Oomura, Y., Ooyama, H., Sugimori, M., Nakamura, T., Yamada, Y. (1974) Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature* 247:284-286.
- Pace, C., Delmo, C., Lee, R. (1989) Altered electrical responses of B-cells after goldthioglucose-induced lesions in the ventromedial hypothalamus. *Res. Comm. Chem. Pathol. Pharmacol.* 65:401-404.
- Penick, S.P., Hinkle, L.E. (1961) Depression of food intake induced in healthy subjects by glucagon. *New Engl. J. Med.* 264:893-897.
- Perkins, M.N., Rothwell, N.J., Stock, M.J., Stone, T.W. (1981) Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* 289:401-402.
- Perry, J.H., Liebelt, R.A. (1961) Extra-hypothalamic lesions associated with gold-thioglucose induced obesity. *Proc. Soc. Exp. Biol. Med.* 106:55-57.
- Powley, T.L., Opsahl, C.A. (1974) Ventromedial hypothalamic obesity abolished by subdiaphragmatic vagotomy. *Am. J. Physiol.* 226:25-33.
- Powley, T.L., Plocher, T.A. (1980) Hypophysectomy blocks the weight gain and obesity produced by goldthioglucose lesions. *Behav. Neur. Biol.* 28:300-318.

Powley, T.L., Prechtl, J.C. (1986) Gold thioglucose selectively damages dorsal vagal nuclei. *Brain Res.* 367:192-200.

Preston, E., Triandafillou, J., Haas, N. (1989) Colchicine lesions of ventromedial hypothalamus: effects on regulatory thermogenesis in the rat. *Pharmacol. Biochem. Behav.* 32:301-307.

Rial, E., Poustie, A., Nicholls, D.G. (1983). Brown-adipose-tissue mitochondria: the regulation of the 32000-Mr uncoupling protein by fatty acids and purine nucleotides. *FEBS Lett.* 137:197-203.

Ricquier, D., Mory, G., Bouillaud, F., Thibault, J., Weissenbach, J. (1984) Rapid increase of mitochondrial uncoupling protein and its mRNA in stimulated brown adipose tissue. Use of a cDNA probe. *FEBS Lett.* 178:240-244.

Ricquier, D., Bouillaud, F. (1986). The brown adipose tissue mitochondrial uncoupling protein, In *Brown adipose tissue*, pp.86-104, Trayhurn, P. and Nicholls, D.G. (eds) Edward Arnold, London.

Romsos, D.R., Vander Tuig, J.G., Kerner, J., Grogan, C.K. (1987) Energy balance in rats with obesity-producing hypothalamic knife cuts: effects of adrenalectomy. *J. Nutr.* 117:1121-1128.

Rothwell, N.J., Stock, M.J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281:31-35.

Rothwell, N.J., Stock, M.J. (1981) A role for insulin in the diet-induced thermogenesis of cafeteria-fed rats. *Metabolism* 30:673-678.

Rothwell, N.J., Stock, M.J. (1984) Sympathetic and adrenocorticoid influences on diet-induced thermogenesis and brown fat activity in the rat. *Comp. Biochem. Physiol.* 79A:575-579.

Rothwell, N.J., Stock, M.J., Warwick, B.P., Winter, P.D.O'B. (1983) Diurnal variations in circulating hormone levels and brown adipose tissue activity in "cafeteria"-fed rats. *Comp. Biochem. Physiol.* 75A:461-465.

Saggerson, E.D., McAllister, T.W.J., Baht, H.S. (1988) Lipogenesis in rat brown adipocytes. Effects of insulin and noradrenaline, contributions from glucose and lactate as precursors and comparisons with white adipocytes. *Biochem. J.* 251:701-709.

Saito, M., Bray, G.A. (1983) Diurnal rhythm for corticosterone in obese (ob/ob) diabetes (db/db) and gold-thioglucose-induced obesity in mice. *Endocrinology* 113:2181-2185.

Saito, M., Bray, G.A. (1984) Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. *Am. J. Physiol.* 246:R20-R25.

Saito, M., Minokoshi, Y., Shimazu, T. (1989) Accelerated norepinephrine turnover in peripheral tissues after ventromedial hypothalamic stimulation in rats. *Brain Res.* 481:298-303.

Sakaguchi, T., Bray, G.A. (1987) The effect of intrahypothalamic injections of glucose on sympathetic efferent firing rate. *Brain Res. Bull.* 18:591-595.

Sakaguchi, T., Bray, G.A., Eddlestone, G. (1988) Sympathetic activity following paraventricular or ventromedial hypothalamic lesions in rats. *Brain Res. Bull.* 20:461-465.

Saphier, D., Feldman, S. (1988) Ionophoretic application of glucocorticoids inhibits identified neurones in the paraventricular nucleus. *Brain Res.* 453:183-190.

Schacterle, G.R., Pollack, R.L. (1973) A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal. Biochem.* 51:654-655.

Schindler, W.J., Liebelt, R.A. (1967) Thyroid activity in mice with hypothalamic lesions induced by goldthioglucose. *Endocrinology* 80:387-398.

Sclafani, A., Kirchgessner, A.L. (1986) The role of the medial hypothalamus in the control of food intake: an update. In: *Neural and humoral controls of food intake* pp.27-66, Ritter, R.S., Ritter, S. and Barnes, C.D. (eds) Academic Press.

Seydoux, J., Trimble, E.R., Bouillaud, F., Assimacopoulos-Jeannet, F., Bas, S., Ricquier, D., Giacobino, J.P., Girardier, L. (1984) Modulation of β -oxidation and proton conductance pathway of brown adipose tissue in hypo- and hyperinsulinemic states. *FEBS Lett.* 166:141-145.

Seitz, H.J., Krone, W., Wilke, H., Tarnowski, W. (1981) Rapid rise in plasma glucagon induced by acute cold exposure in man and rat. *Pflügers Arch.* 389:115-120.

Shimizu, H., Shargill, N.S., Bray, G.A. (1989) Adrenalectomy and response to corticosterone and MSH in the genetically obese yellow mouse. *Am. J. Physiol.* 256:R494-R500.

Shimomura, G.A., Bray, G.A., Lee, M. (1987) Adrenalectomy and steroid treatment in obese (ob/ob) and diabetic (db/db) mice. *Horm., Metabol. Res.* 19:295-299.

Silva, J.E. (1988) Full expression of uncoupling protein gene requires the concurrence of norepinephrine and triiodothyronine. *Mol. Endocrinol.* 2:706-713.

Silva, J.E., Larsen, P.R. (1983) Adrenergic activation of triiodothyronine production in brown adipose tissue. *Nature* 305:712-713.

Silva, J.E., Larsen, P.R. (1986) Hormonal regulation of iodothyronine 5'-deiodinase in rat brown adipose tissue. *Am. J. Physiol.* 251:E639-E643.

Silver, A.J., Flood, J.F., Song, A.M., Morley, J.E. (1989) Evidence for a physiological role for CCK in the regulation of food intake in mice. *Am. J. Physiol.* 256:R646-R652.

Sinha, Y.N., Salocks, C.B., Vanderlaan, W.P. (1975) Prolactin and growth hormone secretion in chemically induced and genetically obese mice. *Endocrinology* 97:1386-1393.

Skala, J.P. (1984) Mechanisms of hormonal regulations in brown adipose tissue during acclimation to cold. *Can. J. Biochem. Cell Biol.* 62:637-647.

Slinde, E., Pedersen, J.I., Flatmark, T. (1975) Sedimentation coefficient and buoyant density of brown adipose tissue mitochondria from guinea pigs. *Anal. Biochem.* 65:581-585.

Smith, P.E. (1927) The disabilities caused by hypophysectomy and their repair. *J. Am. Med. Assoc.* 88:158-161.

Smith, R.E., Horwitz, B.A. (1969) Brown fat and thermogenesis. *Physiol. Rev.* 49:330-425.

Smith, R.E., Roberts, J.C. (1964) Thermogenesis of brown adipose tissue in cold-acclimated rats. *Am. J. Physiol.* 206:143-148.

Smith, C.K., Romsos, D.R. (1985) Effects of adrenalectomy on energy balance of obese mice are diet dependent. *Am. J. Physiol.* 249:R13-R22.

Smith, S.A., Young, P., Cawthorne, M.A. (1986) Quantification in vivo of the effects of insulin on glucose utilization in individual tissues of warm- and cold-acclimated rats. *Biochem. J.* 237:789-795.

Stellar, E. (1954) The physiology of motivation. *Psychol. Rev.* 61:5-22.

Stern, J.S., Hirsch, J., Drewnowski, A., Sullivan, A.C., Johnson, P.R., Cohn, C.K. (1983) Glycerol kinase activity in adipose tissue of obese rats and mice: effects of diet composition. *J. Nutr.* 113:714-720.

Swick, A.G., Swick, R.W. (1986) Rapid changes in number of GDP binding sites on brown adipose tissue mitochondria. *Am. J. Physiol.* 251:E192-E195.

Tanti, J.F., Grémaux, T., Brandeburg, D., van Obberghen, E., LeMarchand-Brustel, Y. (1986) Brown adipose tissue in lean and obese mice. Insulin-receptor binding and tyrosine kinase activity. *Diabetes* 35:1243-1248.

Tokunaga, K., Fukushima, M., Lupien, J.R., Bray, G.A., Kemnitz, J.W., Schemmel, R. (1989) Effects of food restriction and adrenalectomy in rats with VMH or PVH lesions. *Physiol. Behav.* 45:1131-1137.

Tokuyama, K., Himms-Hagen, J. (1986) Brown adipose tissue thermogenesis, torpor, and obesity of glutamate-treated mice. *Am. J. Physiol.* 251:E407-E415.

Tokuyama, K., Himms-Hagen, J. (1987) Increased sensitivity of the genetically obese mouse to corticosterone. *Am. J. Physiol.* 252:E202-E208.

Tokuyama, K., Himms-Hagen, J. (1989a) Adrenalectomy prevents obesity in glutamate-treated mice. *Am. J. Physiol.* 257:E139-E144.

Tokuyama, K., Himms-Hagen, J. (1989b) Enhanced acute response to corticosterone in genetically obese (ob/ob) mice. *Am. J. Physiol.* 257:E133-E138.

Trayhurn, P., Jennings, G. (1986) Evidence that fasting can induce a selective loss of uncoupling protein from brown adipose tissue mitochondria of mice. *Biosci. Rep.* 6:805-810.

Trayhurn, P., Ashwell, M., Jennings, G., Richard, D., Stirling, D.M. (1987) Effect of warm or cold exposure on GDP binding and uncoupling protein in rat brown fat. *Am. J. Physiol.* 252:E237-E243.

Trayhurn, P., Milner, R.E. (1989) A commentary on the interpretation of in vitro biochemical measures of brown adipose tissue thermogenesis. *Can. J. Physiol. Pharmacol.* 67:811-819.

Tse, C.S., Debons, A.F. (1986) Anorexia after adrenalectomy in gold thioglucose-treated obese mice: Role of adipose tissue mass. *Physiol. Behav.* 38:105-110.

Vallerand, A.L., Pérusse, F., Bukowiecki, L.J. (1987) Cold exposure potentiates the effect of insulin on in vivo glucose uptake. *Am. J. Physiol.* 253:E179-E186.

Visser, T.J., Leonard, J.L., Kaplan, M.M., Larsen, P.R. (1982) Kinetic evidence suggesting two mechanisms for iodothyronine 5'-deiodination in rat cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.* 79:5080-5084.

- Wickler, S.J., Horwitz, B.A., Stern, J.S. (1986) Blood flow to brown fat in lean and obese adrenalectomized Zucker rats. *Am. J. Physiol.* 251:R851-R858.
- Wiepkema, P.R. (1966) Aurothioglucose sensitivity of CBA mice injected at two different times of day. *Nature* 209:937.
- Woods, S.C., Porte, Jr., D. (1974) Neural control of the endocrine pancreas. *Physiol. Rev.* 54:596-619.
- Yim, G.K.W., Lowy, M.T. (1984) Opioids, feeding and anorexias. *Fed. Proc.* 43:2893-2897.
- Yonetani, T., Ray, G.S. (1965) Studies on cytochrome oxidase. VI. Kinetics of the aerobic oxidation of ferrocytochrome c by cytochrome oxidase. *J. Biol. Chem.* 240:3392-3398.
- York, D.A., Holt, S.J., Marchington, D. (1985) Regulation of brown adipose tissue thermogenesis by corticosterone in obese fa/fa rats. *Int. J. Obesity* 9:suppl.2, 89-95.
- Yoshida, T., Kemnitz, J.W., Bray, G.A. (1983) Lateral hypothalamic lesions and norepinephrine turnover in rat. *J. Clin. Invest.* 72:919-927.
- Yoshida, T., Nishioka, H., Nakamura, Y., Kondo, M. (1984) Reduced norepinephrine turnover in mice with monosodium glutamate-induced obesity. *Metabolism* 33:1060-1063.
- Young, J.B., Landsberg, L. (1980) Impaired suppression of sympathetic activity during fasting in the gold thioglucose-treated mouse. *J. Clin. Invest.* 65:1086-1094.
- Young, P., Cawthorne, M.A., Smith, S.A. (1985) Brown adipose tissue is a major site of glucose utilisation in C57Bl/6 ob/ob mice treated with a thermogenic β -adrenoceptor agonist. *Biochem. Biophys. Res. Comm.* 130:241-248.
- Zaror-Behrens, G., Himms-Hagen, J. (1984) Turnover of noradrenaline in brown adipose tissue of genetically (ob/ob) and goldthioglucose (GTG) obese mice. *Fed. Proc.* 43:1061.
- Zaror-Behrens, G., Himms-Hagen, J. (1989) Diet and noradrenaline turnover in brown adipose tissue of goldthioglucose-obese mice. *Int. J. Obesity*, submitted.

APPENDIX A
LIST OF ABBREVIATIONS

ADP	adenosine 5'-diphosphate
adx	adrenalectomized
ATP	adenosine 5'-triphosphate
BAT	brown adipose tissue
BSA	bovine serum albumin
cAMP	cyclic adenosine 5'-monophosphate
CI	curie
CIT	cold-induced thermogenesis
CRF	corticotropin-releasing-factor
DIT	diet-induced thermogenesis
EDTA	ethylenediaminetetraacetic acid
GDP	guanine 5'-diphosphate
GTG	gold thioglucose
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HSL	hormone sensitive lipase
LH	lateral hypothalamus
LPL	lipoprotein lipase
mRNA	messenger RNA
MSG	monosodium glutamate
PBS	phosphate buffer saline
PVN	paraventricular nucleus

S.E.M.	standard error of the mean
T3	triiodothyronine
T4	thyroxine (tetraiodothyronine)
TCA	trichloroacetic acid
TES	N-tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid
UCP	uncoupling protein
VMH	ventromedial hypothalamus
WAT	white adipose tissue

APPENDIX B
CURRICULUM VITAE

NAME: Christiane Villemure

DATE OF BIRTH: October 11th, 1964

PLACE OF BIRTH: Amos, Québec

CITIZENSHIP: Canadian

EDUCATION: University of Ottawa
Ottawa, Ontario
M. Sc. (Biochemistry)
registered Jan. 1988-present

University of Ottawa
Ottawa, Ontario
B. Sc. (Biochemistry) 1987

Ecole secondaire Saint-Joseph de Hull
Hull, Québec (sec I à V)

AWARDS: Bourse d'études supérieures du Fonds FCAR
(Fonds pour la formation de chercheurs et
l'aide à la recherche) 1988-1989.

University of Ottawa Supplemental
Research Scholarship 1988-1989.

EXPERIENCE: Summer student
Department of Biochemistry
University of Ottawa, summer 1987
Supervisor Dr. L. Kleine

Lab demonstrator - Biochemistry 3946
University of Ottawa, 1988

PUBLICATIONS:

paper:

Mezl, V.A., Villemure, C., Garber, P., Rakusan, K. (1988) Aortic stenosis produces hypertrophy of the rat heart without causing an accumulation of N¹-acetylspermidine. *Biochem. Cell Biol.* 66:1018-1021.

abstract:

Villemure, C., Garber, P., Mezl, V.A., Rakusan, K. (1987) Chez le rat une sténose de l'aorte produit une hypertrophie du coeur sans affecter la concentration de N¹-acétylspermidine. *Les Annales de l'ACFAS* 55:21.

TABLE 4: GTG EXPERIMENT: GROUPING OF MICE ACCORDING TO THEIR SERUM CORTICOSTERONE LEVELS.

Serum corticosterone levels were measured as described in chapter two (section 10). Mice were divided in different groups of similar serum corticosterone levels in order to be able to do a statistical analysis of the data. The table shows the mean levels \pm S.E.M. The number of animals in each group is indicated in parentheses. Also included in this table is the lowest and highest values for each group. Some mice had a non-detectable level of corticosterone in their serum even though they received a corticosterone implant. It was therefore assumed that those animals received corticosterone at a level that was below the sensitivity of the assay and they were then considered as corticosterone-replaced adx mice separately from adx, non-replaced mice of the same type (group 1). The abbreviation "N.D." means "not detectable".

APPENDIX C

TABLE 4

CONTROL GROUP

mean corticosterone ($\mu\text{g/dl}$)	lowest value ($\mu\text{g/dl}$)	highest value ($\mu\text{g/dl}$)
1) 0.00 ± 0.00 (13)	-	-
2) 0.10 ± 0.05 (16)	N.D.	0.61
3) 3.50 ± 0.37 (16)	1.26	5.60
4) 6.67 ± 0.18 (5)	6.28	7.05
5) 8.86 ± 0.48 (5)	7.70	9.73
6) 12.13 ± 0.39 (5)	11.02	13.12

GTG-OBESE GROUP

1) 0.00 ± 0.00 (5)	-	-
2) 1.25 ± 0.24 (18)	N.D.	3.18
3) 5.45 ± 0.27 (12)	4.15	6.72
4) 8.55 ± 0.30 (7)	7.53	9.86