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CONTROL OF BROWN ADIPOSE TISSUE GROWTH AND FUNCTION IN NORMAL AND MYOPATHIC HAMSTERS

by

S. Lynn Sigurdson

A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirement for the degree of Doctor of Philosophy

Department of Biochemistry
Faculty of Health Sciences
University of Ottawa
Ottawa, Canada
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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

This thesis is dedicated to family and friends who supported me all the way, and to Wade, who always knew I could do it.

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ABSTRACT:

When this research was initiated control of brown adipose tissue (BAT) growth in the hamster was not well understood. Growth can be induced by cold exposure and feeding a high fat diet but is not mediated by noradrenaline, as in the case of the rat. In addition, unlike the rat, short photoperiod can induce BAT growth in the hamster. The myopathic hamster has a reduced amount of BAT, which fails to grow in response to either diet or photoperiod stimulation. The objective of this research was to study the apparent defect in control of myopathic hamster BAT. Since brown fat growth is not well understood in the normal hamster it was necessary to establish how growth and function are regulated in normal hamsters before comparisons with myopathic hamsters could be made. The results show that although noradrenaline seems to mediate acute thermogenic responses to cold, it is not the sole factor involved in promoting growth. Continuous sympathetic stimulation of BAT is not required for growth yet an intact innervation is necessary. This points to possible involvement of other neurotransmitters and/or neuropeptides. Cold acclimated hamsters have a high BAT thyroxine 5'-deiodinase activity and serum T_3 level, and thyroid-induced thermogenesis can partially replace nonshivering thermogenesis in BAT. Neither high fat diet nor short photoperiod stimulate sympathetic or deiodinase activity in BAT, suggesting that neither noradrenaline alone, nor increased local T_3 production are sufficient to induce growth. Failure of myopathic hamster BAT to grow in response to high fat diet or short photoperiod is not due to defective stimulation of deiodinase or sympathetic activity. Myopathic hamsters are hypothyroid

and somewhat T_4 resistant, which may partially explain their cold sensitivity and failure of BAT responses to T_3. These animals are very active and along with increased muscle protein turnover, heat generated at other sites may suppress BAT.

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LIST OF ABBREVIATIONS:

ADP	adenosine diphosphate
ATP	adenosine triphosphate
BAT	brown adipose tissue
BMR	basal metabolic rate
BSA	bovine serum albumin
CGRP	calcitonin-gene-related-peptide
CRF	corticotropin-releasing factor
DIT	diet-induced thermogenesis
EDTA	ethylenediamine tetraacetic acid
GABA	gamma aminobutyric acid
GDP	guanosine diphosphate
GTP	guanosine triphosphate
HEPES	hydroxyethylpiperazine-N'-2-ethane sulfonic acid
HPLC	high pressure liquid chromatography
i.p.	intraperitoneal
kcal	kilocalorie
kD	kilodalton
L:D	hours of light:hours of darkness
MOC	minimal oxygen consumption
MOPS	(3-(N-morpholino)propane sulfonic acid
M_r	relative molecular weight
NA	noradrenaline (norepinephrine)
NaCl	sodium chloride
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate

NGF	nerve growth factor
NPY	neuropeptide Y (Y=tyrosine)
NST	nonshivering thermogenesis
PBS	phosphate buffered saline
PCA	perchloric acid
PPO	2,5-diphenyloxazole
PVN	paraventricular nucleus
s.c.	subcutaneous
SCG	superior cervical ganglion
SCN	suprachiasmatic nucleus
SDS	sodium dodecyl sulfate
T_3	triiodothyronine
T_4	thyroxine
T5'D	thyroxine 5'-deiodinase
TCA	trichloroacetic acid
TES	Tris(hydroxymethyl)-2-aminoethane sulfonic acid
TH	tyrosine hydroxylase
TSH	thyrotropin (thyroid-stimulating hormone)
TRIS	Tris(hydroxymethyl)aminomethane
TRH	thyrotropin-releasing-hormone
UCP	uncoupling protein
VMH	ventromedial hypothalamus

INTRODUCTION:

Brown adipose tissue (BAT) was first described in the 16th century and was thought to be associated with hibernation (see Smith and Horwitz, 1969). Its function was still speculative as of the late 1950's, although much work had been published on brown adipose tissue morphology (see Johansson, 1959). Ten years later brown adipose tissue became recognized as an important site of heat production in neonatal animals, animals living in the cold, and hibernators (Smith and Horwitz, 1969). The quantitative contribution of brown fat to heat production in cold acclimated animals remained controversial until Foster and Frydman identified it as the major site of heat production in the cold acclimated rat (Foster and Frydman, 1978a, b, 1979). At about the same time Rothwell and Stock (1979) reported that BAT could play an important role as an energy buffer in energy balance regulation in overfed rats. Since that time, the field of brown adipose tissue research has expanded enormously and now encompasses a wide variety of disciplines from physiology and behavior to biochemistry and molecular biology.

Brown adipose tissue is not only turned on and off to meet acute thermogenic demands but it can also grow as part of an adaptive response. This adaptive growth is termed the trophic response. The control of brown fat growth and function have been studied mostly in the laboratory rat and to a lesser extent in other species. The Syrian hamster, the subject of this thesis, is a hibernator with a different stategy for survival in the cold than the rat, and a different control

of brown adipose tissue function and growth from that in the rat (see Himms-Hagen, 1984).

The hamster with inherited muscular dystrophy is known to have a reduced amount of brown adipose tissue (Himms-Hagen and Gwilliam, 1980) and defective trophic responses of BAT to growth-promoting stimuli (Triandafillou et al, 1984a, b). The major goals of the research described in this thesis were to study the defective control of brown adipose tissue growth and function in the myopathic hamster in order to understand why this animal has less BAT than normal hamsters. However, very little information was available on the regulation of brown adipose tissue growth and function in the normal Syrian hamster when this project was initiated, therefore much of the work described was carried out to establish how BAT growth and thermogenic function is regulated in normal Syrian hamsters and how it differs from that in the rat.

1. THERMOGENESIS:

In order to understand the function of brown adipose tissue, which is heat production, a preliminary discussion of thermogenesis is warranted.

Total overall thermogenesis is the sum of two broad categories of heat production: obligatory thermogenesis and facultative thermogenesis.

Obligatory Thermogenesis:

Obligatory thermogenesis refers to energy expenditure associated with metabolic processes essential to cellular existence, with maintenance of endothermy (warm bloodedness), and with processing of food (postprandial thermogenesis) (see Danforth and Burger, 1981;

Himms-Hagen, 1983, 1989 in press). The thermic effect of food is defined as the obligatory energy expenditure for digestive activity, absorption, and metabolic processing of food (see Himms-Hagen, 1989).

Obligatory thermogenesis is equivalent to basal metabolic rate (BMR) and is measured as oxygen consumption under conditions of rest, at thermoneutrality, and in a post-absorptive state (Guernsey and Edelman, 1983; Van Hardeveld, 1986; Himms-Hagen, 1983). Basal metabolic rate and minimal oxygen consumption (MOC) are equivalent when, instead of a resting, conscious animal kept at thermoneutrality, an anesthetized animal whose body temperature is maintained at the same temperature by external heat sources, is utilized (Denckla, 1970, 1974; Denckla and Marcum, 1973). Basal metabolic rate or MOC can be measured as oxygen consumption because regardless of fuel type (fat, protein, or carbohydrate), the amount of heat produced for a quantity of oxygen being reduced to water is nearly constant (approximately 5.0 Kcal/L O_2, see Guernsey and Edelman, 1983). Obligatory thermogenesis occurs in all organs, with the primary controller of basal oxygen consumption being thyroid hormones which regulate obligatory thermogenesis (Guernsey and Edelman, 1983; Van Hardeveld, 1986). Hormonally-mediated changes in obligatory thermogenesis are quite slow, requiring hours or even days to manifest themselves (Danforth and Burger, 1981; Van Hardeveld, 1986).

Facultative Thermogenesis:

Heat production over and above obligatory thermogenesis is known as facultative thermogenesis and its occurrence depends primarily on environmental conditions and the particular species involved (see

Himms-Hagen, 1983, 1986, 1989). Facultative thermogenesis can be induced by temperatures below thermoneutrality (cold-induced thermogenesis, shivering or nonshivering), prolonged overfeeding (diet-induced thermogenesis), and voluntary physical activity (exercise-induced thermogenesis). As opposed to obligatory thermogenesis, facultative thermogenesis occurs only in skeletal muscle and brown adipose tissue and is controlled primarily by the nervous system, mediated by acetylcholine from motor nerves in muscle, and noradrenaline from sympathetic nerves in brown adipose tissue. An important characteristic of facultative thermogenesis is that it can be switched on or off very rapidly (see Néchad, 1986; Himms-Hagen, 1983, 1989).

Nonshivering Thermogenesis:

Nonshivering thermogenesis (NST) can be thought of as cold-induced metabolic heat production that is not due to muscle activity (Himms-Hagen, 1976). When an animal is exposed to cold it increases heat production in order to maintain body temperature, first through shivering, then after 2-3 weeks in the cold, shivering thermogenesis is replaced by nonshivering thermogenesis in brown adipose tissue. The capacity for nonshivering thermogenesis is high in cold-adapted animals, newborns, and hibernators (Jansky, 1965; Heldmaier, 1971; Himms-Hagen, 1976). It declines with age but can be re-established by cold acclimation (Himms-Hagen, 1976). Brown adipose tissue has been associated with hibernation and temperature regulation for many years (see Smith and Horwitz, 1969) but not until Foster and Frydman (1978a, b, 1979) used radioactive microspheres to measure blood flow to various

tissues in cold-acclimated rats, and arteriovenous differences in blood oxygen across brown fat, was the major site of NST firmly established as BAT. Administration of noradrenaline also induces a calorigenic increase in oxygen consumption (see Smith and Horwitz, 1969) that was assumed to be equivalent to nonshivering thermogenesis (Foster and Frydman, 1978) and has been demonstrated to occur in brown adipose tissue (Foster and Frydman, 1979).

Diet-Induced Thermogenesis:

Diet-induced thermogenesis (DIT) includes both a neurally-mediated cephalic phase associated with insulin secretion and sympathetic nervous system activation, and an adaptive phase associated with increased sympathetic activity in BAT (see Himms-Hagen, 1989). Usage of the term DIT here will refer only to the adaptive changes in energy expenditure that dissipate excess consumed energy and act to decrease metabolic efficiency (Rothwell and Stock, 1979, 1983). Metabolic efficiency is associated with utilization of absorbed nutrients and is expressed as the energy gained per unit of energy consumed above maintenance (Rothwell and Stock, 1983). It can be modulated by diet through alteration of energy expenditure. The concept of diet-induced thermogenesis is not new (see Rothwell and Stock, 1983) and the similarity between DIT in the overfed rat and NST thermogenesis in the cold acclimated rat was pointed out some twenty years ago (Stirling and Stock, 1968). Rats induced to overeat when offered a highly palatable "cafeteria" diet consumed 80% more energy but only gained 27% more weight than control rats (Rothwell and Stock, 1979). Metabolic efficiency was reduced but resting metabolic rate and respiratory

response to noradrenaline, measured as oxygen consumption, were elevated. Accompanying these changes were increases in brown adipose tissue weight and protein content (Rothwell and Stock, 1979), thermogenic activation of BAT (Brooks et al, 1980), increased noradrenaline turnover (Young et al, 1982), and enhanced blood flow to brown fat following noradrenaline infusion (Rothwell and Stock, 1981).

Behavioral Thermoregulation:

The way in which an animal behaves can also contribute to its overall thermogenic state. Hamsters, like other rodents, are not able to thermoregulate at birth, but have a finely-tuned ability to sense and respond to temperature gradients. Newborn hamsters will rapidly move towards warmth and will adjust their position continually in order to maintain a constant surface temperature (Schoenfeld and Leonard, 1986). In the nest they thermoregulate by trying to stay in the centre of the litter. Near the end of the first post-natal week they can tolerate a wider range of temperatures as they begin to develop physiological regulation of core temperature (Schoenfeld and Leonard, 1986).

Adult hamsters when given a choice of ambient temperatures prefer the lower range of their thermoneutral zone, approximately 28°C (Gordon et al, 1986). At this temperature resting metabolic rate is minimal and near that of the lower critical or thermoneutral temperature. In group housing experiments hamsters caged together increased somatic growth, reduced resting metabolic rate, and became more obese than hamsters housed individually at 20-22°C (Borer et al, 1988). The authors postulate that the energy savings generated by huddling could

be spent on increasing the synthesis of body mass, and that some aspect of body contact appears to remove neural restraints over the rate of somatic growth. They further suggest that given the lifespan of a hamster of 18 months on average, growth-stimulated body contact may be a feature by which hamsters attain a mature body size during the first overwintering in the litter (Borer et al, 1988). Unfortunately, these workers did not study the effects of caging on thermogenic parameters such as brown adipose tissue size and activity.

In a similar study using mice, Jennings et al (1986) found decreasing thermogenic activity of brown adipose tissue with increasing number of mice per cage at 23 or 4°C. In that case social thermoregulation reduced the need for nonshivering thermogenesis. One could hypothesize a similar phenomenon occurring in the hamster.

In sharp contrast to the hamster (and the mouse) which prefers ambient temperatures near thermoneutral, the rat when offered a choice, prefers an ambient temperature of about 17-20°C, considerably cooler than its thermoneutral zone (Gordon, 1988). At this cooler temperature the rat is more active and has a higher metabolic rate than one kept at 29°C, its lower critical temperature. It would also have a larger amount of active brown adipose tissue. Thus differences in thermogenic strategy between the rat and the hamster are even reflected at the behavioral level, where the hamster avoids activating nonshivering thermogenesis, while the rat seeks out cooler temperatures.

2. BROWN ADIPOSE TISSUE:

Structure:

BAT has been best characterized in the laboratory rat and to a

lesser degree in the Syrian hamster and other small mammals (see Smith and Horwitz, 1969; Afzelius, 1970), therefore the general description of brown adipose tissue structure and function presented here is for the rat. Where significant species differences are known they will be discussed.

Brown adipose tissue is distributed as discrete depots in many locations throughout the body. The major sites are in the cervical and thoracic regions overlying large vessels and nerve branches, the interscapular and subscapular areas and around associated blood vessels, and along the aorta and perirenal areas, often covering the renal vasculature (Smith and Roberts, 1964; Smith and Horwitz, 1969). The relative distribution of BAT deposits varies with species and BAT is more abundant in hibernators like the hamster (Smith and Horwitz, 1969).

The strategic location of brown adipose tissue in areas vital for survival in the cold is further emphasized by its rich and topologically unique vasculature. Most BAT depots are internal and therefore supply heat to critical organs by direct conductance through major vessels. The interscapular depot however is superficial and exports most of its heat by vascular convection (Néchad, 1986). The interscapular pads have been the most intensively studied because of their location. They are served by paired thoracodorsal veins and arteries, and also by a single large vein, Sulzer's vein, which transports warmed blood directly to the heart. This arrangement of closely apposed veins and arteries allows a countercurrent transfer of heat to arteries supplying the brain and spinal cord. The central

location of Sulzer's vein permits rapid removal of heated blood by utilizing the body core as a heat sink (Smith and Roberts, 1964). When stimulated, brown adipose tissue blood flow can be extremely high (Foster, 1984; Foster and Frydman, 1978a, b, 1979), enabling heat transfer and oxygen delivery to occur at a very high rate (Smith and Roberts, 1964; Néchad, 1986). In addition to the known configuration of arteries and veins, Nnodim and Lever (1988) have recently observed direct anastomoses within the interscapular BAT pad of the rat. They postulate that these vascular arrangements play a major role in increasing blood flow in response to noradrenaline administration or sympathetic stimulation by enabling a greater fraction of arterial blood to reach to BAT parenchymal capillary beds.

Brown adipose tissue has a high noradrenaline content and dense sympathetic innervation of both the blood vessels and the adipocytes themselves (Derry et al, 1969; Cottle and Cottle, 1970; Cottle, 1970; Barnard et al, 1980; Girardier and Seydoux, 1986). Each interscapular brown fat pad is innervated by five nerve fibre bundles arising through the intercostal muscles under the pad to enter at the ventromedial surface (Girardier and Seydoux, 1986). Morphological heterogeneity of fibres entering and leaving the interscapular pad has been reported (Flaim et al, 1976), the significance of which is unclear. The origin of the neuronal cell bodies from which the sympathetic nerves arise has been somewhat controversial (see Néchad, 1986). The technique of retrograde axonal transport of horseradish peroxidase was used to trace the origin of the five intercostal nerves (Seydoux et al, 1984). Labelling was found from the middle and inferior cervical sympathetic

ganglia to the first five thoracic ganglia but no label was detected in the superior cervical ganglia or central nervous system (Seydoux et al, 1984; Girardier and Seydoux, 1986).

Differentiated brown adipose tissue consists of mainly polygonally-shaped adipocytes characterized by numerous triglyceride droplets dispersed in the cytoplasmic space (Smith and Horwitz, 1969; Afzelius, 1970; Néchad, 1986). The remainder of the tissue is made up of various cell types including endothelial cells, perivascular mesenchymal cells, preadipocytes, mast cells, and Schwann cells, which are all small and rather difficult to detect at the light microscopic level (Néchad, 1986). The colour of BAT is derived largely from blood hemoglobin and from high levels of cytochromes and flavins in the abundant mitochondria (see Smith and Horwitz, 1969). Ultrastructurally, brown adipocytes are characterized by their high content of mitochondria and centrally located nuclei. These features, along with multiple lipid droplets easily distinguish the active brown adipocyte from the white fat cell (Smith and Horwitz, 1969; Afzelius, 1970; Néchad, 1986).

Function:

Brown adipose tissue functions to oxidize fuels and dissipate the energy as heat. Noradrenaline interacting with β-adrenergic receptors on the plasma membrane of the brown adipocyte initiates the thermogenic process. Hormone sensitive lipase is stimulated through activation of adenylate cyclase and the subsequent increase in intracellular cAMP levels. Lipolysis is then stimulated and the resulting fatty acids are activated to acyl CoA and transported into the mitochondria by the

carnitine shuttle for oxidation (see Nedergaard and Lindberg, 1982; Nicholls and Locke, 1984; Cannon and Nedergaard, 1985). Fatty acids are also involved in the process of uncoupling oxidative phosphorylation from electron transport in the mitochondria, which is part of the thermogenic mechanism of brown adipose tissue (see below). Thus fatty acids play the dual role of being both the fuel and the match.

Activation of brown adipose tissue is mediated via both α- and β-adrenergic receptors. Noradrenaline action at α-receptors causes an initial transient temperature decrease in BAT due to vasoconstriction of the blood vessels supplying the tissue (Flaim et al, 1977). Subsequently, BAT temperature rises (Flaim et al, 1977) and blood flow is increased as a result of stimulated metabolism by noradrenaline (Foster, 1986).

In intact BAT cells, alpha$_1$-adrenoceptor-mediated events can account for 20% of the noradrenaline-stimulated respiration in hamster brown adipocytes (Mohell et al, 1983b; Schimmel et al, 1983). This component does not involve adenylate cyclase but rather an ATPase type of control mechanism (Mohell et al, 1987). The ATPase nature of the α_1 mechanism is likely required for ion pumping in order to restore ionic gradients disrupted by ion channel opening in response to stimulation (Schneider-Picard et al, 1985; Néchad, 1986). Noradrenaline binding to α_1-receptors leads to a transient depolarization of the plasma membrane followed by β-receptor mediated hyperpolarization and subsequent prolonged depolarization (Schneider-Picard et al, 1985; Girardier and Seydoux, 1986). Brown adipocytes are electrically coupled by tight

junctions (Schneider-Picard et al, 1984; Girardier and Seydoux, 1986) and this is likely the means by which α_1-mediated depolarization is propagated throughout the tissue. Electrical coupling could also be responsible for the phenomenon of α_1-stimulated potentiation of thermogenesis in rat brown adipose tissue in response to β-adrenergic stimulation observed in vivo (Ma and Foster, 1984) but not in vitro (Mohell et al, 1983a; Schimmel et al, 1983).

Alpha$_1$ activation of isolated hamster brown adipocytes causes rapid mobilization of calcium and increased turnover of phosphatidyl inositol bisphosphate (Connolly et al, 1984; Nanberg and Putney, 1986; Schimmel et al, 1983, 1986) which has been implicated in cellular proliferation (Berridge, 1984). There may be a similar role for α_1-adrenergic stimulation in brown adipose tissue.

Alpha$_1$ receptors have been identified in hamster BAT membranes and isolated cells (Mohell et al, 1983a, b). The number of alpha receptors is increased in BAT from cold acclimated rats and hamsters (Raasmaja et al, 1985). In addition to α_1-receptors in BAT α_2-receptors have been identified in rat (Sundin and Fain, 1983) but not in hamster BAT (McMahon and Schimmel, 1982). Alpha$_2$ receptors are inhibitory to β-receptor mediated responses (Johansson, 1984).

Thermogenic Mechanism:

In thermogenically active brown adipocytes, mitochondrial oxidative processes are predominant, therefore tissue respiration should reflect the rate of heat production. In that case, thermogenesis will be controlled by the activity of the rate-limiting step in the overall sequence of oxidative reactions. Normally

mitochondrial respiration is tightly controlled. That is, there is obligatory coupling between electron flow down the respiratory chain and proton extrusion across the inner membrane on one hand, and proton re-entry through the ATP synthase and ATP production on the other (see Nicholls and Locke, 1984; Nicholls et al, 1986). ATP is continually removed for cytosolic ATP-requiring reactions while the electrochemical potential difference across the inner mitochondrial membrane is constantly replenished by the respiratory electron transfer chains. Since the inner membrane is impermeable to protons other than through the ATP synthase, respiratory control is automatic. When the demand for cellular ATP rises the proton flux increases and respiration is elevated (see Nicholls and Locke, 1984; Nicholls, 1982). In the case of brown adipose tissue, respiration rates can be extremely high. How then can respiration be increased beyond the constraints of normal cellular ATP turnover? The mechanism which enables brown adipose tissue to sustain such high respiratory rates would have to fulfil several criteria: 1) The uncoupling mechanism or signal should be unique to brown adipose tissue. 2) The mechanism should be necessary for thermogenesis, therefore present in active BAT mitochondria. 3) The capacity of the thermogenic pathway should be able to account for observed in vivo respiration rates. 4) There should be a demonstratable structural basis for uncoupling. 5) Uncoupling should be reversible and not damage the mitochondria. 6) There should exist a plausible messenger for acute regulation of thermogenesis (see Nicholls and Locke, 1984).

Mitochondria isolated from brown adipose tissue possess an

unusually high proton permeability but relatively low amount and activity of ATP synthase (Cannon and Vogel, 1977; Grav et al, 1970; Nicholls and Lindberg, 1973; Nicholls, 1974). Thus respiration can be stimulated maximally, unconstrained by flux through the ATP synthase complex. Respiratory control can be restored only if albumin is added to remove endogenous fatty acids and millimolar concentrations of ATP or GTP are also present (see Nicholls and Locke, 1984). Purine nucleotide binding to brown adipose tissue mitochondria plays a key role in regulation of thermogenesis. Purine nucleotides (di- and triphosphates) reduce membrane proton conductance by binding to the outer surface of the inner membrane in a non-covalent fashion (Nicholls, 1976). The nucleotide binding site is identical to the inner membrane component that regulates proton conductance and is unique to brown adipose tissue (Nicholls, 1976; Cannon et al, 1982). Furthermore, purine nucleotide binding affinity and pH dependency correspond well with inhibition of proton conductance (Nicholls, 1976; Bieber et al, 1975; Klingenberg, 1988). Photoaffinity labelling of a purine nucleotide analogue plus incubation with atractyloside to inhibit the adenine nucleotide translocator allowed identification of a 32 kD M_r band in brown adipose tissue mitochondrial preparations (Heaton et al, 1978). The relative amount of a 32 kD M_r polypeptide from rat brown adipose tissue mitochondria was observed to increase in cold acclimated animals (Ricquier and Kader, 1976). This suggested that changes in the abundance of this uncoupling protein (or thermogenin) as it became known, might be responsible for some of the changes during cold acclimation.

Brown adipose tissue uncoupling protein (UCP) has been purified (Ricquier et al, 1979; Lin and Klingenberg, 1980) and studies indicate that it behaves like a dimer in the inner membrane (Lin et al, 1980; Lin and Klingenberg, 1982; Klingenberg, 1984) and is related to the mitochondrial ADP/ATP carrier (Aquila et al, 1985).

Measurement:

Thermogenic activity and capacity must be quantified in order to estimate differences between basal and stimulated states, and to determine whether a particular treatment has had an effect on the thermogenic function of BAT. Blood flow measurements in brown adipose tissue are the only quantitative means by which thermogenesis can be determined, as blood flow is directly related to thermogenic state (see Foster and Frydman, 1978a, b, 1979; Foster, 1984a, 1986). However, this method is technically demanding and time consuming, and therefore not routinely used.

Production of specific antisera has led to the development of immunoassays for uncoupling protein (see Ricquier and Bouillaud, 1986; Ridley et al, 1986). The concentration of uncoupling protein will determine thermogenic capacity (see Rial and Nicholls, 1984). Another way in which thermogenesis can be estimated is by measuring specific binding of purine nucleotides to isolated BAT mitochondria. GDP is most often used because it binds with high affinity and is not translocated into the matrix (Nicholls, 1976), although ATP is the probable physiological regulator (Nicholls and Locke, 1984). GDP-binding is an index of BAT thermogenic activity but it does not measure thermogenic capacity unless the amount of UCP is also known. GDP-

binding can increase unaccompanied by changes in amount of UCP. This dissociation between level of binding and amount of UCP is referred to as unmasking/masking of binding sites. The immediate increase in GDP-binding observable 1-4 h after exposing rats to cold reflects unmasking of binding sites already present (Desautels et al, 1978), and returning cold acclimated rats to a warm temperature results in a rapid fall in GDP-binding without a change in UCP (Swick and Swick, 1986; Trayhurn et al, 1988; Peachy et al, 1988). The hamster differs from rats and mice in that it retains a relatively high level of GDP-binding even in the warm adapted state (Locke et al, 1982; Himms-Hagen and Gwilliam, 1980; Trayhurn et al, 1983) but can increase binding in response to cold although the changes are smaller than those observed in rats or mice (Trayhurn et al, 1983).

3. GROWTH OF BROWN ADIPOSE TISSUE:

Measurement:

Growth of brown adipose tissue can be quantified in several ways. Weighing the tissue is not sufficient as some treatments (eg. thyroid hormone administration) stimulate lipid accumulation in the absence of tissue activation and growth (Sundin, 1981; Triandafillou et al, 1982). A better index of tissue growth is protein content which is often, but not always, accompanied by changes in BAT DNA content. DNA content is a measure of hyperplasia or an increase in cellularity, but since only 40% of cells are brown adipocytes, DNA does not measure true tissue size. Oxidative enzymes reflect mitochondrial content and thus metabolic mass and oxidative capacity of the tissue. One such commonly measured enzyme is cytochrome oxidase. Therefore protein content and

cytochrome oxidase activity are better measures of size because brown adipocytes are larger and contain most of the tissue's mitochondria. The total level of uncoupling protein in brown adipose tissue can be measured by a variety of immunoassays and reflects specific changes that occur in brown fat mitochondria during growth.

Developmental Growth:

In the rat, brown adipose tissue develops late in fetal life, from about 6 days before birth (Barnard and Skala, 1970). At this time the tissue is loosely organized in areas destined to become BAT depots and cells are small and undifferentiated. Ultrastructurally, brown adipocytes arise from mesenchymal cells associated with the capillary endothelium. Between 6 days ante partum and a period up to 2 weeks after birth the most intensive developmental activity takes place. Mitochondria proliferate and increase in size, and the number and packing of cristae also increases. Within a few hours of birth mRNA levels for UCP rise sharply (Obregon et al, 1987). Lipid droplet size and number also increase, except during the period around birth, when they are depleted when the tissue is activated. From 2 weeks until almost a month of age, development plateaus; after 30 d of age the tissue begins to involute and respiratory activity declines. This stage occurs as thermoregulatory mechanisms are maturing in the rat. At the end of differentiation brown fat is functionally innervated, which indicates that sympathetic innervation occurs at the same time as differentiation (Barnard and Skala, 1970; Néchad, 1986; Nnodim, 1987).

In contrast to the rat, hamster brown adipose tissue differentiation occurs postnatally instead of prenatally (Néchad and

Barnard, 1979). In addition, the sequence of morphological changes differs from the pattern exemplified by the rat. Adipose tissue precursor cells of unknown origin appear in the interscapular area at about 12 d of gestation (Smalley, 1970). Shortly after birth the precursor cells begin to accumulate lipid as a single droplet and take on the appearance of unilocular white fat cells. At 3-4 d *post partum* a second cell type appears in close association with capillary endothelial cells, from which it appears to arise. This new cell type, the pericyte, will ultimately give rise to a mature multilocular brown adipocyte in the generally accepted manner described for the rat. In addition to this pathway another, unique to the hamster, also exists. The unilocular adipocytes that had developed perinatally are transformed into multilocular adipocytes indistinguishable from those formed from pericytes (Néchad and Barnard, 1979). During the peak of brown adipocyte transformation noradrenaline content rises rapidly, indicating development of NA storage sites in the tissue. The innervation of the brown fat parenchyma parallels development of multilocularity but innervation of the blood vessels develops somewhat later, during tissue differentiation (Néchad and Barnard, 1979). Sympathetic innervation of hamster BAT is involved in regulation of differentiation but is not obligatory for it to occur (Néchad and Olson, 1983). Thermogenic activity of developing hamster BAT has also been studied using GDP-binding (Sundin *et al*, 1981). Binding was low at 12 d after birth but increased almost four fold, peaking at 20 d of age. A slow decline to adult values followed and this pattern corresponded well to tissue oxygen consumption and ability to maintain

body temperature (Sundin et al, 1981). The age at which UCP appears has not been established for the hamster.

Cold-Induced Growth:

Exposure of a warm-acclimated rat to cold induces a number of acute changes in brown adipose tissue resulting in increased heat production. Continued exposure to cold for several weeks results in cold acclimation in connection with gradual adaptive changes in brown adipose tissue.

Acute exposure of rats to 4°C increases oxygen consumption (Foster and Frydman, 1979), brown adipose tissue temperature (Szelenyi, 1968), and tissue blood flow (Foster and Frydman, 1979). Accompanying these changes are depletion of lipid droplets (Cameron and Smith, 1964; Géloen et al, 1988) and increased brown fat noradrenaline turnover (Young et al, 1982), consistent with sympathetic-mediated stimulation of lipolysis and thermogenesis.

The inner mitochondrial membrane rearranges into a more parallel configuration (Desautels and Himms-Hagen 1980). Within one hour of cold exposure GDP-binding to isolated BAT mitochondria increases through unmasking of purine nucleotide binding sites (Desautels et al, 1978). Acute cold exposure stimulates uncoupling protein mRNA synthesis within minutes which peaks within 6-24 h and is followed by increased UCP synthesis (Ricquier et al, 1983; Ricquier et al, 1986). Cold exposure increases the activity of brown adipose tissue thyroxine 5'-deiodinase markedly through the action of noradrenaline on α_1- and possibly also β-adrenergic receptors on the brown adipocyte plasma membrane (Silva and Larsen, 1983, 1985, 1986). The increase is due to

a specific increase in mRNA synthesis and consequent synthesis of the enzyme (Silva and Larsen, 1986; Jones et al, 1986; Obregon et al, 1987a). T_3 generated by deiodination of T_4 in BAT is thought to facilitate β-receptor mediated UCP mRNA synthesis and therefore play a role in optimizing the response of BAT to cold (see Silva and Larsen, 1985; Bianco and Silva, 1987a, b).

Within 2-4 h of cold exposure, lipoprotein lipase on the surface of brown adipocytes is activated in response to acute cold exposure (Blanchette-Mackie and Scow, 1983; Carneheim et al, 1984; Carneheim et al, 1988) and permits BAT to take up fatty acids from blood lipoproteins in addition to those derived from triglyceride breakdown within the brown fat cell.

Exposure of rats to cold for 48 h stimulates proliferation of interstitial cells and preadipocytes (Cameron and Smith, 1964; Bukowiecki et al, 1982; Bukowiecki et al, 1986) with attendant growth of vasculature and sympathetic innervation. Work with cultured brown fat cells has suggested that noradrenaline promotes but does not initiate the differentiation of precursor cells and preadipocytes but can stimulate mitochondriogenesis (Néchad et al, 1987). Mediation of the hyperplastic response to cold is via sympathetic stimulation and noradrenaline release as noradrenaline administration can mimic the effect of cold on BAT protein and DNA in rats (Mory et al, 1984; Ricquier et al, 1984; Bouillaud et al, 1984; Géloen et al, 1988).

The effects of acute cold exposure on hamster brown adipose tissue have not been as well characterized as they have been in the rat. Rapid changes in BAT function do occur during arousal from hibernation.

Hamsters in hibernation have reduced BAT mitochondrial GDP-binding compared to cold acclimated nonhibernating hamsters but within 80-90 minutes of initiation of arousal GDP-binding doubles, unaccompanied by changes in protein content. This indicates that purine nucleotide binding sites become unmasked or that unmasked sites become available for binding (Horwitz et al, 1985). In addition, during arousal there is a decrease in lipid content of brown adipose tissue which if combusted, can deliver 90-100% of the heat required for arousal (Nedergaard and Cannon, 1984). During the nonshivering phase of arousal the contribution of brown adipose tissue to total heat production is similar to that in the newborn or cold acclimated animal (Nedergaard and Cannon, 1984).

Acute exposure of hamsters to cold for 17 h results in a decrease in BAT weight, but increases in protein content and mitochondrial GDP-binding (Triandafillou et al, 1984a). Acclimating hamsters to cold for longer periods of time (10 d to 3 mo) induces growth of brown fat measured by increases in protein and DNA contents (Triandafillou et al, 1984a; Himms-Hagen and Gwilliam, 1980; Desautels and Dulos, 1986), increased content of mitochondria (Sundin et al, 1986), and elevated total UCP content (Desautels and Dulos, 1986; Sundin et al, 1986). The mechanism and mediator(s) of hamster brown adipocyte proliferation and BAT growth are currently unknown.

Diet-Induced Growth:

Rats fed a cafeteria diet exhibit hypertrophied brown adipose tissue, increased thermogenic capacity, and greater blood flow to brown fat following NA infusion (see section on diet-induced thermogenesis;

Rothwell and Stock, 1979, 1980, 1981, 1982, 1984; Brooks et al, 1980, 1982). BAT noradrenaline turnover is also stimulated by a palatable diet in rats (Young et al, 1982). The mechanism of hyperplasia is unclear but is likely to occur in a somewhat similar manner to cold stimulated growth (Bukowiecki et al, 1982). During the initial phase of the response to a palatable diet, BAT is increased solely by adipocyte enlargement but with increasing time on the diet brown adipocyte numbers rise, reflected as increases in protein and DNA contents (Tulp et al, 1980). There are differences however, between cold-induced and diet-induced growth of BAT in rats. For instance, the increase in UCP mRNA and UCP levels are much less in cafeteria fed rats than in those adapted to cold (Falcou et al, 1985). Brown adipose tissue lipoprotein lipase activity is not stimulated by diet but is markedly elevated by cold (Bertin et al, 1985; Radomski and Orme, 1971; Carneheim et al, 1984). In addition, there is no response of BAT thyroxine 5'-deiodinase to cafeteria diet whereas enzyme activity is stimulated by cold (Kopecky et al, 1986a; Eley and Himms-Hagen, 1988, in press; Kates et al, 1986). Taken together, these results indicate that other factors are involved in mediating trophic responses of BAT to diet, which may include insulin and/or some signal derived from diet composition.

In contrast to rats, hamsters do not generally overeat when offered a palatable high fat diet but they become obese (Wade, 1982, 1983; Wade and Bartness, 1983, 1984; Triandafillou et al, 1984a, b; Hamilton et al, 1986). Their brown adipose tissue nonetheless grows in response to the diet but thermogenesis may or may not be activated

(Triandafillou et al, 1984a, b; Schimmel and McCarthy, 1985; Hamilton et al, 1986). Sympathetic nervous system activity in brown adipose tissue of high fat diet-fed hamsters is dissociated from growth but thermogenic capacity is increased as measured by noradrenaline-stimulated oxygen consumption (Hamilton et al, 1986; Wade, 1982). In other words, BAT growth is not necessarily coupled to thermogenesis or increased sympathetic activity. Neither the mechanism of diet-induced brown adipose tissue growth nor its mediator(s) are known in the hamster.

Short Photoperiod-Induced Growth:

Many small animals are sensitive to changes in photoperiod, including two species of hamster, the Syrian golden hamster (Mesocricetus auratus) and the Siberian or Djungarian hamster (Phodopus sungorus). Exposure of both types of hamster to less than 12.5 hours of light per day induces growth of brown adipose tissue and increases thermogenic capacity (Hoffman et al, 1965; Reiter, 1975; Rafael et al, 1981; Heldmaier et al, 1981). Pinealectomy prevents photoperiod-induced BAT hypertrophy (Reiter, 1975) and administration of the pineal hormone melatonin can mimic the effects of short photoperiod on brown adipose tissue (Heldmaier and Hoffman, 1974). Sympathetic nervous system activity in BAT of Syrian hamsters (the most studied of the two species) is either unchanged (Viswanathan et al, 1986; McElroy and Wade, 1986) or suppressed by short photoperiod (Viswanathan et al, 1985). Thermogenic activation as measured by GDP-binding was unchanged by short photoperiod in one study (Triandafillou et al, 1984b) but stimulated in another (McElroy and Wade, 1986).

Short photoperiod has no effect on BAT growth in rats and cold-induced growth of the tissue is not affected by pinealectomy (Kott and Horwitz, 1983).

4. ATROPHY OF BROWN ADIPOSE TISSUE:

Brown adipose tissue can not only grow when stimulated but it can also atrophy when the appropriate stimuli are absent, reduced, or overridden by other signals.

Denervation:

Surgical denervation of interscapular BAT in rats and mice leads to decreases in protein and mitochondrial uncoupling protein but no loss of tissue cellularity (DNA) (Desautels et al, 1986); in rats cytochrome oxidase activity is also reduced (Bartness et al, 1986). Park and Himms-Hagen (1988, in press) found that denervation decreases UCP and deiodinase activity as well as protein content of BAT in rats.

Cutting the nerve supply to hamster interscapular BAT decreases protein and uncoupling protein contents, and GDP-binding in cold-exposed animals but does not alter DNA content of the tissue (Triandafillou et al, 1984b; Desautels and Dulos, 1988). In all cases, it seems that an intact nerve supply to brown adipose tissue is required in order to maintain the tissue in a functional state.

Thermoneutrality:

Mice maintained at 33°C have atrophied brown adipose tissue characterized by decreased protein content and cellularity (DNA content), reduced amount of UCP, and lower GDP-binding and cytochrome oxidase activity (Desautels et al, 1986; Ashwell et al, 1983).

Returning cold acclimated rats to a warm environment reduces the

stimulated GDP-binding by 50% within the first 2 d, without changing the UCP concentration (Peachey et al, 1988). However, uncoupling protein mRNA levels decreased after only 8 h of deacclimation to reach a minimum after 1-2 d at 28°C (Reichling et al, 1987).

Brown adipose tissue from hamsters kept at 35°C is lighter in colour, has a reduced protein content and consists mainly of unilocular adipocytes. In addition, the activity of several mitochondrial enzymes is decreased (Rabi and Cassuto, 1976). GDP-binding and UCP are reduced by housing hamsters at 30°C (Trayhurn et al, 1983) and there is a lower sympathetic activity in spleen and heart of heat-acclimated hamsters (Jones and Musacchia, 1976).

Fasting:

Depriving mice of food leads to losses of protein and cytochrome oxidase activity from BAT which may (Trayhurn and Jennings, 1986, 1988) or may not (Desautels et al, 1985) be accompanied by selective loss of UCP. Fasting also reduces both GDP-binding and thermogenic capacity (Trayhurn and Jennings, 1988). Cardiac but not brown adipose tissue noradrenaline turnover rate is decreased by fasting mice for 24 h (Young and Landsberg, 1980; Knehans and Romsos, 1983).

Food restricting or fasting rats results in decreased BAT mass and protein, and lower thermogenic activity (Rothwell and Stock, 1982; Rothwell et al, 1984). Noradrenaline turnover in BAT is also suppressed in the fasted rat (Young et al, 1982; Young and Landsberg, 1977; Rothwell and Stock, 1982; Rothwell et al, 1984).

Depriving hamsters completely of food for 36-48 h at 21°C causes loss of tissue cellularity in addition to losses in mass, protein, and

uncoupling protein; 30% food restriction does not cause DNA loss (Desautels et al, 1986). The extent of protein and DNA depletion depends on the original tissue mass and severity of food deprivation (Desautels et al, 1986). However, Levin and Trayhurn (1987) found thermogenic capacity to be impaired in fasted hamsters housed at 24°C, as a consequence of BAT mitochondrial loss rather than by selective loss of UCP.

Exercise:

Almost all the work on exercise and brown adipose tissue has been done in the rat.

Exercising rats stimulates the sympathetic nervous system (LeBlanc et al, 1982) but despite this, exercise trained warm acclimated rats exhibit no changes in BAT mitochondrial GDP-binding, thermogenic response to noradrenaline, or NA-induced changes in blood flow to BAT (see Himms-Hagen, 1989). Exercise training in the cold reduces or negates cold-induced BAT hyperplasia (Arnold and Richard, 1987a), and exercise can even attenuate thermogenic and trophic responses to a high fat diet (Arnold and Richard, 1987b). Thus during running metabolic heat generated by exercise can replace shivering thermogenesis in 24°C acclimated rats during acute exposure to 4°C, and nonshivering thermogenesis in BAT of cold acclimated rats (Arnold et al, 1986).

Voluntary exercise increases growth and weight gain in hamsters (Borer, 1974) but studies of the effects of exercise on hamster BAT and thermogenesis are lacking.

Pregnancy and Lactation:

During pregnancy there are large weight gains and deposition of

fat which is later mobilized during lactation.

Pregnant rats and mice accumulate lipid in brown adipose tissue but the tissue is relatively unchanged, with no alterations in cytochrome oxidase or protein content during much of the pregnancy. Late in the term BAT protein content is reduced but it is during lactation the most of the changes in brown adipose tissue occur (Villarroya et al, 1986; Trayhurn et al, 1982; Trayhurn and Richard, 1985). BAT from lactating rats and mice is atrophied and inactive, likely as a result of reduced sympathetic nervous activity (Villarroya et al, 1987; Trayhurn et al, 1982; Trayhurn and Wusteman, 1987) and heat production due to milk synthesis in the active mammary gland, which would suppress thermogenesis elsewhere (Trayhurn and Richard, 1985; see Himms-Hagen, 1989).

In contrast to rats, BAT in both commonly studied hamster species (Syrian and Siberian) atrophies during pregnancy and is not further altered during lactation (Wade et al, 1986; Schneider and Wade, 1987). Atrophy appears to result from mitochondrial loss with no selective loss of UCP. Sympathetic nervous system activity is not changed by pregnancy (Trayhurn and Wusteman, 1987). The cause of pregnancy-induced brown adipose tissue atrophy in the hamster is not known but does not seem to directly involve the sympathetic nervous system.

5. CONTROL OF BROWN ADIPOSE TISSUE:

Neural Control:

Central Regulation:

Nonshivering thermogenesis is regulated by peripheral (skin) and central (preoptic area of hypothalamus) thermoreceptors which relay

information to integrative centres in paraventricular, ventromedial, and other nuclei of the hypothalamus (Boulant and Dean, 1986; Gordon and Heath, 1986; Brück and Zeisberger, 1987). The neural circuits involved include the subcoeruleus region and raphé nuclei (Gordon and Heath, 1986) but the pathways by which the hypothalamus stimulates thermogenesis in the periphery are complex and relatively unknown. They have been partially elucidated in the rat and guinea pig but may vary with species and are still highly speculative (Brück and Zeisberger, 1987). In the widely studied guinea pig, monoaminergic systems are involved in modulation of adaptive changes in thermoregulation. These brain circuits receive information from several sensory systems and deliver additional neuromodulatory signals to integrative centres. Serotonin and noradrenaline act as antagonists; the systems are interconnected and influence each other as well as being regulated by neurohormonal feedback from the periphery (Brück and Zeisberger, 1987). Thus, monoaminergic pathways influence both thermosensitive and thermointegrative hypothalamic neurons. Similar circuitry could also be involved in mediating behavioral and social thermoregulation (see section 1, behavioral thermoregulation). Other pathways that influence BAT thermogenesis may include GABAergic and corticotropin-releasing factor (CRF)-containing neurons (see Himms-Hagen, 1989).

Central control of diet-induced thermogenesis is even less well understood (see Rothwell and Stock, 1982a). The ventromedial hypothalamus (VMH) is an important regulatory centre as recognition of signals generated by diet composition and food ingestion seems to occur

there (see Himms-Hagen, 1989). VMH lesions in the rat impair DIT but not NST (Hogan et al, 1982; see Himms-Hagen, 1989).

VMH lesions in hamsters produce hyperphagia through greater meal size, and increase body length and fat content (Rowland et al, 1986). When hamsters lesioned in the VMH or paraventricular nucleus (PVN) are offered a palatable diet they either do (Marks and Miller, 1972) or do not (Rowland et al, 1986) show a preference for the high fat diet. These contradictory results may have arisen from differences in lesion placement or in the diet offered, as hamsters seem to have a preference for a high fat diet and this is not likely to be altered by the lesion. The effects of hypothalamic lesions on hamster brown adipose tissue have not been reported.

Peripheral Regulation:

The sympathetic innervation of brown adipose tissue is the primary if not only mediator of the acute thermogenic response to cold or diet in the rat, and the major mediator of trophic responses to chronic stimulation (Néchad, 1986; Girardier and Seydoux, 1986; Ricquier and Bouillaud, 1986). Denervation entirely prevents the cold induced increase in blood flow to BAT (Foster et al, 1982) and almost entirely prevents the increase in GDP-binding caused by acute cold exposure (Park and Himms-Hagen, 1988 in press). Nerve stimulation mimics the effects of cold exposure (Flaim et al, 1976; Girardier and Seydoux, 1986) and it is generally assumed that noradrenaline is the mediator, although neuropeptide Y (NPY) is present in BAT vascular innervation but not in parenchymal fibres (Cannon et al, 1986).

While noradrenaline is generally accepted to be the mediator of

responses to acute cold exposure in hamsters (Triandafillou et al, 1984a) the picture becomes more complicated when examining trophic responses. Chronic administration of noradrenaline to hamsters does not promote growth of BAT (Triandafillou et al, 1984a; Desautels and Dulos, 1988) although it does increase GDP-binding (Desautels and Dulos, 1988). Denervation of hamster BAT produces atrophy with decreases in UCP and GDP-binding, and prevents the cold-induced increase in GDP-binding (Desautels and Dulos, 1988) but has little effect on DNA or mitochondrial content (Triandafillou et al, 1984a; Desautels and Dulos, 1988). This has led to the suggestion that factors other than or in addition to noradrenaline mediate trophic responses in hamster BAT.

Hormonal Control:

Brown adipose tissue function and growth are influenced by a wide variety of hormones. Most hormonal studies on brown adipose tissue have been performed on rats with little comparative work on hamsters. Therefore, for the purposes of this thesis, these studies will be discussed in a general manner only.

Glucocorticoids have a largely suppressive effect on brown adipose tissue, exerted indirectly by a central influence on the sympathetic nervous system (see Himms-Hagen, 1989).

Insulin exerts both direct and indirect effects and has a variable trophic influence on BAT (Himms-Hagen, 1989). Glucose entry into brown adipocytes is facilitated by insulin (Smith et al, 1986; Vallerand et al, 1987; Greco-Perotto et al, 1987) and lipogenesis is promoted via effects on glucose uptake and on key lipogenic enzymes (Saggerson et

al, 1988; see Himms-Hagen, 1989). Insulin may also influence diet-induced thermogenesis via the hypothalamus and responses to dietary carbohydrate (see Rothwell and Stock 1988; Himms-Hagen, 1989).

Glucagon also has a direct action on rat brown adipose tissue. It is implicated in increased thermogenesis and growth in response to cold (Kuroshima et al, 1984), and chronic administration produces BAT hyperplasia similar to that induced by cold (Billington et al, 1987).

Pituitary hormones do not appear to be directly required for growth of BAT in response to cold, as hypophysectomy does not impair trophic responses in rats during cold acclimation (Fellenz et al, 1982). However, there may be other roles for pituitary hormones in control of BAT. Hypophysectomized rats housed at 26-28°C have hypertrophied and thermogenically active BAT, which is thought to result from greater cold stress experienced by these animals at even such a warm temperature (Fellenz et al, 1982; Laury et al, 1984). Others have suggested that corticotropin-releasing factor (CRF) secretion in the hypothalamus is elevated as a consequence of removal of the inhibitory influence of glucocorticoids (Liposits et al, 1987) and stimulates sympathetic activity in BAT (LeFeuvre et al, 1987; Holt et al, 1988).

Sex hormones do not seem to have much of an effect on brown adipose tissue. Excess progesterone and prolactin have slight suppressive influences on GDP-binding (see Himms-Hagen, 1989). Castration of Syrian hamsters has no effect on BAT (Kott et al, 1986) but in male rats there is some increase in protein (Rothwell and Stock, 1986).

The effects of thyroid hormones and melatonin on hamster brown adipose tissue have been studied in more detail and therefore will be discussed at greater length below.

Thyroid Hormones:

During cold exposure both metabolic rate and serum T_3 levels rise (Bernal and Escobar del Rey, 1975; Balsam and Sexton, 1975; Van Hardeveld et al, 1979), and because BAT is also thermogenic interest has grown in the role of thyroid hormones on brown adipose tissue function and growth.

Thyroid hormones are synthesized by and released into the circulation from the thyroid gland. Hormone synthesis and secretion are regulated by both extrathyroidal and intrathyroidal mechanisms with control by pituitary thyrotropin (TSH) being the major one (see Greenspan and Rapoport, 1986). TSH is produced and secreted by the thyrotrophs in the anterior pituitary and controlled by both stimulatory (TRH) and inhibitory (somatostatin) influences from the hypothalamus, and modulated by thyroid hormone feedback inhibition (see Spira and Gordon, 1986; Greenspan and Rapoport, 1986). Hypothalamic thyrotropin-releasing hormone (TRH) is the signal for TSH release from the pituitary. It is secreted into the hypothalamic-hypophyseal portal system, and in the pituitary binds to specific receptors on the thyrotrophs to stimulate TSH synthesis and release.

The principal hormones secreted from the thyroid gland, thyroxine (T_4) and triiodothyronine (T_3) are transported in the blood largely bound to protein but it is the unbound or "free" hormones that are biologically active (see Greenspan and Rapoport, 1986). T_4 is the

major secretory product of the thyroid gland but almost all biological effects are exerted by T_3 rather than by T_4. Most of the T_3 in circulation results from monodeiodination of T_4 in the periphery by the enzyme thyroxine 5'-deiodinase. There are two types of 5'-monodeiodinase, one in liver and kidney (type I) and another in BAT, pituitary, cortex, and pineal (type II) (Visser et al, 1982; Leonard et al, 1983; Silva et al, 1987; Murakami et al, 1988; Guerrero et al, 1988; Puig-Domingo et al, 1988). Specific binding sites for thyroid hormones have been identified in cell membranes, cytoplasm, nucleus, and mitochondrial inner membrane (Barsano and DeGroot, 1983; Hoffenberg and Ramsden, 1983). Most tissues are thyroid hormone sensitive with the exceptions of spleen, testis, and adult rat brain (Barsano and DeGroot, 1983). Thyroid hormones are involved in growth and development as well as in the control of metabolism but it is this latter function that will be considered here.

Thyroid hormones not only have a long term effect by influencing metabolic rate as previously discussed, but they can also have more immediate effects by modifying sympathetic activity and potentiating the action of catecholamines. In turn, noradrenaline can modulate thyroid activity and peripheral deiodination (see Himms-Hagen, 1983).

Hypothyroid rats have a reduced rate of β-adrenergic-stimulated oxygen consumption and are unable to survive in the cold unless provided with a maintenance dose of T_4 (Fregly et al, 1979; Sellers and You, 1950; Sellers et al, 1974). Their brown adipose tissue is less responsive to noradrenaline or β-adrenergic agonist stimulation (Sundin et al, 1984; Woodward and Saggerson, 1986), and short term cold

exposure neither mobilizes lipid nor increases blood flow (Mory et al, 1981; Hemon et al, 1976; Kuroshima et al, 1967). While T_3 has no direct thermogenic effect on BAT, it does have a permissive effect on thermogenic responses to noradrenaline at both receptor and post receptor levels (Sundin et al, 1984). Chronic treatment of rats with T_4 increases the calorigenic response to noradrenaline and causes BAT hypertrophy but it is mostly due to lipid deposition rather than increased protein content (LeBlanc and Villemaire, 1970; Triandafillou et al, 1982). Thermogenesis in BAT is suppressed by T_4 treatment, as evidenced by reductions in GDP-binding (Triandafillou et al, 1982; Sundin, 1981) and BAT noradrenaline turnover (Knehans and Romsos, 1984). This results from increased basal metabolic rate obviating the requirement for extra regulatory heat production (Sundin, 1981).

The recent discovery of the enzyme thyroxine 5'-deiodinase in BAT has pointed to a greater role of thyroid hormones in the thermogenic responses of BAT to cold than the permissive role generally ascribed to them (Danforth and Burger, 1981; Himms-Hagen, 1983).

BAT deiodinase converts thyroxine (T_4) to thermogenically active triiodothyronine (T_3) and thus can provide the tissue with locally produced hormone as well as contributing to the systemic pool (Fernandez et al, 1987; Bianco and Silva, 1987a). Activation of deiodinase is believed to be via NA acting predominantly on α-adrenergic receptors (Silva and Larsen, 1983). The role of increased intracellular T_3 in BAT trophic responses is not entirely clear. High T_3 levels are correlated with increased mitochondrial UCP concentration and it has been suggested that T_3 might be involved in control of UCP

gene transcription stimulated by noradrenaline (Bianco and Silva, 1987a, b). Brown adipose tissue nuclear T_3 receptors require endogenously produced hormone for full saturation (Bianco and Silva, 1987c) and this may allow an optimum trophic response of BAT (Bianco and Silva, 1987a, b).

The role of thyroid hormones in responses to diet is less clear as cafeteria feeding of rats and mice stimulates BAT hypertrophy but not thyroxine deiodinase activity (Wu et al, 1987; Eley and Himms-Hagen, 1988, in press; Kates et al, 1986). On the other hand, cold increases both deiodinase activity and stimulates BAT growth by NA-mediated processes. This suggests that factors other than sympathetic nervous system activity must also be involved in control of BAT deiodinase.

Melatonin:

Most animals living in temperate climates are sensitive to seasonally changing environmental conditions and adjust their behavior and metabolism in order to maximize survival. Strategies may include migration to a more favourable climate for part of the year, hibernation, molting and colour change, and timing of reproduction to assure young are born in a favourable season. The major environmental cues are temperature and daylength or photoperiod. Changes in photoperiod are the most dependable markers of seasonal progression and the most widely used to regulate reproductive cycles (Goldman, 1983). Photosensitive species respond to changes in lighting by altering physiological functions and include the Syrian hamster, the Siberian hamster, and the white-footed mouse (Peromyscus leucopus) among many others.

The pineal gland is the transducer of information from the environment to the brain where it can be integrated and the appropriate neuroendocrine responses generated. Photic information from the retina reaches the pineal gland in a rather circuitous pathway involving hypothalamic tracts projecting to the superior cervical ganglion (SCG) of the sympathetic chain, and by post-SCG nerves to the pineal (Ralph et al, 1977). The suprachiasmatic nucleus of the anterior hypothalamus (SCN) contains a major circadian pacemaker, and light acting through the eyes synchronizes the SCN with environmental lighting by inhibiting transmission of signals from the SCN to the pineal (Goldman, 1983). Noradrenaline released from neurons originating in the SCG acts at pinealocyte α_1- and β_1-adrenergic receptors to stimulate synthesis of melatonin from tryptophan and its release from the pineal (Cardinali and Vacas, 1987). Melatonin production is much greater during the dark phase of the daily lighting cycle in both nocturnal and diurnal animals, in keeping with its role as mediator of photoperiodic information. The site of melatonin action is believed to be within the hypothalamus, but sites in the SCN, median eminence, and pituitary have also been identified, depending on the probe used (Reiter et al, 1981; Vanecek et al, 1987; Anton-Tay et al, 1988; Duncan et al, 1988; Hastings et al, 1988).

In such long day breeders as Syrian and Siberian hamsters, and the white-footed mouse, photoperiod regulates reproduction. The Syrian hamster is the most widely studied model for short photoperiod-induced gonadal regression. Less than 12.5 h light per day or melatonin injection during the latter portion of the light phase (Gaston and

Menaker, 1967; Steger et al, 1985; Tamarkin et al, 1977; Tamarkin et al, 1976) produces gonadal atrophy, then several months later the animals become photorefractory to short photoperiod and the gonads "spontaneously" recrudesce in preparation for the spring breeding season (Nelson and Zucker, 1987).

Syrian and Siberian hamsters differ in energy balance and thermoregulatory strategies. The Siberian hamster, which does not hibernate, loses weight in response to short photoperiod to reduce energy requirements for thermoregulation, while the Syrian hamster, a hibernator, gains weight mainly by fat deposition (Bartness and Wade, 1985).

The pineal gland also contributes to thermoregulation in several ways. It is involved in 1) regulation of body temperature level, 2) control of diurnal variations of body temperature, and 3) control of thermoregulatory effectors and thermogenesis (Heldmaier and Lynch, 1986). Only the role in thermogenesis will be discussed here.

Short photoperiods or appropriately timed melatonin injections increase capacity for nonshivering thermogenesis in both Siberian and Syrian hamsters (Steinlechner and Heldmaier, 1981; Heldmaier et al, 1981; Wade and Bartness, 1984) and cause BAT hypertrophy (Hoffman et al, 1965; Heldmaier et al, 1981; Heldmaier and Hoffmann, 1974; Reiter, 1975; Wade and Bartness, 1984; Triandafillou et al, 1984b; McElroy and Wade, 1986; Heldmaier et al, 1985). Melatonin does not stimulate growth of brown adipose tissue in the rat (Kott and Horwitz, 1983).

Pinealectomy reduces or prevents hibernation, and in the Siberian hamster, melatonin may be necessary for entry into shallow daily torpor

(see Heldmaier and Lynch, 1986). In the Syrian hamster melatonin levels are at a minimum during hibernation and increase slowly during arousal. It has been suggested that in this species melatonin may be involved in control of hibernation bout length and periodic arousal (see Heldmaier and Lynch, 1986). It is not clear whether adjustments in reproduction and thermoregulation in response to photoperiod are independently controlled (see Heldmaier and Lynch, 1986).

Recently, melatonin treatment was found to stimulate BAT deiodinase activity in the Richardson's ground squirrel (Spermophilus richardsonii), pointing to another regulatory role of this indole in brown adipose tissue growth and function (Puig-Domingo et al, 1988). This has not been studied in hamsters.

6. MYOPATHIC HAMSTERS:

Hamsters with inherited muscular dystrophy have been used as a model for studying various types of muscular dystrophy, including Duchenne muscular dystrophy, a progressive, lethal muscle wasting disease inherited as an X-linked trait (Hunter et al, 1984). In the hamster model the disease is inherited in an autosomal recessive manner, having arisen spontaneously during systematic inbreeding of Syrian golden hamsters (Homburger et al, 1962; Homburger, 1979). The BIO 14.6 line, derived from the original mutant line is the most widely used. These animals live approximately a year. The strain of hamsters used in the research described in this thesis, CHF 146, is a line developed at Canadian Hybrid Farms, Nova Scotia, and is derived from the BIO 14.6 line (Hunter et al, 1984). Other lines have been developed, such as the UM-X7.1, from the University of Montreal (Hunter

et al, 1984). Each line differs slightly in the course of the disease and lifespan of the animal.

Hamster muscular dystrophy is characterized by progressive muscle wasting involving the heart and skeletal muscles (Homburger, 1979; Jasmin and Proschek, 1982; Hunter et al, 1984). The disease is first detectable at about 20 days of age as scattered lesions of focal myolysis in skeletal muscle. The lesions occur in scattered foci of altered and necrotic cells in association with calcifying lesions, surrounded by normal tissue (Burbach, 1987; Hunter et al, 1984; Jasmin and Proschek, 1982). In altered myocytes there is cellular edema, sarcoplasmic reticulum distension and abnormalities in the mitochondria. Calcium deposition within the extruded mitochondria and on organelle debris occurs after cell disruption and often remains in the interstitium, detectable as highly calcified lesions (Burbach, 1987). The disease progresses in severity up to the approximate age of 150 days, after which it subsides. Cardiac lesions begin to appear at about 30-40 days of age, and myolysis and calcification are more severe than in skeletal muscle. After 80 d of age there is healing but scarring is extensive and most animals die of congestive heart failure (Jasmin and Proschek, 1982; Proschek and Jasmin, 1982).

The nature of the primary lesion in muscular dystrophy has not been fully elucidated but of the three major hypotheses proposed, the membrane defect hypothesis seems the most attractive. This theory suggests that muscular dystrophy is an inherited disorder of membranes with widespread tissue involvement (Elbrink and Malhotta 1985; Elbrink et al, 1987; Roses and Appel, 1978). The postulated final common

pathway of muscle necrosis for myopathy is overloading of mitochondria with calcium and consequent energy depletion (Elbrink and Malhotta, 1985).

In addition to the well characterized lesions and congestive heart failure, the myopathic hamster possesses a number of defects in its thermoregulatory system. It has a reduced calorigenic response to infusion of the β-adrenergic agonist isoproterenol, a reflection of its diminished ability to generate heat via nonshivering thermogenesis (Horwitz and Hanes, 1974; Wickler and Horwitz, 1983, 1984). Subsequently, dystrophic hamsters were found to have a reduced amount of brown adipose tissue (Himms-Hagen and Gwilliam, 1980). Reduction of respiratory response to noradrenaline in isolated brown adipocytes from myopathic hamsters reflects reduced cell size, protein content per cell and enzyme activity (Horwitz and Wickler, 1983). Myopathic BAT does grow in response to cold but to a lesser extent than normal (Himms-Hagen and Gwilliam, 1980); it does not hypertrophy in response to either a high fat diet or short photoperiod (Triandafillou et al, 1984b). Therefore it has been postulated that there is a defect in the control of brown adipose tissue growth (Triandafillou et al, 1984a, b).

Recently several groups have reported the identification and partial sequencing of the gene product of the human Duchenne muscular dystrophy (DMD) locus (Hoffman et al, 1987a, b; Lev et al, 1987; Nudel et al, 1988; Zubrzycka-Gaarn et al, 1988). The 400 kD protein, named dystrophin, is present in normal skeletal muscle but not in muscle from boys with DMD (Slater et al, 1987). Using cDNA clones as probes, Lev et al, (1987) did not find the dystrophin transcript outside cell lines

of muscle lineage but Nudel et al (1988) reported the DMD gene product expressed in rat, mouse, and rabbit brain, in addition to myogenic cell cultures, and smooth and striated muscle. Slater and workers (1987) found dystrophin associated with muscle triads and noted that the predicted amino acid sequence suggests the presence of a large portion of α-helix in the N-terminal region, consistent with an affinity for membranes or such helical proteins as myosin. They hypothesize that dystrophin binds contractile filaments to the internal membrane system, thus linking the membrane from which calcium is released on excitation to contractile proteins. Absence of dystrophin may somehow disrupt calcium homeostasis and cause tissue damage. In another study, antibodies directed against synthetic peptides derived from N-terminal region cDNA reacted with normal muscle sarcolemma, not triads; antigen was reduced or absent in DMD muscle fibres (Zubrzycka-Gaarn et al, 1988). This group argues that dystrophin is associated with the sarcolemma and speculates that dystrophin strengthens the sarcolemma by anchoring elements of the internal cytoskeleton to the surface membrane. The amino acid sequence of dystrophin is however not homologous with the anchoring proteins α-actinin or spectrin (Zubrzycka-Gaarn et al, 1988).

OBJECTIVES:

When this project was initiated in 1983 the control of growth of hamster brown adipose tissue was not well understood. The finding that noradrenaline administration to hamsters failed to promote BAT growth pointed to major differences in the way this tissue is controlled compared to the rat. (see Introduction section 3, cold-induced growth) To further complicate matters, diet-induced growth of hamster brown fat was not necessarily coupled to thermogenic activation (see Introduction section 3, diet-induced growth), and short photoperiod could also produce growth of BAT without activation (see Introduction section 3, short photoperiod-induced growth). Rats do not exhibit short photoperiod-induced growth of BAT.

Myopathic hamsters were known to have less brown adipose tissue than normal and the tissue failed to grow in response to either palatable diet or short photoperiod, suggesting a defective control of BAT growth in this animal (see Introduction section 6, myopathic hamsters).

The purpose of the experiments was:

1) To study the apparent defect in the control of BAT growth in the myopathic hamster.

2) Since brown adipose tissue growth was not well understood in the normal hamster it was necessary to establish how BAT growth and function are regulated in normal Syrian hamsters before comparisons with myopathic animals could be made.

At roughly the same time as this research was being carried out, the work of a number of others proceeded in parallel. This work includes several papers by Wade, Trayhurn, and Desautels. These reports will be discussed in relation to the findings of this thesis.

MATERIALS AND METHODS:

1. ANIMALS:

Male Syrian golden hamsters (Mesocricetus auratus) and cardiomyopathic (CHF 146) hamsters were obtained from Canadian Hybrid Farms (Halls Harbor, Nova Scotia) at 4-5 weeks of age. They were housed individually in plastic cages at 24°C ±1°C under 14:10 (L:D) lighting (lights on at 8:00 am). Hamsters were allowed to become accustomed to their surroundings for at least one week before being used in experiments. New Zealand white rabbits were used for production of antibodies to uncoupling protein, and were bled from the ear vein to obtain preimmune serum.

2. FOOD:

Hamsters were fed Purina rat chow (5012) and water ad lib. In some experiments hamsters were also offered unroasted, unsalted sunflower seeds. When food intakes involving sunflower seeds were measured, the bedding was removed from the cages for the 24 hour intake period to make it easier to retrieve the leftover seeds. In these experiments chow was also placed inside the cages. When the intake of chow alone was being measured, it was placed in the food hoppers in the cage lids as there was no difference between intake when chow was inside the cage versus in the hopper.

Sunflower seeds were unsalted, unroasted and had a caloric density of 6.01 kcal/g with 13.2% of kcal as carbohydrate, 70.8% as fat, and 16% as protein (Bowes and Church, 1980). The fatty acid composition of the sunflower seeds was 66.2% linoleic acid, 25.1% oleic acid, 5.6%

palmitic acid, 2.2% stearic acid, and 0.9% arachidic acid (Altmann and Dittmer, 1972).

3. SURGERY AND INJECTIONS:

For implantation of miniosmotic pumps (Alzet), hamsters were anesthetized with a mixture of halothane and oxygen. An area about 3-4 cm around a middorsal incision site was shaved and swabbed with disinfectant. A small incision was made and a pocket was formed under the skin into which the filled pump was placed. Two or three wound clips closed the incision. All procedures were performed aseptically. The animals were checked daily to monitor healing.

For BAT denervation studies, the hamsters were anesthetized as described above and the interscapular brown fat was exposed and lifted to reveal five intercostal nerves innervating each pad. These nerves were cut on one side in unilateral operations or on both sides for bilateral denervations. For sham operated animals, the nerves were exposed and touched but not cut. The incisions were closed with wound clips and the animals monitored daily during recovery.

All injections were made through plastic rodent restraint cones (Ealing) with the animal held firmly in position. Subcutaneous injections were made on the back over the hindquarters, and intraperitoneal injections were made into the lower right quadrant of the animal.

The immunization schedule used for rabbits was that of Fernandez et al (1987). One hundred micrograms of purified UCP in Freund's complete adjuvant were injected intramuscularly into the hind legs and subcutaneously on the back. In the second week 100 µg UCP in Freund's

incomplete adjuvant was injected subcutaneously at several sites on the back. The next week a booster of 100 µg UCP in 0.9% saline was given into the ear vein and the following week the rabbit was bled by cardiac puncture. Blood was left to clot overnight at 4°C then centrifuged at 2500 x g for 30 minutes and the serum recentrifuged at 2500 x g for 15 minutes.

4. OXYGEN CONSUMPTION STUDIES:

In order to measure minimal oxygen consumption (MOC), hamsters were anesthetized with sodium pentobarbital (80 mg/kg i.p.). Once under anesthetic a rectal probe (YSI) connected to a proportional temperature controller (YSI model 72) was inserted about 4 cm. The hamster's body temperature was maintained at 36.7°C ± 0.5°C (Sigurdson and Himms-Hagen, 1988) by means of the controller and two 60 W light bulbs. Maintenance of body temperature is important in anesthetized animals and eliminates thermoregulatory thermogenesis (Denckla and Marcum, 1973). Oxygen consumption was measured using a hood and an open system. Air was drawn through the hood at 83.5 ml/min and passed over silica gel to dry it before entry into the oxygen analyzer (Thermox I). Room air at ambient temperature (22-25°C) was dried and served as the reference gas.

Recordings were made when a stable rate of oxygen consumption was achieved and continued for approximately 20-40 minutes. Data from animals not fully anesthetized or from those which awoke during recording were not used. Oxygen consumption was expressed as ml O_2 consumed/min/$kg^{0.75}$ to allow an appropriate comparison between normal and myopathic hamsters.

5. TISSUE PREPARATIONS:

Hamsters were killed by rapid decapitation and trunk blood collected into centrifuge tubes and placed on ice for 20-30 minutes to clot. If body temperature was being measured, it was recorded immediately after blood collection by insertion of a temperature probe 4-5 cm into the rectum and using a digital readout thermometer (Bailey Instruments, model BAT-8). Brown adipose tissue from the interscapular depot (unless specified otherwise) was removed and cleaned of muscle, connective tissue and white fat and homogenized in 10 volumes/weight isolation medium [0.25 M sucrose, 1.0 mM N-2-hydroxyethylpiperazine-N'-2 ethane sulfonic acid (HEPES), 0.2 mM ethylenediamine tetraacetic acid (EDTA) potassium salt, pH 7.2] using a glass-teflon homogenizer. Aliquots were removed for assay of protein and thyroxine 5'-deiodinase activity. Ten microlitre samples for protein determination were precipitated in 12.5% trichloroacetic acid (TCA) at 4°C overnight and assayed the next day. Samples to be assayed for thyroxine 5'-deiodinase activity were rehomogenized by hand with a glass-glass homogenizer and two 125 µl aliquots were immediately frozen in liquid nitrogen and stored at -80°C for no more than two months.

In some experiments the weights of other organs were recorded. Gonadal white adipose tissue from around the testes and epididymis was used as an index of general adiposity. Paired testes weights gave an indication of the response to short photoperiod as less than 12.5 h light per day causes gonadal regression (Gaston and Menaker, 1967). Hearts were weighed in experiments where hypertrophy might be expected such as during cold acclimation. In all cases the organs were cleaned

of connective tissue and blotted dry before weighing.

Mitochondria were isolated using the method of Cannon and Lindberg (1979). All centrifugations were carried out at 4°C. After an initial centrifugation at 8,500 x g (10,000 rpm Sorvall HB4 rotor, Sorvall RC2B or 5B centrifuge) to sediment cellular debris, nuclei, and mitochondria, the pellet was carefully resuspended in isolation medium and recentrifuged at 650 x g for 5 minutes. The supernatant containing mitochondria was filtered through two layers of cheesecloth into clean tubes and pelleted at 8,500 x g for 10 minutes. Following two washes in isolation medium (8500 x g, 10 minutes), the final mitochondrial pellet was suspended in a small volume of isolation medium (less than 1 ml) and kept on ice for less than two hours prior to measurement of GDP-binding. Five microlitres of mitochondrial protein were placed directly into 0.5 N sodium hydroxide (NaOH), bypassing the precipitation step necessary with homogenates, and protein content determined.

6. ASSAYS:

6.1. Protein Determination:

Brown adipose tissue (BAT) homogenate and mitochondrial protein were determined using the modified Lowry assay of Schacterle and Pollack (1973). The modified version makes use of a single more stable alkaline copper reagent and shorter assay time than the original method (Lowry *et al*, 1951). Bovine serum albumin (BSA) fraction V was used as the standard, and colour intensity was read at 650 nm.

6.2. DNA Determination:

Brown adipose tissue homogenate DNA content was determined using

diphenylamine in a colorimetric assay as described by Burton (1968) and modified by Giles and Myers (1965). Calf thymus DNA was used as the standard. DNA was extracted in 5% trichloroacetic acid (TCA) at 90°C for 10 minutes. These conditions result in less than 5% destruction of the deoxyribose (Burton, 1968). After extraction and cooling, 2.25 N perchloric acid (PCA) was added and the extract filtered through a Millipore membrane filter, pore size 3.0 µm. After addition of 4% diphenylamine in glacial acetic acid, colour development was for 15 hours at 30°C. To eliminate the effects of impurities the differences between optical density readings at 595 and 700 µm were calculated, resulting in a blank reading of zero and a linear calibration line (Giles and Myers, 1965).

6.3. GDP-Binding:

The extent to which purine nucleotides bind to isolated brown fat mitochondria can be used as an index of the tissue's thermogenic activity. The more active the tissue, the greater the binding. The method was first described by Nicholls (1976) and modified by Desautels et al (1978). In these studies GDP-binding was determined using the method of Desautels et al (1978) with further minor modifications: One hundred micrograms of freshly prepared mitochondrial protein (in 20 µl) was added to 280 µl incubation medium [100 mM sucrose, 20 mM N-Tris(hydroxymethyl)-2-aminoethane sulfonate (TES), 10 mM choline chloride, 1 mM EDTA disodium salt (to inhibit adenylate kinase activity), 5 µM rotenone, 100 µM potassium atractyloside (to inhibit the adenine nucleotide translocator), 10 µM [^{3}H]GDP (Amersham, 13.1 Ci/mmol) and 0.1 µCi/ml [^{14}C]sucrose (Amersham, 540 mCi/mmol), with or

without 100 μM ADP for estimation of nonspecific binding]. After vortexing, incubation was for 2 minutes at room temperature. Mitochondria were then separated by centrifugation for 2 minutes in an Eppendorf microfuge. The supernatant was aspirated and the pellet dissolved in NCS tissue solubilizer (Amersham) overnight at 55°C. The dissolved pellet was counted in a cocktail containing 50 μl 10% ascorbic acid and 10 ml toluene containing 0.7% 2,5-diphenyloxazole (PPO) in a Beckman LSC6800 liquid scintillation counter. Specific binding was calculated as the difference between binding in presence and absence of 30 mM ADP, after correction for the amount of incubation medium trapped in the pellet, as estimated from the [^{14}C]sucrose values. Binding was expressed as pmol GDP bound per mg protein.

6.4. Thyroxine 5'-Deiodinase Assay:

Brown adipose tissue thyroxine 5'-monodeiodinase (T5'D) type II (E.C.3.8.1.4) converts thyroxine (T_4) to triiodothyronine (T_3) with liberation of free iodine from the outer ring (Leonard et al, 1983). In this assay the release of free (radioactive) iodine is a measure of enzyme activity with greater release reflecting increased enzyme activity. The original assay was described by Visser et al (1982) and has been modified (Kopecky et al, 1986) and minor refinements made. [^{125}I]T_4 (Amersham, >1200 μCi/μg) was repurified by paper electrophoresis to reduce the amount of free $^{125}I^-$ contamination due to autoradiolysis. Electrophoresis was performed using 50 mM ammonium acetate buffer. After extraction in methanol:ammonium hydroxide (99:1), the purified [^{125}I]T_4 was concentrated under nitrogen on ice. The incubation medium consisted of (final concentration): potassium

phosphate-EDTA buffer (10 mM potassium phosphate, 1 mM EDTA) pH 7.0, 10 mM dithiothreitol (DTT, in 0.25 M sucrose, 1 mM HEPES, 0.1 mM EDTA, pH 7.2 as a source of thiol groups for enzyme activity), 2.57 nM T_4 (in 0.05 N NaOH + 0.1% BSA), 1 mM propylthiouracil (PTU, in 0.05 N NaOH to inhibit type I T5'D activity), and approximately 50,000 cpm purified [^{125}I]T_4 per assay tube. The reaction was started by the addition of 5 µg BAT homogenate protein. After mixing and flushing with nitrogen, tubes were incubated at 37°C in a shaking waterbath for 20 minutes. Blanks containing phosphate buffer were incubated along with samples and later used to correct for any autoradiolysis that occurred during the incubation period. Reactions were terminated by the addition of thyroid hormone-free normal human serum containing thyroid hormone binding proteins (AMF Biologicals and Diagnostics). The protein-bound substrate was precipitated with ice-cold 12.5% TCA. The free iodine was separated by passing 500 µl of the supernatant through small Dowex 50W-X2 (Biorad) columns (bed volume 1.2 ml) equilibrated with 10% acetic acid. $^{125}I^-$ was eluted with three 1 ml washes of 10% acetic acid. Under these conditions greater than 99% of the substrate remained bound to the column (Visser et al, 1982). The eluate was counted in a Beckman 5500 gamma counter with an efficiency of 72.3%. Correction was made for the decay of ^{125}I. Counts per minute were converted to fmol $^{125}I^-$ and the results expressed as pmol/h/mg (specific activity) and pmol/h (total activity).

6.5. Serum Thyroid Hormone Determination:

Serum T_3 and T_4 were determined in samples stored at -20°C that had been thawed no more than twice. Measurements were made using commercially available kits (Amerlex from Amersham) following the manufacturer's instructions. The validity of using the kits for hamster serum was checked by running the human standards supplied with hamster standards (made using thyroid hormone-stripped hamster serum and added known amounts of T_3 and T_4). Hamster serum was stripped of thyroid hormone by using approximately 50,000 cpm [^{125}I]T_4/ml serum and 0.023 g Norit A charcoal (Sigma) per ml. This mixture was stirred overnight at 4°C, then the charcoal pelleted at 100,000 x g (35,000 rpm Beckman Ti 55 rotor, Beckman L8-55M ultracentrifuge) for 30 minutes at 4°C. The supernatant was removed to a clean tube and the same amount of charcoal was added and stirred overnight. Following centrifugation the procedure was repeated. An aliquot of the final supernatant was counted and the serum was considered essentially thyroid hormone-free if 1-5% of the added counts remained (Larsen, personal communication). The curves were parallel and agreed with results obtained by Tomasi and Horwitz (1987) using similar methods. Values obtained with these kits agreed with values reported using similar kits (Tomasi and Horwitz, 1987; Vriend, 1984).

6.6. Noradrenaline Determination:

Determination of noradrenaline was made utilizing high performance liquid chromatography (HPLC) (Varian 5000 chromatograph) using an MCH-10 guard column (Varian) and 4.0 mm I.D.x 15.0 cm reverse phase column (Varian MCH-S-N CAP, 5 µm particle size). The filtered, degassed

mobile phase consisted of: 0.150 M monochloroacetic acid, 0.10 M NaOH, 2 mM EDTA, and 0.006% octyl sodium sulfate, pH 3.05. Peaks were detected electrochemically (BAS model LC-48) utilizing a glassy-carbon electrode (TL-5) and silver-silver chloride reference electrode (BAS RE-1) at an applied potential of +0.70 V. The NA peak was collected into a premixed cocktail (Beckman Redisolv MP) and counted by liquid scintillation counting in a Beckman LS 1801 counter.

The regression coefficient ± sample standard error for logarithmic decline in specific activity of NA in BAT was calculated by linear regression analysis (Snedecor, 1956) as described by Himms-Hagen et al (1986). The rate constant was calculated from the slope of the regression line and the noradrenaline turnover rate was the product of the rate constant and NA content. The standard error of the turnover rate was calculated as described by Chase and Rabanowitz (1969).

6.7. Uncoupling Protein Assay:

Protein Purification:

Uncoupling protein (UCP) was derived from cold-acclimated hamster brown adipose tissue mitochondria, purified according to the method of Lin and Klingenberg (1982), except that the sucrose density gradient centrifugation step was omitted. The purification was done by Dr. Gloria Zaror-Behrens and is outlined as follows:

Mitochondria were isolated by differential centrifugation in isolation medium. The final pellet was resuspended at a concentration of 100 mg/ml in a "standard buffer" consisting of : 20 mM MOPS (3-(N-morpholino)propane sulfonic acid), 20 mM Na_2SO_4, 1 mM EDTA, pH 6.7, frozen in liquid nitrogen, and stored at -80°C.

Five hundred milligrams of protein were incubated with 3.2% Lubrol WX for 30 minutes at 0°C to remove soluble and peripheral membrane proteins. The resulting membranes were pelleted at 100,000 x g for 30 minutes then washed once in 0.3 M sucrose containing 10 mM Tris-Cl pH 7.2, and 2 mM EDTA, and resuspended in standard buffer containing 5% Triton X 100 and incubated for 30 minutes at 0°C. The supernatant from the final 100,000 x g centrifugation contained UCP-Triton micelles.

Chromatography on hydroxyapatite is the major purification step. The UCP-Triton extract was applied to a 3 x 10 cm hydroxyapatite column equilibrated with standard buffer and the protein eluted at room temperature with the same buffer. One millilitre fractions were collected and the UCP peak determined using a modified Lowry assay containing 1% SDS. The peak fractions were then pooled. UCP is eluted whereas most of the other solubilized proteins are adsorbed onto the hydroxyapatite. The major contaminant, the ADP/ATP carrier protein is not stable at room temperature and when degraded, is retained by the column. The amount of Triton in the purified UCP was determined spectrophotometrically at 276 nm.

Samples of the purified UCP (1-3 µg) were run on SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) and either stained with 0.05% Coomassie Blue (R-250, Bio Rad) or electrophoretically transferred to nitrocellulose (0.2-0.4 pore size, Bio Rad) using 60 V for 3 h at 4°C with stirring to assist even transfer. The nitrocellulose was washed in PBS + 0.05% Tween 20 for 30 minutes at room temperature in a shaking bath then incubated with rabbit anti-rat

UCP antiserum diluted to 1:800 in PBS-Tween 20 for 2 h at room temperature in a shaking waterbath. After four washes in PBS-Tween 20 [^{125}I] protein A was used for detection. The blot was incubated with 100,000 cpm/ml [^{125}I] protein A in PBS-Tween 20 for 1 h at room temperature in a shaking bath, then washed overnight in PBS-Tween 20 in several changes of buffer. The blot was dried between sheets of filter paper and subjected to autoradiography overnight at -80°C using Kodak X-ray film. Both the stained gel and the western blot showed only one band. Purified UCP was aliquoted and stored at -80°C.

Antibody Production:

For immunization schedule and bleeding of rabbits see METHODS (section 1. ANIMALS and 3. SURGERY AND INJECTIONS). To adsorb trace contaminating antibodies to other mitochondrial proteins in the antiserum 500 µl aliquots of antiserum were incubated at 18°C for 2 h with 50 µl sonicated hamster liver mitochondria (40-50 mg/ml), centrifuged at 11,000 x g for 2 minutes then used. It was found that untreated and treated antisera gave identical results in the assay; subsequently only untreated antisera were used. Antisera were stored at -80°C until use, then stored at -20°C in glycerol 1:1 and freshly diluted just before addition to the assay. Binding of preimmune serum to UCP-coated plates was less than 1%.

Sample Preparation:

Aliquots of hamster BAT homogenate were diluted to 1 mg/ml protein and extracted with 5% Triton X-100 in PBS as described by York (personal communication). Two hundred microlitres of homogenate was added to 22 µl of 5% Triton X-100, mixed well and incubated at room

temperature for 30 minutes. Following incubation, 880 µl PBS were added and the samples centrifuged for 2 minutes in an Eppendorf microfuge. The infranatant was removed and stored at -20°C until assay.

Uncoupling Protein Assay:

A solid phase radioimmunoassay was employed.

Flexible 96-well microtitre plates (Falcon 3912) were initially soaked for 30 min in Pierce RBS 35 detergent then thoroughly washed with distilled water, in order to remove residues from the manufacturing process and to increase protein binding to the plates. Washed plates were coated with 10 µg/ml hamster UCP (50 µl/well). Wells used for nonspecific binding contained 50 µl PBS + 0.02% Triton. The plates were incubated for 2 h at 37°C. Following incubation, the liquid was discarded and the plates washed with PBS + 1% BSA, 0.1% sodium azide (PBS-BSA). After the first wash the plates were incubated with PBS-BSA for 10 min at 37°C to block sites not bound with UCP. After blocking, the plates were washed 3 times with PBS-BSA and drained well.

Samples to be assayed were diluted 5 times in PBS to bring the Triton concentration to 0.02%. Standards consisted of hamster UCP diluted in PBS to give a concentration range of 2-67 ng UCP. The samples were added to give a final homogenate protein concentration of 0.80 µg/well. Rows of wells for total and nonspecific binding were included, along with wells for standard and sample nonspecific binding. After addition of standards and samples, 10 µl/well of freshly diluted rabbit anti-hamster UCP antiserum was added to all the wells (final

dilution 1:6000 in PBS). The plates were covered with parafilm and incubated overnight at 4°C.

The next day the plates were washed 5 times with PBS-BSA and 60,000-70,000 cpm/well of [^{125}]I protein A (ICN, 0.922 mCi/ml, 32.7 µCi/µg) in PBS (50 µl/well) was added, and the plates incubated for 1½ h at room temperature. After washing the plates 5 times with PBS-BSA the wells were cut out and counted using a Beckman 5500 gamma counter. Following correction for nonspecific binding, the results were calculated from the linear regression line of logit-ln UCP and expressed as µg UCP/mg protein.

7. NORADRENALINE TURNOVER:

Radioactive noradrenaline (NA), when given in a tracer dose, mixes rapidly and uniformly with endogenous NA stores in sympathetic nerve endings (Neff et al, 1968). Sympathetic activity can therefore be estimated from the decline in specific radioactivity of the labelled amine over time, where an increase in slope of the regression line indicates increased activity in the tissue under study (Neff et al, 1968).

The hamsters were injected i.p. at lights on with a tracer dose (0.5 µg NA/kg) of L-[2,5,6-^{3}H]NA (48 Ci/mmol, New England Nuclear) diluted to 75 µCi/ml in 0.9% NaCl at a dose of 150 µCi/kg. At exactly timed intervals after injection the animals were killed by decapitation. Interscapular, subscapular, and cervical BAT were quickly removed, cleaned and immediately frozen either on dry ice or in liquid nitrogen. Samples were stored at -80°C for no longer than one month prior to assay.

Frozen tissues were homogenized in 4 ml 1.0% perchloric acid (PCA) with 150 ng dihydroxybenzylamine (DHBA, Sigma) as internal standard, using a Polytron homogenizer at maximum output for 10 seconds (ST-7 microprobe, Brinkmann Polytron). Homogenates were centrifuged at 19,000 xg (10,000 rpm, Sorvall HS-4 rotor, Sorvall RC5B centrifuge) for 30 minutes at 4°C, and resulting supernatants were filtered through glass wool and frozen at -80°C. Blanks were prepared with 90 ng noradrenaline plus 150 ng DHBA in PCA, and processed along with the samples. These were later used to calibrate the HPLC and correct for recovery of NA.

Less than one month later noradrenaline was extracted by addition of 1.3 ml Tris-EDTA buffer (0.2 M Tris, 5.2% EDTA, pH 8.6), 50 µl freshly prepared 11% sodium metabisulfite, and approximately 100 mg acid-washed alumina (Baker). Samples and blanks were mixed for 5 minutes then centrifuged for 2 minutes at 2,000 rpm (Sorvall GLC-1 benchtop centrifuge). Supernatants were aspirated and the alumina washed 3 times with glass-distilled water. After final washing, NA was desorbed from the alumina with 0.4 ml 0.2 N PCA and mixing for 5 minutes. After pelleting the alumina as described above, the NA-containing supernatant was recentrifuged in an Eppendorf microfuge to remove any particles that might block the HPLC column.

8. IMMUNOHISTOCHEMISTRY:

Hamsters were deeply anesthetized with sodium pentobarbital and the chest cavity carefully opened. Animals were perfused through the right ventricle with approximately 50 ml of normal saline (0.9% NaCl). Brown adipose tissue was rapidly excised and placed in a modified

Zamboni's fixative (Zamboni and De Martino, 1967) for 24 hours at 4°C. The fixative consisted of (per litre): 40 g paraformaldehyde and 140 ml saturated picric acid in 0.16 M phosphate buffer, pH 6.9. After fixation the tissues were stored in 10% sucrose in 100 mM phosphate buffer pH 7.4 at 4°C. Fourteen µm sections were cut on a cryostat (Bright Instrument Company) at -25-33°C onto gelatin-chrome alum coated slides. Some sections were stored at -80°C prior to staining with no decrease in intensity of either histochemical or immunohistochemical reactions as compared to sections which were not stored. Sections were rinsed in 10 mM phosphate buffered saline (PBS) pH 7.4 for 15 minutes and incubated with one of the following antisera diluted in PBS containing 0.3% Triton X-100 overnight at 4°C in a humid environment: nerve growth factor 1:200 (NGF, Collaborative Research), calcitonin-gene-related-peptide 1:200 (CGRP, Amersham), neuropeptide Y 1:400 (NPY, Amersham), tyrosine hydroxylase 1:50, 1:200; 1:400 (TH, Eugene Tech, different lots of antisera). All of the antisera had been raised in rabbits.

After the primary antisera were washed off in a 15 minute rinse in PBS the secondary antibody was added. For immunofluorescence the secondary was fluorescein-linked donkey antirabbit immunoglobulin (Amersham) 1:20 in PBS containing 0.3% Triton X-100. Incubation was for 30 minutes at 37°C in a covered, humid box. After a final 15 minute rinse in PBS, the slides were coverslipped with a glycerol:PBS (3:1) mounting medium containing 50 mg p-phenylendiamine to inhibit fading of the fluorescence. Sections were examined under a Zeiss fluorescence microscope using appropriate filters (excitation 450-490

nm, long pass 515 nm) and later stored at -80°C to preserve fluorescence.

Some sections were reacted for the histochemical demonstration of cytochrome oxidase activity. These were reacted in a solution containing the following: 5mg diaminobenzadine, 3 mg cytochrome c type 3, (Sigma) 400 mg sucrose, and 9 ml 0.1 M phosphate buffer, for 1½ hours at 37°C in the dark. Following staining the sections were rinsed in PBS and coverslipped using 50 mM Tris:glycerol 3:1 or dried in an alcohol series and xylene and coverslipped with Permount.

Fluorescent photomicrographs were taken using Kodak Tri-X film (ISO 400). Cytochrome oxidase reacted sections were photographed using Kodak Pan X (ISO 32).

RESULTS AND DISCUSSION:

1. GROWTH OF BROWN ADIPOSE TISSUE:

1A: EFFECT OF COLD ON BAT:

Background:

When this experiment was initiated it was known that in rats cold acclimation induces hyperplastic growth of brown adipose tissue (see INTRODUCTION, section 3 cold-induced growth) and raises serum T_3 levels (see INTRODUCTION, section 5 thyroid hormones). Cold acclimated hamsters likewise grow more brown fat but the mediator is unknown (see INTRODUCTION, section 3 cold-induced growth).

Thyroxine 5'-deiodinase (T5'D) had been recently described in rat brown adipose tissue (Leonard et al, 1983; Silva and Larsen, 1983) and it was thought to play a role in cold-induced BAT growth.

Myopathic hamsters have less BAT than normal hamsters. They can grow more BAT in response to cold but it remains smaller than normal (Himms-Hagen and Gwilliam, 1980).

Objectives:

The objectives of these experiments were to study the deiodinase enzyme in hamster brown adipose tissue to determine if its activity is changed by cold, and whether T5'D is abnormal in the myopathic hamster.

Methods:

Normal male Syrian golden and CHF 146 cardiomyopathic hamsters aged 4-5 weeks were obtained from Canadian Hybrid Farms. They were housed individually in plastic cages and fed as described in METHODS. The experiments described were done between October and January.

One group of normal hamsters was exposed to 4°C for 2, 10, 24, or

72 h at 8-12 weeks of age. These are referred to as cold exposed hamsters. For cold acclimation experiments, hamsters were to be transferred to 4°C for 30-40 d. Myopathic hamsters moved directly from 24° to 4° had a high mortality rate. For this reason all hamsters were first adapted to 14°C for 12 d then transfered to 4°C for 30-40 d. Even so, 3 myopathic hamsters died soon after transfer from 14°C. No normal hamsters died. Normal and myopathic hamsters treated in this way are referred to as cold acclimated hamsters. In order to study cold deacclimation, some cold acclimated hamsters were returned to 24°C for 2, 10, or 24 h.

Hamsters were killed by decapitation, trunk blood collected for thyroid hormone assay, and in some cases body temperature was measured as described in METHODS. Interscapular brown adipose tissue was cleaned, weighed, then removed into ice-cold isolation medium and homogenized. Aliquots were taken for protein determination and deiodinase assay, and the remainder was used for isolation of mitochondria for GDP-binding and UCP assay. Procedures are described in METHODS. Hearts were also removed, blotted dry and weighed to determine extent of cold-induced hypertrophy.

The thyroxine 5'-deiodinase assay in these experiments was performed by Dr. Jan Kopecky, who introduced the method into the lab. The assay was performed on homogenates as described in the METHODS section, but using an incubation time of 1 hour and a $[T_4]$ of 2.4 nM (Kopecky et al, 1986a).

Mitochondrial UCP was measured using polyacrylamide gel electrophoresis followed by blotting to nitrocellulose. UCP was

detected using rabbit antirat UCP antiserum and ^{125}I protein A as described by Kopecky et al (1986a).

Data were analyzed using analysis of variance or unpaired Student's t-test. The level of significance was 0.05.

Results:

Body Weight:

Hamsters exposed to cold for 3 d lost weight relative to those kept at 24°C, however by the time they were fully cold acclimated (30-40 d), they had regained most of the weight (table 1, upper panel). Deacclimation for 24 h had no effect on body weight (table 1, lower panel). Myopathic hamsters kept at either 24°C or cold acclimated weighed significantly less than normal (table 3).

Body Temperature and Heart Weight:

Both cold exposure and acclimation lowered body temperature in normal but not myopathic hamsters (table 3). Cold acclimation increased cardiac weight in normal and myopathic hamsters (table 3).

Brown Adipose Tissue:

Size:

Brown adipose tissue weight was reduced by cold exposure while acclimation increased wet weight significantly. Protein content was decreased after 10 h of cold exposure but cold acclimation more than doubled it (table 1, upper panel). Brown fat weight and protein were unchanged after 24 h deacclimation (table 1, lower panel). Myopathic hamsters always had less BAT than normal and its protein content did increase upon cold acclimation although not to normal levels (table 2).

GDP-binding and UCP:

During the course of cold acclimation GDP-binding rose slowly to reach a significantly higher value after 24 h of cold exposure. The greatest increase in binding was observed between 24 h and 3 d, with the level remaining high for the rest of the acclimation period (figure 1). Cold acclimation nearly doubled the extent of GDP-binding to isolated BAT mitochondria in both normal and myopathic hamsters (table 4). There were no differences between the two animal types. Cold acclimation increased the amount of UCP nearly two-fold in normal hamsters. UCP levels were not significantly different between warm acclimated normal and myopathic hamsters but the increase in UCP cold acclimation produced in myopathic hamsters was less than that seen in normal animals (table 4).

The ratio of GDP bound/UCP was greater in cold acclimated myopathic hamsters than in cold acclimated normal hamsters (table 4).

Thyroxine 5'-Deiodinase Activity:

In normal hamsters, a significant elevation in deiodinase activity was seen after 10 h of cold exposure and increased rapidly until it reached a peak at 3 d of cold exposure (figure 2). Specific activity then declined somewhat in fully cold acclimated animals but was still significantly above basal levels. Total BAT deiodinase activity increased with increasing length of cold exposure as tissue grew, especially between 3 and 30-40 days. Transfer of cold acclimated hamsters back to 24°C resulted in a rapid drop in both specific and total deiodinase activities within 24 h, though not to warm acclimated levels (figure 3). Large increases in specific deiodinase activity were seen after 1 d of cold exposure in both normal and myopathic

hamsters, although in myopathic hamsters the increase was less than in normal animals (figure 4, upper panel). After cold acclimation, specific activity in normal hamsters was somewhat reduced, and at the same elevated level as in myopathic hamsters. Total BAT deiodinase activity was increased with increasing length of cold exposure in both types of animal. Total deiodinase activity in myopathic BAT was less than normal because of the reduced amount of tissue in these animals (figure 4, lower panel).

Serum Thyroid Hormones:

In normal hamsters serum T_3 rose significantly within 10 h of cold exposure and continued to do so during the course of cold acclimation (figure 5, lower panel). Serum T_4 was initially increased at 2 h of cold exposure, then after 24 h fell below warm acclimated values (figure 5, lower panel). As a result, the ratio of T_3/T_4 rose during cold acclimation (figure 5, upper panel). After removal of cold acclimated hamsters to the warm, serum T_3 rapidly dropped to basal levels within 24 h (figure 6, lower panel). Serum T_4 decreased significantly at 10 and 24 h deacclimation (figure 6, lower panel). Likewise, the T_3/T_4 ratio fell during the deacclimation period (figure 6, upper panel). Myopathic hamsters had a lower than normal level of T_3 but could respond to cold, although serum T_3 was not normalized (figure 7, upper panel). Serum T_4 levels were the same in normal and myopathic hamsters kept at 24°C but myopathic hamsters failed to increase T_4 levels in response to 1 d cold exposure (figure 7, middle panel). Cold acclimation reduced serum T4 in normal hamsters only. T_3/T_4 ratios were increased by cold acclimation in normal hamsters, and

cold exposure and acclimation in myopathic hamsters. The ratio was always lower in the myopathic animals (figure 7, lower panel).

Discussion:

The major finding of these experiments was that acute and chronic cold exposure greatly stimulated thyroxine 5'-deiodinase in normal hamster brown adipose tissue. The increase in specific deiodinase activity during cold acclimation showed an initial lag phase of at least 2 h, which was followed by a relatively slow increase in activity that peaked at 3 d of cold exposure. Specific activity then decreased somewhat in the cold acclimated state, though it remained significantly elevated over warm acclimated values. This pattern of activation with an initial lag phase and rapid changes in activity thereafter suggests the stimulated synthesis of a protein with a relatively short half life (Kopecky et al, 1986a).

The timecourse of deiodinase activation is very different in rats, where during the initial stages of acclimation the increase in activity is much more rapid (Silva and Larsen, 1986; Kopecky et al, 1986a). The increase in GDP-binding in response to cold also occurs very early during exposure in rats (Desautels et al, 1978), whereas at least 24 h are required before the increase becomes significant in hamsters. The slowness of these responses in the hamster suggests that perhaps there is either a slower response to noradrenaline secreted by the nerves or a slower or lesser activation of the sympathetic nervous system by cold. The magnitude of change also differs between the two species. The increase in specific deiodinase activity during cold acclimation is almost 3 times greater in hamsters than in rats, although the peak

occurs later (Kopecky et al, 1986a). In contrast, in cold acclimated rats there can be a five-fold increase in GDP-binding over that in warm acclimated animals (Desautels et al, 1979), whereas GDP-binding usually no more than doubles in cold acclimated hamsters (Himms-Hagen and Gwilliam, 1980; Triandafillou et al, 1984a; these experiments).

Neither during cold acclimation nor during 24 h of deacclimation did changes in deiodinase activity parallel changes in tissue protein in hamsters. There was an association between deiodinase activity and GDP-binding and UCP concentration, in that enzyme activity is increased by cold and remains high in cold acclimated animals. Mitochondrial uncoupling protein concentration was doubled by cold acclimation. This result agrees with work by Trayhurn et al (1983) and Sundin et al (1987) using hamsters, and with Trayhurn et al, (1987) using rats. However, Desautels and Dulos (1986) cold acclimated normal and myopathic hamsters but were unable to demonstrate a specific increase in UCP. The reason for this discrepancy is unclear.

Myopathic hamsters had normal body temperatures and when exposed to cold were able to maintain them. This is in contrast to the finding of Desautels et al (1985) that myopathic hamsters housed at 21°C thermoregulate at a lower body temperature. Cold acclimated myopathic hamsters were able to grow more brown adipose tissue as evidenced by increases in weight and protein content, although not to levels seen in normal hamsters. The deiodinase from myopathic hamsters was less responsive to cold than normal during the early phase of acclimation. Both specific and total deiodinase activities were less after 1 d of cold exposure, and although specific activity of the enzyme was at a

normal level in cold acclimated myopathic animals, total activity remained lower because of the smaller tissue size. Myopathic hamsters had less T_3 in their blood than normal but were able to raise levels in response to cold. However serum T_3 was not normalized by cold. The reduced serum T_3 could reflect reduced total capacity for peripheral deiodination in brown adipose tissue (Kopecky et al, 1986b). Myopathic hamsters are reported to be resistant to several actions of T_4 on their heart (Hegyvary et al, 1979), which could be related to reduced deiodination capacity and hypothyroidism. How this resistance develops and what its relationship is, if any, to muscular dystrophy in these animals is currently unknown.

Uncoupling protein levels and GDP-binding were the same in BAT mitochondria from normal and myopathic hamsters in an unstimulated state. This means that brown adipose tissue of myopathic hamsters has the same basic thermogenic properties as that of normal hamsters. Indeed, the sympathetic innervation to the tissue is normal in these animals (Triandafillou et al, 1984b; see 3B). Upon cold acclimation GDP-binding increased to the same extent in both animal types but the concentration of UCP was increased to a greater extent in the normal hamsters than in the myopathic hamsters.

These results provide no evidence for a defect in deiodinase response to sympathetic stimulation playing a role in reduced trophic responses of BAT to cold in myopathic hamsters.

Table 1

Effect of Cold Acclimation and Deacclimation on Body Weight and Brown Adipose Tissue of Normal Hamsters

ACCLIMATION				
Time at 4°C	n	Body Weight (g)	Brown Adipose Tissue weight (mg)	protein (mg)
0 h	14	96.8±2.0	251± 6.7	19.9±1.4
2 h	10	89.3±2.5*	190±14.7*	17.9±2.3
10 h	4	84.0±7.3	134±14.7*	13.2±2.1*
24 h	9	90.2±3.2	186±14.5*	24.7±3.9
3 d	4	81.6±2.8*	202±24.0	19.8±3.9
30-40 d	5	95.9±3.0	354±13.3*	51.2±2.9*
DEACCLIMATION				
Time at 24°C	n	Body Weight (g)	Brown Adipose Tissue weight (mg)	protein (mg)
0 h	5	110.2±5.3	295±13.0	50.7±5.4
2 h	4	110.3±5.5	284±27.4	48.2±2.7
10 h	4	105.0±3.1	341±35.7	53.5±6.4
24 h	4	113.3±8.4	380±41.7	49.4±5.5

Values are means ± SE for number of animals (n). * indicates significant difference from corresponding value at time 0 ($p<0.05$). Acclimation was for the indicated times in hours or days.

Table 2

Effect of Cold Exposure on Brown Adipose Tissue in Normal and Myopathic Hamsters

		NORMAL		MYOPATHIC
Control (24°C)	**n**		**n**	
BAT weight (mg)	14	251± 6.7	4	108± 5.7†
BAT protein (mg)		19.9±1.4		13.0±2.3†
Cold Exposed				
BAT weight (mg)	9	186±14.5*	5	102±12.2†
BAT protein (mg)		24.7±3.9		12.3±1.4†
Cold Acclimated				
BAT weight (mg)	5	354±13.3*	5	256±20.5†*
BAT protein(mg)		51.2±2.9*		36.3±4.8†*

Values are means ± SE for number of animals (n). Cold exposure was for 1 day and cold acclimation was for 30-40 days. * indicates significant effect of cold and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

Table 3

Body Weight and Temperature of Cold Exposed or Cold Acclimated Normal and Myopathic Hamsters

		NORMAL		MYOPATHIC
Control (24°C)	**n**		**n**	
Body weight (g)	15	109.0±4.5	4	81.2±4.4†
Temperature (°C)	4	36.2±0.1	3	35.4±0.3
Heart weight				
(mg)	5	350±16.8	4	303±31.5
(mg/100g)	5	357±20.3	4	370±21.7
Cold Exposed				
Body weight (g)	9	90.2±3.2	5	84.0±2.6
Temperature (°C)	5	33.5±0.3*	5	35.6±0.5†
Heart weight				
(mg)	5	340±7.1	5	306±16.9
(mg/100g)	5	348±3.9	5	364±15.0
Cold Acclimated				
Body weight (g)	5	95.9±3.0	5	86.2±2.3†
Temperature (°C)	4	34.0±0.6*	5	34.5±0.5
Heart weight				
(mg)	5	434±22.1*	5	386±22.1
(mg/100g)	5	452±13.6*	5	449±25.1*

Values are means ± SE for number of animals (n). Cold exposure was for 1 day and cold acclimation was for 30-40 days. * indicates significant effect of cold vs control. † indicates significant difference between normal and myopathic hamsters ($p<0.05$). The control group for this experiment differs from that in tables 1 and 2.

Table 4

Effects of Cold Acclimation on Brown Adipose Tissue Mitochondrial GDP-Binding and UCP in Normal and Myopathic Hamsters

		NORMAL		MYOPATHIC
Control 24°C	n		n	
GDP-binding (pmol/mg protein)	5	313±25.5	4	317±36.5
UCP (µg/mg protein)	5	31.1±2.7	4	32.4±0.4
Molar ratio (GDP bound/UCP)	5	0.33±.02	4	0.31±.03
Cold Acclimated				
GDP-binding (pmol/mg protein)	5	535±27.3*	5	521±18.4*
UCP (µg/mg protein)	5	61.6±4.8*	5	46.8±4.1*†
Molar ratio (GDP bound/UCP)	5	0.28±.01	5	0.36±.03†

Values are means ± SE for number of animals (n). Cold acclimation was for 30-40 days. * indicates significant effect of cold and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

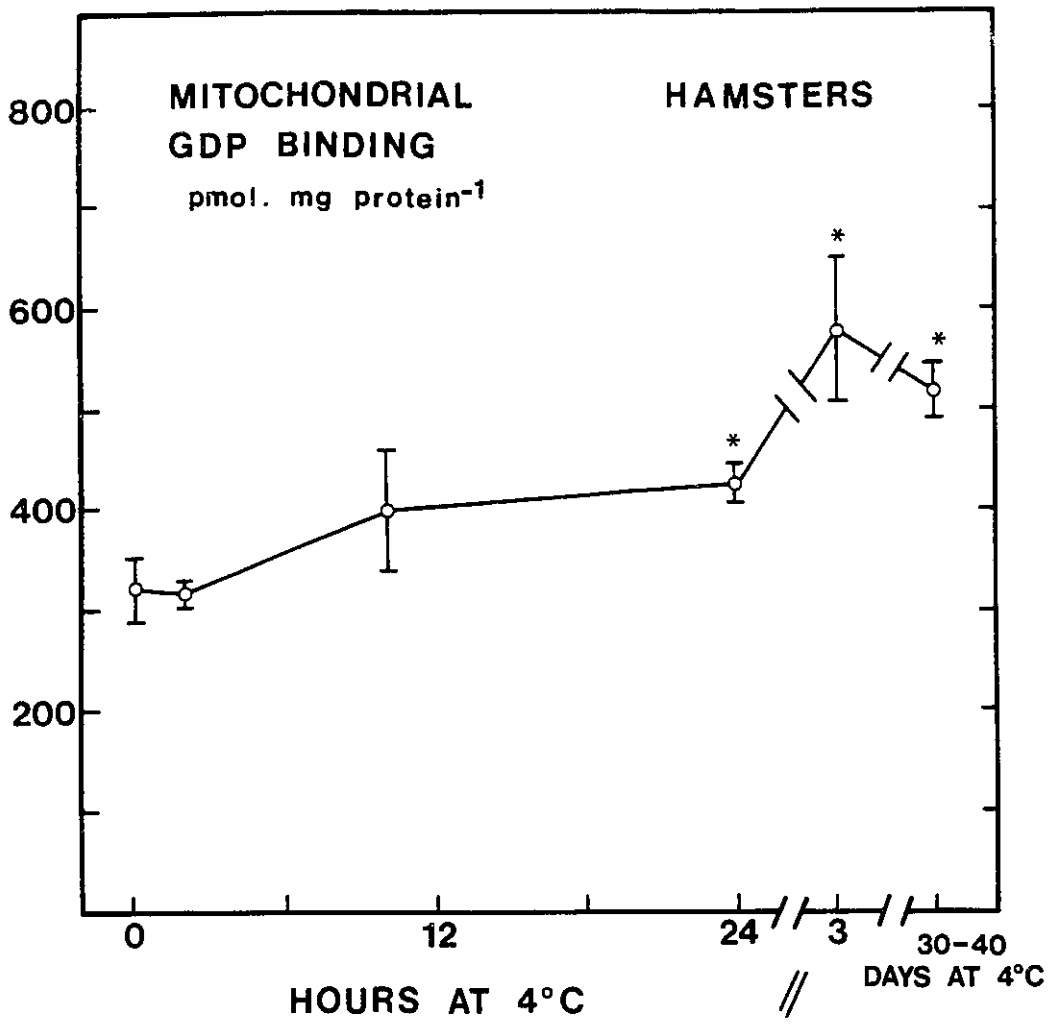


Figure 1. GDP-binding to isolated hamster brown adipose tissue after acute or long term cold exposure. Values are means ± SE for the number of animals given in table 1. Cold-induced increases in binding are significant at 24 h, 3 d, and 30-40 d as indicated by * ($p<0.05$).

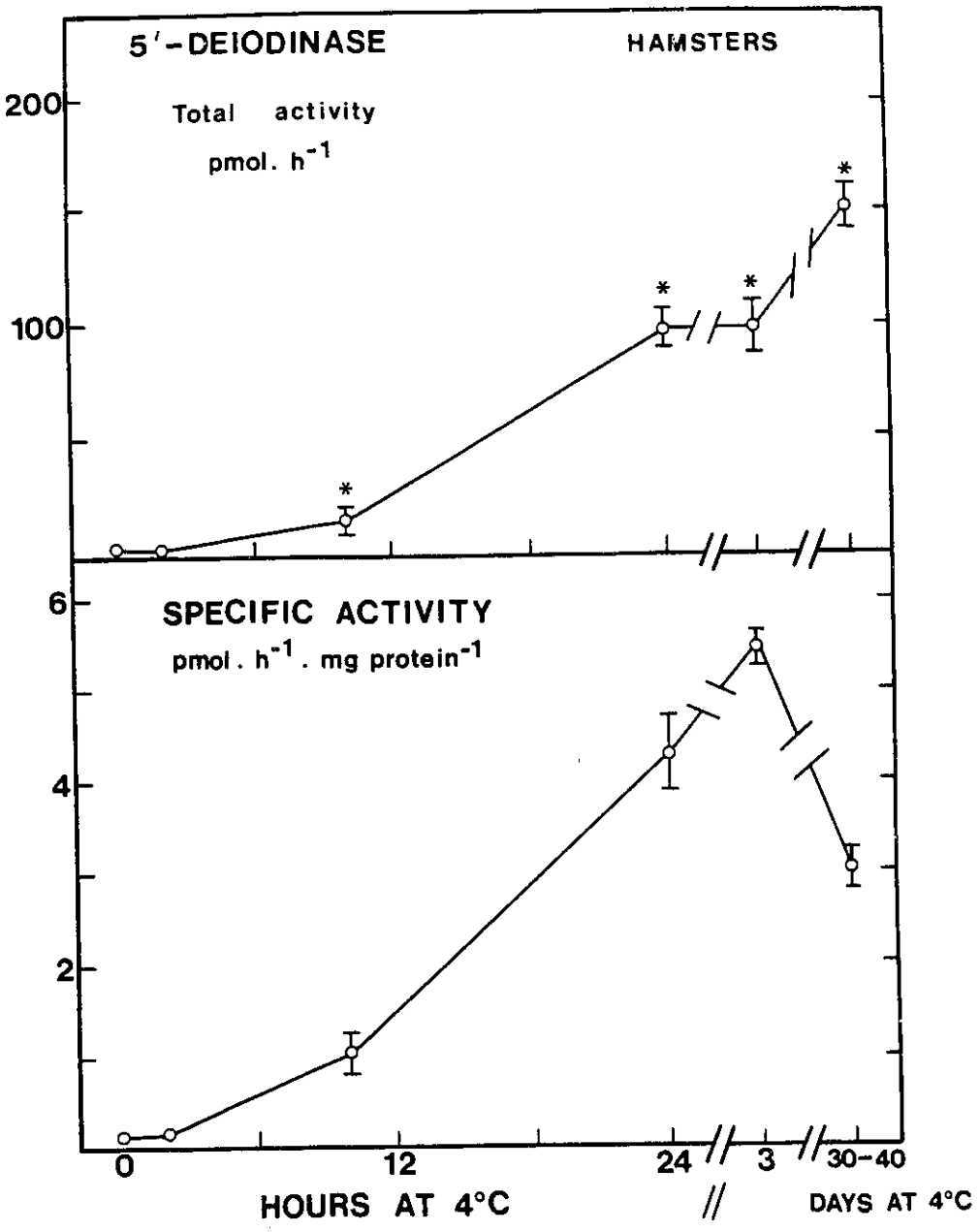


Figure 2. Thyroxine 5'-deiodinase activity in brown adipose tissue from cold exposed or acclimated hamsters. Values are means ± SE for numbers of animals given in table 1. SE for 0 and 2 h are too small to be seen on this scale. Values for specific activity are significantly different from time 0 at 10 h, 24 h, 3 d, and 30-40 d as indicated by * ($p<0.05$). Total deiodinase activity was significantly different from 0 time at 10 h, 24 h, 3 d, and 30-40 d as indicated by * ($p<0.05$).

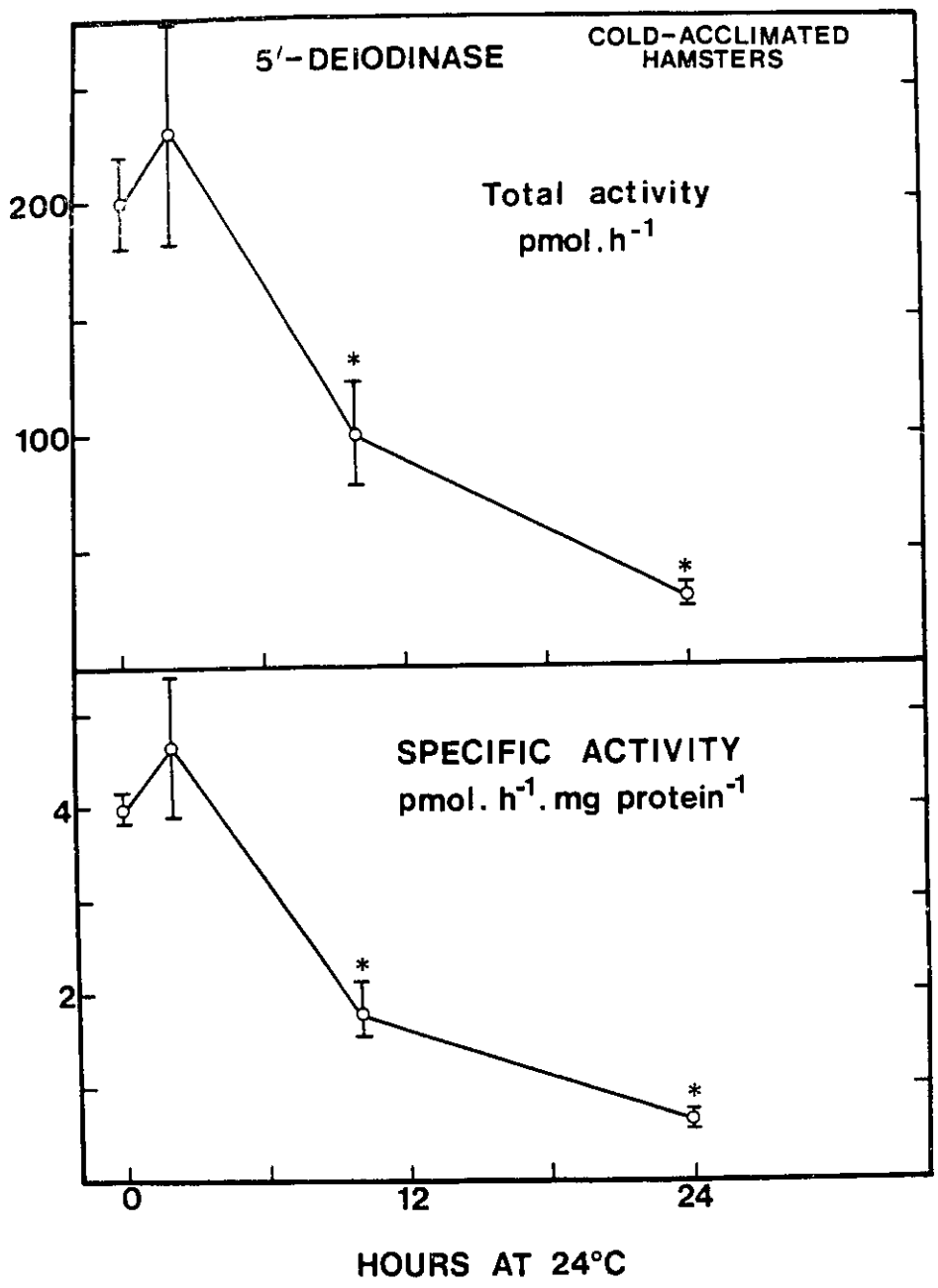


Figure 3. Thyroxine 5'-deiodinase activity in brown adipose tissue of cold acclimated hamsters during deacclimation. Values are means ± SE for numbers of animals given in table 1. Significant decreases in both specific and total deiodinase activities occur at 10 and 24 h as indicated by * ($p<0.05$).

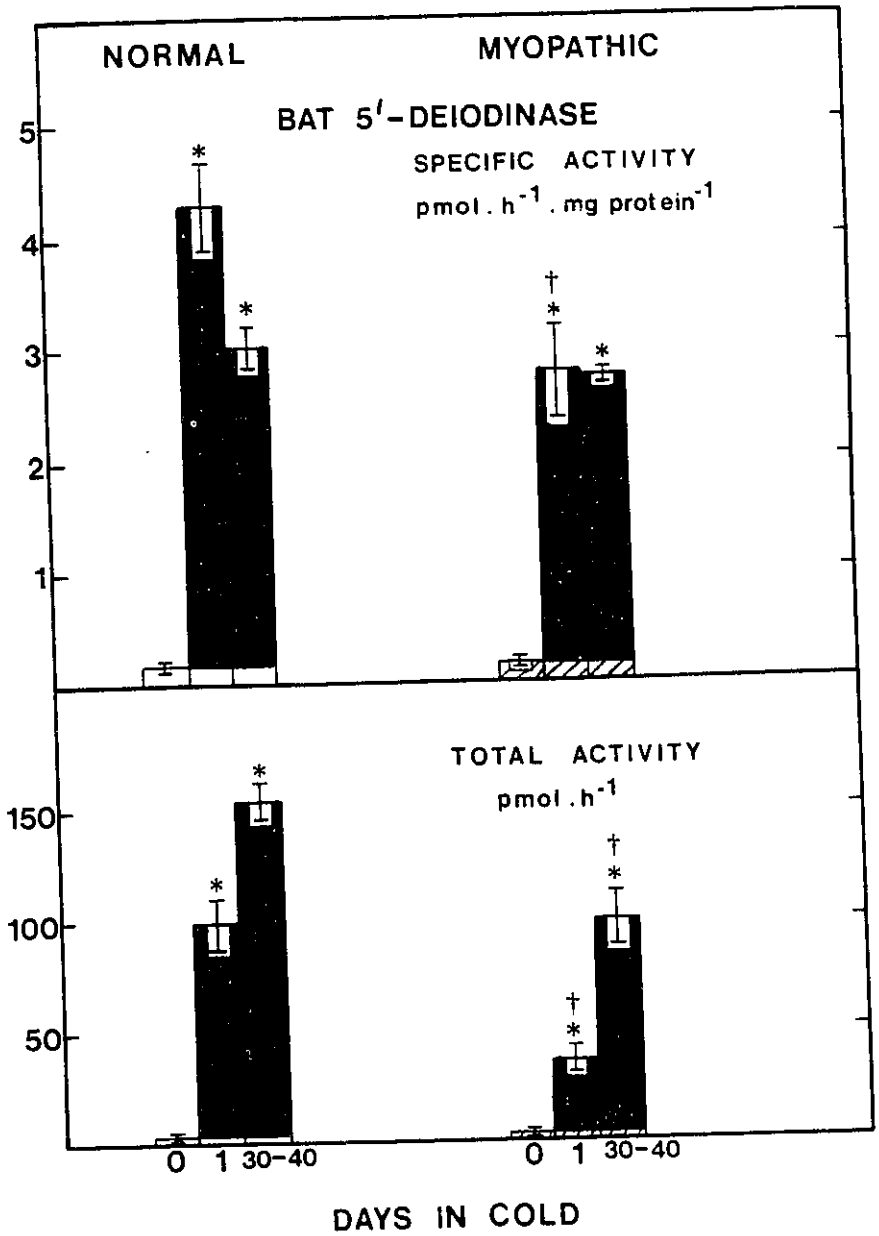


Figure 4. Thyroxine 5'-deiodinase activity in normal and myopathic hamsters acutely or chronically exposed to cold. Values are means ± SE for numbers of animals given in table 3. Open bars represent normal hamsters and hatched bars represent myopathic hamsters. Shaded portions of bars indicate significant effect of cold (*, $p<0.05$). † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

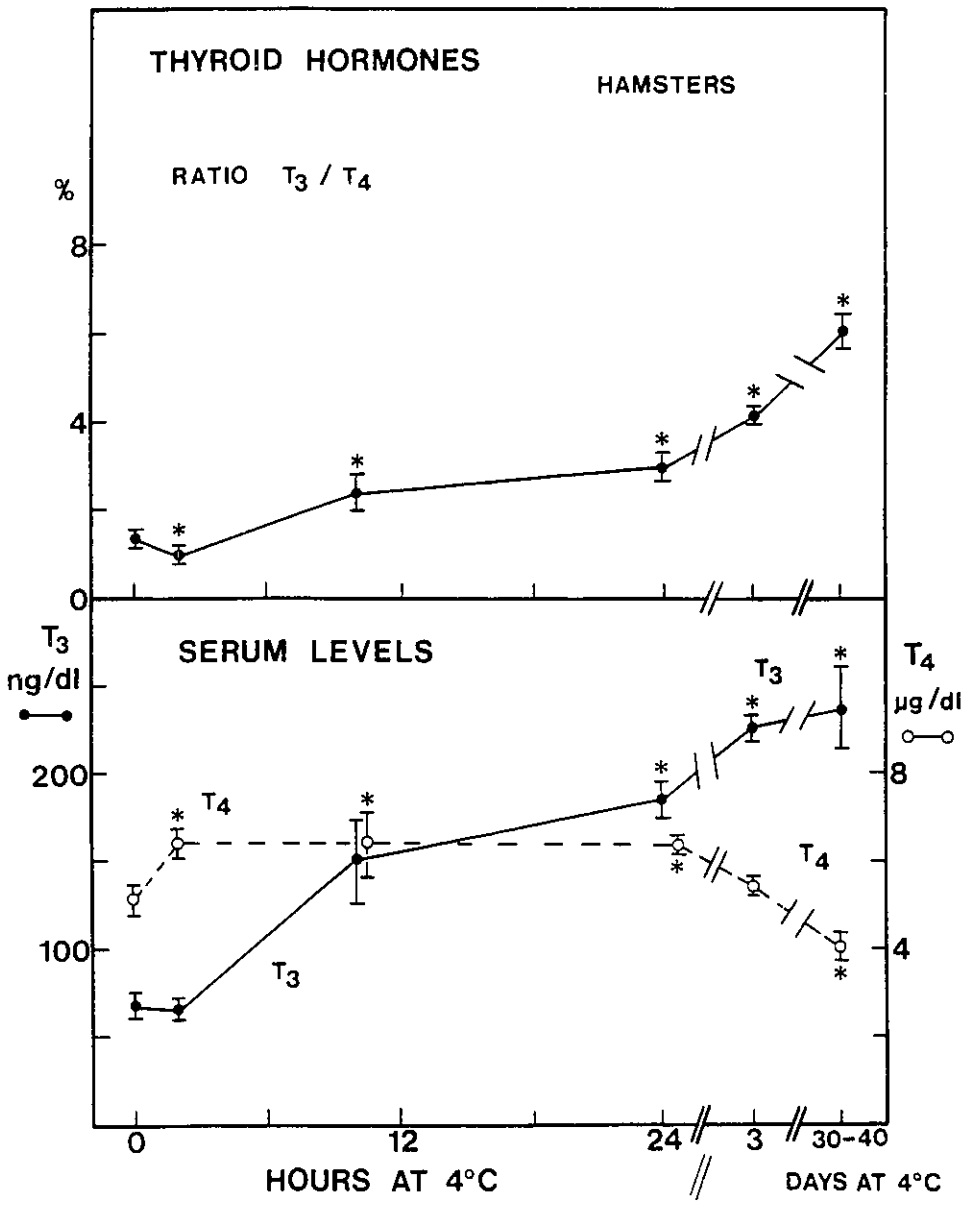


Figure 5. Serum thyroid hormone levels in hamsters during acute or long term cold exposure. Values are means ± SE for animals as indicated in table 1. Cold-induced increases in T_3 are significant at 10 h, 24 h, 3 d, and 30-40 d as indicated by * ($p<0.05$). Cold-induced changes in serum T_4 are significant at 2 h, 24 h, and 3-40 d as indicated by * ($p<0.05$). The ratio of T_3/T_4 (as %) is lower at 2 h but increased at all other times as indicated by * ($p<0.05$).

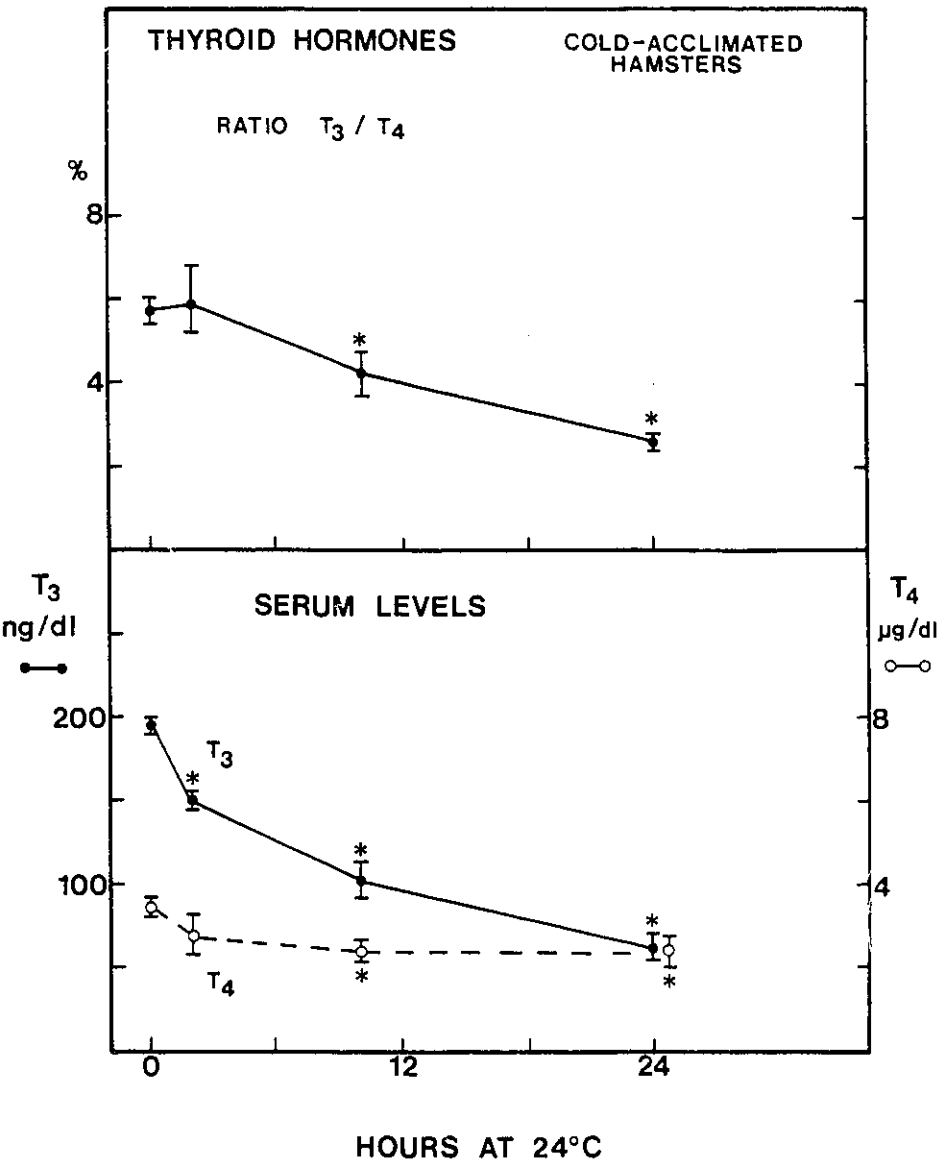


Figure 6. Serum thyroid hormones in cold acclimated hamsters during deacclimation. Values are means ± SE for animals given in table 1. Serum T_3 is decreased at all times compared with time 0 (*, $p<0.05$). Serum T_4 is decreased at 10 and 24 h. T_3/T_4 ratio is significantly reduced at 10 and 24 h (*, $p<0.05$).

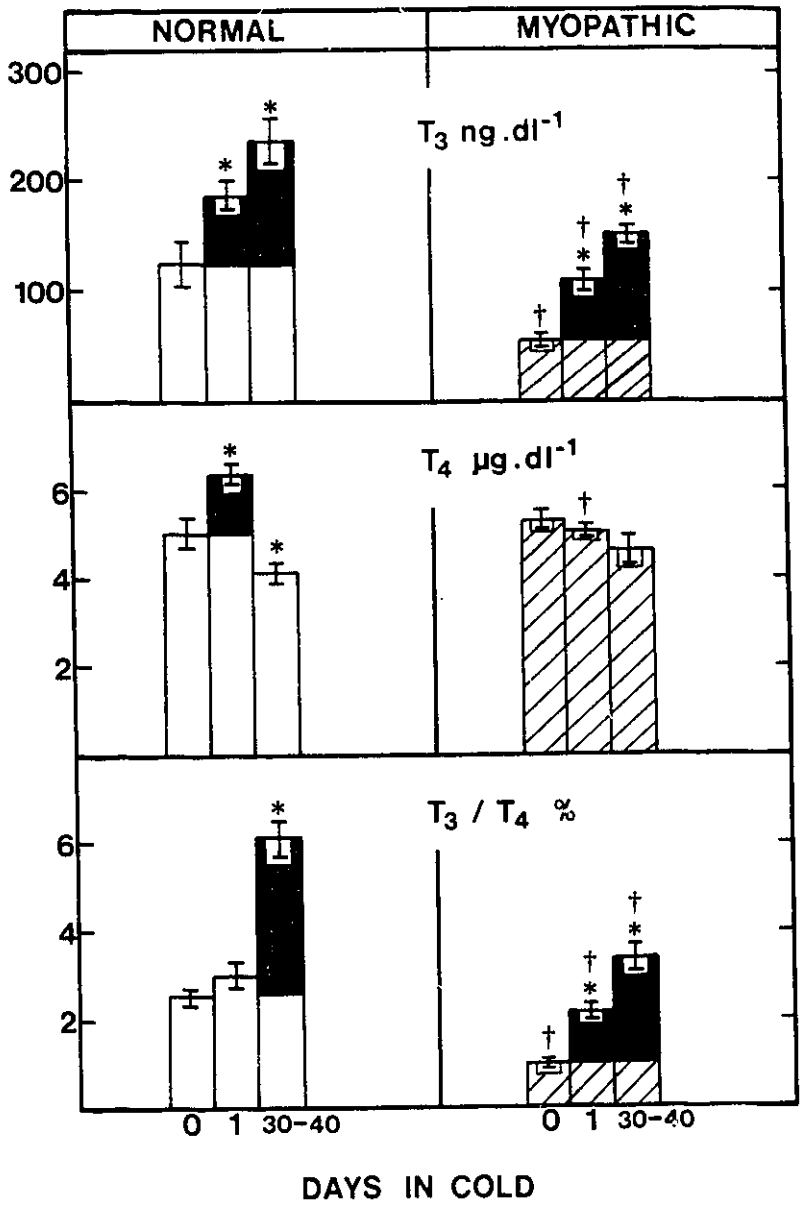


Figure 7. Serum thyroid hormones in normal and myopathic hamsters during acute or long term cold exposure. Values are means ± SE for numbers of animals given in table 3. Open bars represent normal hamsters and shaded bars represent myopathic hamsters. Shaded portions of bars or * indicate significant effect of cold ($p<0.05$). † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

1B: EFFECT OF DIET ON BAT GROWTH:

Background:

At the time this experiment was started it had been reported that hamsters will become obese without overeating (Wade, 1982; Wade and Bartness, 1983; Triandafillou et al, 1984b; Bartness and Wade, 1984a; Hamilton et al, 1986), and exhibit growth of brown adipose tissue (see INTRODUCTION, section 3 diet-induced growth) without thermogenic activation (Triandafillou et al, 1984b). However, myopathic hamsters fail to respond with diet-induced BAT growth (Triandafillou et al, 1984b).

Objectives:

The objectives of this study were to determine if diet-induced growth of BAT involves stimulated deiodinase activity as it did in cold-induced growth (see 1A), and whether the response is abnormal in myopathic hamsters.

Methods:

Normal male golden and cardiomyopathic hamsters were obtained from Canadian Hybrid Farms at 4-5 weeks of age. They were housed individually as described in the METHODS section.

For the high fat diet experiment 16 normal and 16 myopathic hamsters were used at 6-8 weeks of age, and given access to unsalted, unroasted sunflower seeds plus chow. The energy density of the sunflower seeds is given in the METHODS. Equal numbers of normal and myopathic hamsters fed chow alone served as controls. Hamsters were killed after 1, 3, 6, or 12 weeks on the diets.

Food intakes and body weights were recorded weekly.

The hamsters were killed by decapitation shortly after lights on, trunk blood collected, and serum frozen for determination of thyroid hormone levels. Interscapular BAT was removed, cleaned and weighed, then homogenized as described (see METHODS). An aliquot was taken for protein determination and the remainder rehomogenized and frozen for subsequent assay of deiodinase activity. Gonadal white adipose tissue was weighed to estimate body fatness. Assays for protein, deiodinase activity and thyroid hormones were performed as described in the METHODS section. Data were analyzed using two or three way analysis of variance and modified Duncan's multiple range test post hoc. The level of significance was 0.05.

Results :

Food Intake and Body Weight:

Both normal and myopathic hamsters consumed approximately 25% more calories during the first week on the high fat diet than did those on chow alone (table 5). Despite this there were no significant differences in weight gain during that time (table 6). Normal fat-fed hamsters continued to overeat by about 20% up to week 12, and after 6 weeks they gained more weight than controls.

Myopathic hamsters fed sunflower seeds did not overeat after the first week (table 5) nor did they gain more weight compared to controls (table 6).

White Adipose Tissue Weight:

Gonadal white fat was increased by the high fat diet in normal but not in myopathic hamsters, which were in general, leaner than normal (table 6).

Brown Adipose Tissue Weight and Protein Content:

Brown fat weight and protein content increased in fat-fed normal hamsters, reaching significant values by week 6 (table 7). High fat feeding had no effect on brown adipose tissue weight or protein content in myopathic hamsters. In addition, myopathic hamsters had lower BAT weight and protein content than normal.

Deiodinase Activity:

In sunflower seed-fed normal animals there was a trend to decreasing specific deiodinase activity with increasing time on the diet but the differences were not statistically significant. There were no significant differences in deiodinase activity between normal and myopathic hamsters. Total BAT deiodinase activity was unchanged by diet in normal hamsters (table 8). There was a trend (nonsignificant) towards a decrease in total activity in myopathic hamsters on both diets over time. Differences in total activity between normal and myopathic hamsters were not significant.

Serum Thyroid Hormones:

Serum T_3 was unchanged by diet in both normal and myopathic hamsters (table 9). Myopathic hamsters were hypothyroid with respect to T_3 levels. Serum T_4 was depressed in fat-fed normal hamsters at 3 and 6 weeks, and at 1, 3, and 12 weeks in fat-fed myopathic hamsters (table 9).

Discussion:

The major finding of this experiment was lack of effect of diet on deiodinase activity in both normal and myopathic hamsters and lack of change in serum T_3. Decreased serum T_4 with no change or a decrease in

BAT deiodinase activity suggests decreased secretion from the thyroid gland, perhaps as a result of some high fat-derived signal acting at the hypothalamic level. Decreased specific deiodinase activity was observed in another study, where the high fat diet also produced an increase in uncoupling protein (Kopecky et al, 1986b), suggesting that under these circumstances deiodinase activity and subsequent T_3 production are not required to produce increased UCP levels. It can be concluded that increased endogenous T_3 production in BAT by the deiodinase is not an essential accompaniment of the hypertrophy induced by diet.

Thermogenic capacity as measured by noradrenaline-stimulated oxygen consumption is increased in hamsters on a high fat diet, as are BAT weight, protein, and DNA contents (Wade, 1982; present experiments). However thermogenic activity may or may not be stimulated, depending on the diet composition and length of time it is fed (Triandafillou et al, 1984a, b; Kopecky et al, 1986a, b; Schimmel and McCarthy, 1985; Hamilton et al, 1986).

Experiments with hibernating chipmunks have shown that animals consuming a diet rich in polyunsaturated fatty acids such as sunflower seeds (66% linoleic acid, <10% saturated fatty acids) have a lower metabolic rate during torpor, lower mimimum body temperature, and longer torpor bouts than animals on control or saturated fat diets (Geiser and Kenagy, 1987). This would allow the animal to conserve energy during hibernation for use in rewarming during arousal. Data on the natural diet and metabolism of wild hamsters is scarce or lacking so one must speculate on the adaptive significance of the observed

effects of a high fat diet. Hamsters eating a lipid-rich seed diet would increase thermogenic capacity but not thermogenic activity in preparation for winter. They would decrease resting metabolic rate and become obese in order not to place an undue burden on their food hoard, and they would use their increased thermogenic capacity and brown adipose tissue for rewarming from numerous short hibernation bouts.

Myopathic hamsters did not respond to the high fat diet in general as evidenced by failure to gain weight, and in particular by failure of brown adipose tissue growth (increase in protein content and weight) as observed before (Triandafillou et al, 1984a). Deiodinase activity was not significantly different from normal, indicating that failure of BAT growth in response to the diet was not due to a defect in deiodinase per se. The hypothyroidism of myopathic hamsters could result from lower total deiodinase activity in their smaller brown fat, and possibly other tissues such as liver. Alternatively, it could result from impaired feedback responses at one or more control points of thyroid hormone secretion. Hypothyroidism, coupled with resistance to thyroxine actions on certain tissues (Hegyvary et al, 1979) could lead to a decreased sensitivity to the actions of catecholamines, alone or in combination with other factors.

Table 5

Food Intake of Normal and Myopathic Hamsters Fed Chow or a High Fat Diet

		TIME ON DIET (Weeks)			
ANIMAL		1	3	6	12
NORMAL	CHOW	23.8±1.1	22.6±1.6	26.1±0.7	26.6±0.5
	HFD	31.6±.03*	29.9±1.5*	32.1±0.9*	31.2±0.7
MYOPATHIC	CHOW	22.0±.04	27.2±0.6	27.0±1.4	30.5±0.9
	HFD	29.9±2.8*	27.9±0.6	31.2±1.0	32.1±1.0

Values in kcal/d are means ± SE for 4 animals per group. HFD is high fat diet of chow plus sunflower seeds. * indicates significant effect of diet ($p<0.05$). There was no significant difference between normal and myopathic hamsters.

Table 6

Body Weight Gain and White Adipose Tissue in Normal and Myopathic Hamsters Fed Chow or High Fat Diet

		TIME ON DIET (Weeks)			
		1	3	6	12
Gain (g)					
NORMAL	**CHOW**	7.0±1.7	11.1±1.1	25.4±3.1	51.6± 6.7
	HFD	15.4±6.4	26.4±2.8	42.7±8.9*	70.7± 6.6*
MYOPATHIC	**CHOW**	2.6±1.3	12.6±0.8	34.6±7.7	46.9±11.8
	HFD	4.8±0.4	14.8±0.7	25.3±3.2†	54.6± 5.5†
White Adipose Tissue (mg)					
NORMAL	**CHOW**	1300.8± 20.9	1516.8± 83.6	1773.4±284.5	2144.5±212.7
	HFD	1813.7± 85.3	2684.1±150.5*	2905.2±339.4*	4598.7±604.3*
MYOPATHIC	**CHOW**	739.8± 98.4	982.5± 61.3	982.8± 21.6†	938.2±135.0†
	HFD	1110.3±122.3†	1188.7± 95.0†	1092.8± 75.2†	1320.7± 61.2†

Values are means ± SE for 4 animals per group. HFD is high fat diet of chow plus sunflower seeds. * indicates significant effect of diet and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

Table 7

Brown Adipose Tissue in Normal and Myopathic Hamsters Fed Chow or High Fat Diet

		TIME ON DIET (Weeks)			
ANIMAL		1	3	6	12
BAT Weight (mg)					
NORMAL	**CHOW**	236.3±24.2	304.2±32.6	348.2± 9.9	423.5±35.4
	HFD	290.8±32.8	345.9±18.2	438.4±50.2*	599.5±52.8*
MYOPATHIC	**CHOW**	134.3± 6.5†	169.1±11.3†	162.7± 7.3†	180.2±12.1†
	HFD	135.9±12.1†	159.8±15.8†	165.4±15.8†	181.3± 9.1†
BAT Protein (mg)					
NORMAL	**CHOW**	19.0± 1.4	17.. ± 1.8	26.1± 1.7	28.3± 2.8
	HFD	21.1± 5.1	19.1± 2.2	34.0± 3.5*	50.8± 2.7*
MYOPATHIC	**CHOW**	11.4± 0.6†	13.5± 2.8	11.4± 0.6†	12.2± 1.7†
	HFD	11.7± 1.0†	11.8± 0.8	12.8± 1.0†	13.8± 1.0†

Values are means ± SE for 4 animals per group. See table 6 for symbols and abbreviations.

Table 8

Thyroxine 5'-Deiodinase Activity in BAT of Normal and Myopathic Hamsters Fed Chow or High Fat Diet

			TIME ON DIET (Weeks)			
ANIMAL			1	3	6	12
NORMAL	CHOW	SA	0.33±0.03	0.45±0.15	0.18±0.05¶¶	0.21±0.06¶¶
		TA	6.18±0.48	7.70±4.10	3.80±1.30	5.41±0.82
	HFD	SA	0.31±0.10	0.29±0.04	0.20±0.04	0.12±0.04
		TA	5.09±1.51	5.50±1.51	4.95±0.80	6.44±1.95
MYOPATHIC	CHOW	SA	0.36±0.06	0.32±0.09	0.23±0.06	0.16±0.04
		TA	4.24±0.89	5.09±2.02	2.49±0.33	1.94±0.51
	HFD	SA	0.52±0.14	0.32±0.07	0.20±0.02¶	0.18±0.04¶
		TA	6.44±2.15	3.71±0.65	2.59±0.26	2.42±0.52

Values are means ± SE of 4 animals per group. SA is specific activity in pmol/h/mg protein and TA is total activity in pmol/h. HFD is high fat diet of chow plus sunflower seeds. ¶¶ indicates significant difference vs 3 weeks and ¶ indicates significant difference vs 1 week. There were no differences between normal and myopathic hamsters. There were no significant effects of diet.

Table 9

Serum Thyroid Hormones in Normal and Myopathic Hamsters Fed Chow or High Fat Diet

ANIMAL		TIME ON DIET (Weeks) 1	3	6	12
T_3 (ng/dl)					
NORMAL	CHOW	78.2±7.9	82.7± 8.9	86.5±5.7	99.2±4.9
	HFD	75.5±4.6	79.5±10.1	89.0±7.5	80.7±9.7
MYOPATHIC	CHOW	44.2±4.8†	29.7± 3.9†	35.5±3.3†	45.5±2.9†
	HFD	54.5±4.6†	44.7± 4.3†	42.5±3.2†	43.2±5.0†
T_4 (µg/dl)					
NORMAL	CHOW	2.6±0.4	2.7±0.4	3.2±0.4	2.6±0.2
	HFD	1.8±0.4	1.6±0.2*	2.1±0.2*	2.1±0.2
MYOPATHIC	CHOW	2.9±0.2	2.6±0.3	3.1±0.4	3.4±0.3
	HFD	1.5±0.2*	1.6±0.3	1.8±0.1*	2.2±0.2*

Values are means ± SE for 4 animals per group. For abbreviations and symbols see table 6.

1C: EFFECT OF PHOTOPERIOD ON BAT GROWTH:

Background:

It was known when this experiment was begun that exposing hamsters to short photoperiod (less than 12.5 h per d) produces BAT growth (see INTRODUCTION, section 3 short photoperiod-induced growth) and increases thermogenic capacity (Hoffman et al, 1965; Bartness and Wade, 1984; Wade and Bartness, 1984). Myopathic hamsters on the other hand, do not grow more brown fat in response to stimulatory photoperiods (Triandafillou et al, 1984b).

Objectives:

The major objectives of this experiment were to determine whether changes in thyroxine 5'-deiodinase activity accompany trophic responses of BAT to short photoperiods, and if deiodinase activity is abnormal in short photoperiod exposed myopathic hamsters.

Methods:

Normal male golden hamsters and cardiomyopathic hamsters were obtained from Canadian Hybrid Farms at 4-5 weeks of age. They were housed individually as described in METHODS.

Eight normal and 8 myopathic hamsters were housed under 4:20 (L:D, lights on at 8:30) and fed chow for 12 weeks. Eight of each animal type kept under 14:10 (L:D) were the controls. Body weights were recorded weekly.

The hamsters were killed by decapitation shortly after lights on, trunk blood collected, and serum frozen for determination of thyroid hormone levels. Interscapular BAT was removed, cleaned and weighed, then homogenized as described (see METHODS). An aliquot was taken for

protein determination and the remainder rehomogenized and frozen for subsequent assay of deiodinase activity. Gonadal white adipose tissue and testes were weighed to estimate body fatness and to check for the effectiveness of the short photoperiod in causing gonadal regression. Assays for protein, deiodinase activity and thyroid hormones were performed as described in the METHODS section. Data were analyzed using two way analysis of variance and modified Duncan's multiple range test post hoc. The level of significance was 0.05.

Results:

Body Weight:

Short photoperiod had no effect on body weight gain in either animal type (table 10).

White Adipose Tissue and Testes Weights:

White fat weight was decreased by exposure of normal hamsters to short photoperiod but no change was observed in myopathic hamsters. Myopathic animals were generally leaner than normal. Exposure of hamsters to short photoperiod for 12 weeks caused marked testicular regression. Only one myopathic hamster failed to show testicular regression (table 11).

Brown Adipose Tissue Weight and Protein Content:

Short photoperiod exposure was without effect on BAT weight in both normal and myopathic hamsters. It did increase protein content in normal hamsters but not in myopathic hamsters. Myopathic hamsters had less BAT than normal (table 12).

Deiodinase Activity;

Specific deiodinase activity was unchanged by photoperiod in both

animal types, and there was no difference between normal and myopathic hamsters. Total deiodinase activity was increased by short days in normal hamsters but not in myopathic hamsters. Total activity in control myopathic hamsters was less than in normal controls (table 13).

Serum Thyroid Hormones:

Short photoperiod increased serum T_3 levels in both types of animal, but did not normalize the levels in the myopathic hamsters (table 14). Serum T_4 was decreased by short photoperiod in normal hamsters but increased in the myopathic animals (table 14).

Discussion:

The principal result of this experiment was a lack of change in specific deiodinase activity in BAT of normal and myopathic hamsters exposed to short photoperiod for 12 weeks. Total activity was increased in normal hamsters because of tissue growth but not in myopathic hamsters, in which BAT did not grow in response to short photoperiod as seen previously (Triandafillou et al, 1984b). It is concluded therefore, that increased endogenous T_3 production in BAT is not an essential accompaniment to the hypertrophied state induced by short photoperiod. Serum T_3 was increased possibly due to the increased total deiodinase activity but more likely due to increased peripheral deiodination elsewhere, possibly in the pineal (Tanaka et al, 1986; Guerrero et al, 1988; Tanaka et al, 1987). Serum T_4 was depressed in short day-housed normal hamsters and is well documented in both short day-housed and melatonin treated hamsters (Petterborg et al, 1984; Vriend and Reiter, 1977; Vriend et al, 1977; Vriend et al, 1982; Vriend, 1983, 1984, 1985). Short photoperiod or exogenous melatonin

reduces circulating T_4 by a pineal-dependent process, as pinealectomy can reverse the effect (Vriend et al, 1977; Vriend and Reiter, 1977; Vriend and Wilber, 1983). The site of melatonin action is postulated to be within the hypothalamus; autoradiography with labelled melatonin has revealed high affinity binding sites in the suprachiasmatic nucleus and median eminence (Vriend, 1978; Relkin, 1978; Reiter et al, 1981; Vanecek et al, 1987).

Myopathic hamsters are hypothyroid (Kopecky et al, 1986a, b; this experiment) but short photoperiod can raise serum T_3, though not to normal (this study; Sigurdson and Himms-Hagen, 1988), indicating that peripheral deiodination does occur although not in brown adipose tissue.

Failure of trophic responses of BAT to short photoperiod in myopathic hamsters could result from a central defect, but one that does not interfere with melatonin action to induce gonadal regression. Perhaps in myopathic hamsters pineal-thyroid interactions are more sensitive to the effects of the defect than are pineal-gonad interactions.

Table 10

Body Weight Gain in Normal and Myopathic Hamsters Exposed to Long or Short Photoperiod for 12 Weeks

		NORMAL	MYOPATHIC
Weight Gain	**LONG**	49.3±4.3	42.3±2.0
	SHORT	41.2±4.9	37.1±2.8

Values are mean body weight gain (g) ± SE of 8 animals per group. Long photoperiod was 14:10 (L:D); short photoperiod was 4:20 (L:D). There was no significant effect of photoperiod and no difference between normal and myopathic hamsters.

Table 11

Gonadal White Adipose Tissue and Testes Weights of Long and Short Photoperiod Exposed Normal and Myopathic Hamsters

		NORMAL	MYOPATHIC
WAT (mg)	LONG	2327.6±234.9	789.6± 85.2†
	SHORT	1614.9±255.7*	610.9± 31.0†
Testes (mg)	LONG	2932.8±143.1	2836.8±140.7
	SHORT	347.7± 35.2*	650.3±302.5*

White adipose tissue (WAT) and testes weights in normal and myopathic hamsters adapted to long (14:10) or short (4:20) photoperiod for 12 weeks. In one myopathic hamster there was failure of testes to regress with short photoperiod. * indicates significant effect of short photoperiod and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

Table 12

Brown Adipose Tissue Growth in Long and Short Photoperiod Adapted Normal and Myopathic Hamsters

		NORMAL	MYOPATHIC
Weight (mg)	**LONG**	476.2±28.0	158.0±13.7†
	SHORT	549.2±47.6	199.2±14.1†
Protein (mg)	**LONG**	39.4± 3.1	16.9± 1.8†
	SHORT	52.6± 4.8*	20.9± 2.1†

Brown adipose tissue weight and protein content in long and short day housed normal and myopathic hamsters. Values are means ± SE for numbers of animals given in table 10. For details see table 11.

Table 13

Thyroxine 5'-Deiodinase Activity in Normal and Myopathic Hamsters Exposed to Long or Short Photoperiod for 12 Weeks

		NORMAL	MYOPATHIC
DEIODINASE ACTIVITY			
SA	LONG	0.33±0.03	0.35±0.02
	SHORT	0.38±0.05	0.42±0.03
TA	LONG	12.7±1.0	5.8± 0.6†
	SHORT	19.2±2.7*	9.0± 1.4†

Values are means ± SE of 8 animals per group. SA is specific activity in pmol/h/mg protein and TA is total activity in pmol/h. Long photoperiod is 14:10 (L:D) and short photoperiod is 4:20 (L:D). * indicates significant effect of short photoperiod and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

Table 14

Serum Thyroid Hormones in Normal and Myopathic Hamsters Exposed to Long or Short Photoperiod for 12 Weeks

		NORMAL	MYOPATHIC
T_3 (ng/dl)	LONG	68.4±2.6	39.2±2.3†
	SHORT	102.2±8.9*	59.0±3.2*†
T_4 (µg/dl)	LONG	2.1±0.2	2.1±0.1
	SHORT	1.3±0.1*	2.7±0.2*†

Values are means ± SE for 8 animals per group. For details see table 11.

SUMMARY: 1. GROWTH OF BROWN ADIPOSE TISSUE:

Exposure of hamsters to cold stimulates growth of brown adipose tissue and increases GDP-binding, suggesting thermogenic activation. It increases UCP concentration and T5'D concentration suggesting a selective increase in synthesis of these two proteins. In addition the animals rapidly become hyperthyroid and remain so during cold acclimation.

Feeding a palatable high fat diet produces brown adipose tissue hypertrophy without thermogenic activation and without increased deiodinase activity, or alterations of thyroid hormone levels.

Short photoperiod exposure likewise induces a trophic response of hamster brown adipose tissue without stimulation of thermogenesis or any increase in deiodinase activity. Short photoperiod decreases hamster serum T_4 levels.

Myopathic hamster brown adipose tissue can be stimulated to grow by cold but it does not grow to the same extent as in normal hamsters, always remaining smaller than normal. In addition myopathic hamsters are hypothyroid and somewhat cold sensitive, although there does not appear to be a defect in the response of the deiodinase to sympathetic stimulation in the cold. Brown fat from myopathic hamsters does not grow in response to either high fat diet or short photoperiod. Since there is no increase in T5'D in normal BAT in response to these stimuli, the defect in the myopathic hamster cannot be ascribed to a failure to generate T_3 in BAT.

The results of this first part of the research raised a number of interesting questions that warranted further investigation:

1) What, if any, is the role of elevated serum T_3 in the increased thermogenesis in cold acclimated hamsters? Experiments designed to study this are described in section 2.

2) What mediator(s) might be involved in BAT growth and are they the same for diet and photoperiod as for cold? Experiments designed to study this are described in sections 3A, 3B, 3C, 3D, and 3E.

3) Is there a relationship between low serum T_3 level in the myopathic hamster and its blunted thermogenic and trophic responses of BAT to stimulation? Experiments designed to study this are described in section 3F.

2: ROLE OF THYROID HORMONES IN THERMOGENESIS IN THE COLD-ACCLIMATED HAMSTER:

Background:

Cold exposed hamsters rapidly become hyperthyroid (defined as increased T_3) and maintain this condition as long as they remain in the cold (see 1A, figure 5; Kopecky et al, 1986a; Tomasi and Horwitz, 1987). In addition the activity of brown adipose tissue thyroxine 5'-deiodinase is greatly stimulated (1A, figure 2; Kopecky et al, 1986a). When the cold acclimated hamster is returned to a warmer temperature the elevated serum T_3 level declines to normal in about 24 h (see 1A, figure 6; Kopecky et al, 1986a). This decline correlates well with the fall in BAT deiodinase activity observed during deacclimation of cold acclimated hamsters (see 1A, figure 3; Kopecky et al, 1986a). The apparent hyperthyroid state of the cold acclimated hamster suggests that at least some portion of cold-induced nonshivering thermogenesis in this animal may occur as thyroid hormone-induced thermogenesis and in organs other than BAT.

Objectives:

The objectives of this experiment were to assess whether the raised level of T_3 in the serum exerts a thermogenic effect in the cold acclimated hamster, by measuring minimal oxygen consumption, MOC (or basal metabolic rate, BMR) in temperature-maintained anesthetized animals. Under these conditions all thermoregulatory thermogenesis would be suppressed and oxygen consumption influenced only by thyroid status (Denckla and Marcum, 1973) Another objective was to try to correlate the expected decrease in minimal oxygen consumption when cold acclimated hamsters are returned to a warmer temperature with the time

course of T_3 decline described in 1A, figure 6.

Methods:

Male golden hamsters 5-6 weeks of age were housed individually as described. One group was maintained at 4°C for 6-8 weeks. The animals were then transferred from 4°C to 24°C for 12, 24, 48, or 72 h, or studied immediately after removal from the cold. Warm acclimated hamsters were those kept at 24°C and were always studied at the same time as the cold acclimated hamsters.

The hamsters were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and oxygen consumption was measured at an ambient temperature of 22-25°C. Body temperature was maintained at 36.7±0.5°C using a rectal thermister probe, proportional temperature controller and 2 lamps (see METHODS). Recording was started when a stable rate of oxygen consumption was reached, about 20 minutes after pentobarbital injection, and was continued for 20-40 minutes. Data were analyzed by analysis of variance and Duncan's tests with a significance level of 0.05.

Results:

The oxygen consumption of anesthetized cold acclimated hamsters was 9.2 ml O_2/min/kg$^{0.75}$, 59% greater than the warm acclimated value of 5.8 (figure 8). Upon removal to 24°C the elevated metabolic rate slowly declined. Even 3 d after removal from the cold, oxygen consumption was still elevated with respect to the warm acclimated animals.

Discussion:

The main result of this experiment, namely that cold acclimated

hamsters have a markedly elevated minimal oxygen consumption, provides evidence for the suggestion that the high level of T_3 in the serum of cold acclimated hamsters does exert a thermogenic influence. The elevated metabolic rate of the cold acclimated hamster declines rather slowly when it is returned to the warm and lags behind the decrease in serum T_3 and deiodinase activity (see 1A, figures 6 and 3; Kopecky et al, 1986a). Thus, the gradual decline in MOC would be expected given the long term nature of the thermogenic actions of T_3 (Van Hardeveld, 1986).

Increases in anesthetized (Bartness et al, 1984) and resting (Tomasi and Horwitz, 1987) metabolic rates have been reported for cold acclimated hamsters returned to a warmer temperature for 1-2 h. Some studies of conscious cold acclimated hamsters that had been returned to the warm for unspecified periods of time have found increases in oxygen consumption (Vybiral and Jansky, 1974; Vybiral et al, 1975; Adolph and Lawrow, 1951) while others have not (Pohl, 1965; Hissa and Hirsimaki, 1971).

The hamster is capable of increasing its metabolic rate several fold over a range of environmental temperatures (Pohl, 1965). Thermoneutrality is 30-32°C for the Syrian hamster (Pohl, 1965; Tomasi and Horwitz, 1987), therefore even at the relatively warm temperature of 24°C the metabolic rate is increased. Metabolic rate further increases about 60% when the temperature is dropped from 24°C to 4°C (Vybiral and Jansky, 1974; Vybiral et al, 1975; Desautels and Dulos, 1986).

Thus a 59% increase in minimal oxygen consumption in cold

acclimated hamsters might be accounted for by thyroid hormone-induced thermogenesis, and might contribute to part of cold-induced thermogenesis. The decline in the elevated metabolic rate and in serum T_3 upon deacclimation are consistent with the nature of thyroid hormone action.

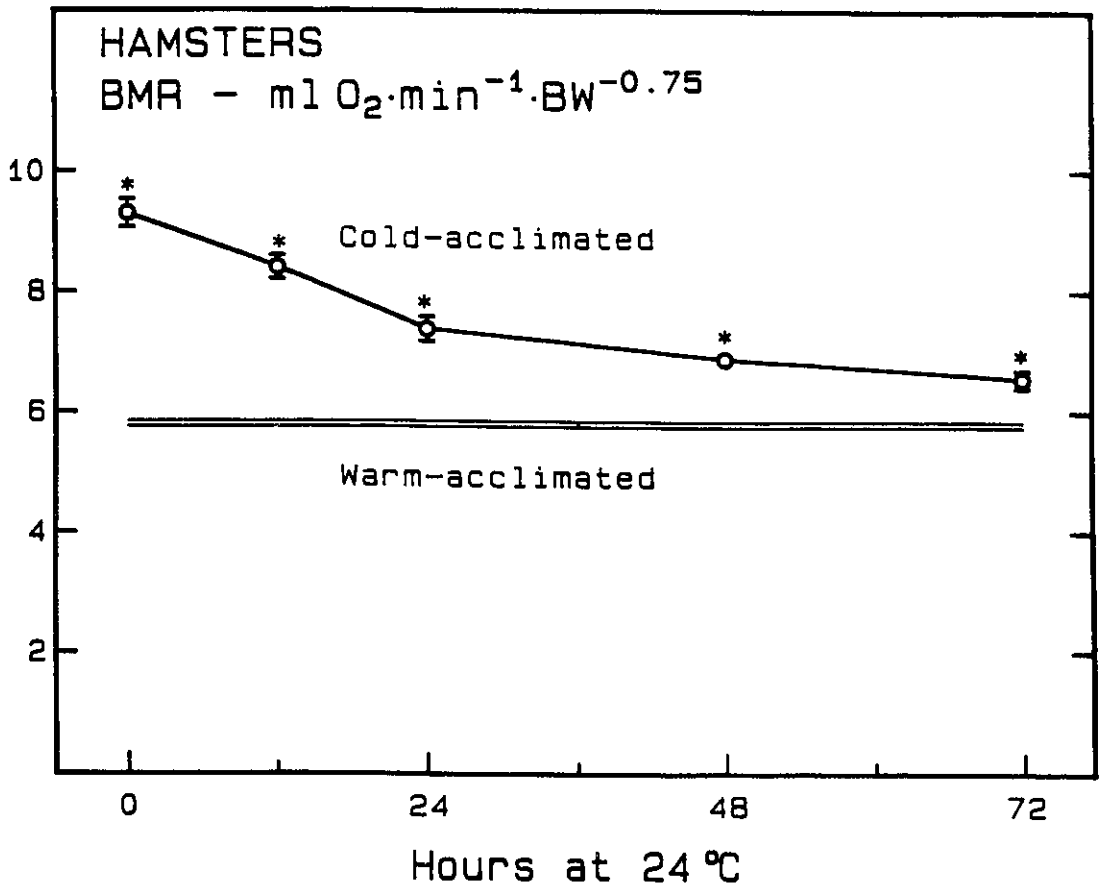


Figure 8. Minimal oxygen consumption of anesthetized warm and cold acclimated hamsters. Values are means ± SE for 7 or 8 hamsters and are expressed in terms of body weight (BW) in $kg^{0.75}$. BMR refers to basal metabolic rate. Where no error bar appears it is too small to be seen. Values for all warm acclimated hamsters were pooled and the 2 lines represent the standard error about the mean of 5.8. At all times the values for cold acclimated hamsters are significanlty greater than values for the warm acclimated animals as indicated by *. The value for 0 h is significantly greater than that for 12 h, which in turn is significantly greater than that for 24 h, but the 24 h value is not significantly different from the 48 or 72 h values.

3. MEDIATORS OF BROWN ADIPOSE TISSUE GROWTH:

3A: ROLE OF NORADRENALINE AS A MEDIATOR OF BAT GROWTH:

Background:

In rats, the rich sympathetic innervation of brown adipose tissue controls acute thermogenic responses to cold, and mediates tissue hypertrophy during acclimation to cold (see INTRODUCTION, section 3 cold-induced growth). Noradrenaline does stimulate respiration in isolated hamster brown adipocytes, through both α_1- and β-adrenergic receptors (Mohell et al, 1980, 1983; Schimmel et al, 1983; Nånberg and Putney, 1986). However, the role of noradrenaline as a mediator of hamster brown adipose tissue growth has not been clearly established, as injections of NA fail to promote tissue hypertrophy (Triandafillou et al, 1984a). Denervation, however, does produce atrophy of hamster BAT. Thus, a role for the nerves seems likely.

The marked increase in hamster BAT deiodinase that occurs in response to cold precedes the increase in GDP-binding and is of greater magnitude than the response of rat brown adipose tissue (Kopecky et al, 1986a). Thus deiodinase activity in hamster BAT can be used as an index of the early phase of response to stimulation. It is known for the rat that this response is mediated by noradrenaline (Silva and Larsen, 1983, 1986).

Objectives:

One objective was to determine whether a single injection of noradrenaline could influence BAT as measured by changes in thyroxine 5'-deiodinase activity, and whether one dose of NA could provide sufficient stimulus for tissue growth. A second objective was to

determine if a continuous infusion of noradrenaline could induce trophic responses of hamster BAT.

Methods:

Male hamsters were housed as described in METHODS. In the first study noradrenaline (NA) bitartrate (Sigma) was injected s.c. into 6 week old hamsters at doses of 0.4 and 1.6 mg free base NA/kg in a peanut oil suspension approximately 1 h after lights on. The animals were killed 24 h later and interscapular BAT was homogenized for protein and deiodinase assays as described (see METHODS). In a separate experiment noradrenaline was injected at a dose of 3.2 mg free base/kg in peanut oil and the hamsters were killed 6 or 24 h later. Interscapular BAT was processed for protein and deiodinase assays as before.

In the second study, noradrenaline bitartrate was dissolved in 0.9% NaCl with 0.02% ascorbic acid as preservative and used to fill mini osmotic pumps (Alzet from Alza). The pumps were implanted under the skin along the dorsal midline and delivered 3.33 or 10 ng/min/g BW for 1 d or 1.66, 3.33, or 10 ng/min/g BW for 5 d, based on 85 g starting weight to give 0, 400, or 1200 µg NA, or for 5 d to give 0, 1000, 2000, or 6000 µg NA in total. Food intake and body weight were recorded. The hamsters were killed by decapitation and brown adipose tissue removed, cleaned and homogenized for protein and deiodinase assays and homogenate uncoupling protein determination. Body temperatures were recorded immediately after death. Heart and white adipose tissue were removed for weighing to assess the effects of noradrenaline on other tissues, and blood was collected for assay of

thyroid hormones.

Data from these studies were analyzed using t-tests whenever group size was greater than 2, and data from the infusion study were analyzed using analysis of variance followed by Duncan's multiple range test at a significance level of 0.05.

Results:

Acute Noradrenaline Injections:

Twenty-four hours after a single injection of 0.4 mg/kg NA, BAT specific deiodinase activity was increased by 31% and total activity increased 34% (table 15). Higher NA doses had no effect on deiodinase activity. Brown adipose tissue protein was not significantly altered within 24 h of injection (table 15). However, the deiodinase values for the control group were higher than expected. This could have resulted from the stress of injection which would have raised sympathetic activity and stimulated T5'D. Hence, the increases seen were smaller than might otherwise have been achieved.

Noradrenaline Infusions:

Food intake decreased during the first 2 days of 400 and 1200 µg/d NA infusion but recovered by the 5th day (table 16). Body weight was reduced by the highest NA dose after 5 days of treatment and overall, hamsters receiving 400 and 1200 µg NA/d lost weight relative to the controls (table 16). Body temperature was not affected by noradrenaline infusion (table 17). White adipose tissue was significantly reduced by the 5 d infusion of NA (table 17). Heart weight was not affected (table 17). BAT weight had a tendency to decrease with increasing NA dose but the differences were not

statistically significant (table 17). The highest NA dose decreased BAT protein after 5 days of infusion (table 18), most likely due to tissue necrosis observed near the infusion pump and in portions of the brown fat. Infusion of 400 µg/d NA for 1 d increased both specific and total BAT deiodinase activities but 6000 µg NA (1200 µg/d for 5 d) increased specific activity only (table 18). Uncoupling protein concentration tended to increase with low doses of NA but the higher doses actually resulted in decreases, presumably because of tissue damage (table 19). Because of small group size none of the differences were statistically significant. Serum T_3 was not significantly changed by noradrenaline infusion but there was a trend towards decreasing T_4 with increasing dose of NA (table 18). The only statistically significant reduction was seen with the dose of 2000 µg NA.

Discussion:

Noradrenaline was effective in increasing BAT deiodinase activity 24 h after a single injection of a 0.4 mg/kg dose. Noradrenaline can thus elicit a response in hamster brown adipose tissue, as measured by the increase in specific and total deiodinase activities, as well as by a rise in tissue temperature upon NA injection (Horowitz et al, 1982). Isolated brown adipocytes from hamsters can also respond to noradrenaline by increasing oxygen consumption (Nedergaard, 1982) so it appears probable that noradrenaline is indeed the mediator of acute thermogenic responses in hamster BAT.

In these studies single doses of NA lower or higher than 0.4 mg/kg did not alter deiodinase activity, therefore a more extensive experiment utilizing a wider range of doses with more animals should be

performed to delineate the sensitivity of the response. In addition a time course experiment should be done to determine when the earliest response of the deiodinase to NA occurs, as has been done in the rat (Silva and Larsen, 1986). Without a more complete time course however it is not possible to say what the temporal pattern of deiodinase stimulation is in hamster BAT and whether it is the same as that during cold exposure.

Single injections of noradrenaline at the doses and times studied were not sufficient to stimulate brown adipose tissue hypertrophy in the hamster, nor was the 5 d infusion sufficient to increase protein content. In fact, too high a NA dose caused tissue damage around the site of infusion.

Mory et al (1984) infused rats with 480 µg NA/d for 9 d (4320 µg total) and were able to produce increases in total BAT protein and UCP, but the doses used in these present studies (3C: 200-1200 µg/d for 5 d in 85 g hamsters = 1000-6000 µg total) had no effect on BAT protein. Lower doses of infused noradrenaline did seem to increase UCP but the sample size was too small to draw any definite conclusions. Clearly a larger number of animals and wider range of noradrenaline doses is required to clarify the interpretation of these results. These NA doses did cause body weight loss and lipid depletion however. The results of this experiment are similar to those of Triandafillou et al (1984a) who injected 0.4 mg/kg NA into hamsters daily for 2 weeks and observed no trophic effect on BAT, but did find weight loss and depletion of lipid stores in treated hamsters.

Table 15

Effect of Acute Noradrenaline Injection on Brown Adipose Tissue

Dose (mg/kg)	CONTROL (pooled)	3.2 (6h)	0.4 (24h)	1.6 (24h)	3.2 (24h)
	(6)	(2)	(4)	(4)	(2)
Protein (mg)	17.4 ±0.9	15.5, 27.4	18.5 ±1.9	19.9 ±0.4	21.3, 23.3
Deiodinase					
SA (pmol/h/mg)	3.7 ±0.4	3.8, 3.1	5.4* ±0.2	4.9 ±0.6	3.5, 3.5
TA (pmol/h)	65.6 ±7.8	58.5, 84.2	99.6* ±10.4	97.7 ±12.2	75.1, 59.1

Effect of acute noradrenaline injection on brown adipose tissue protein and deiodinase activity. Groups of animals were killed 6 or 24 h after injection as indicated and numbers of animals are given in parentheses. Values are means ± SE or actual values in groups of only 2 animals. Some data were not analyzed because of small group size; those analyzed were done by t-test. * indicates significant effect of noradrenaline ($p<0.05$). SA is specific deiodinase activity and TA is total deiodinase activity.

Table 16

Effect of 5 Day Noradrenaline Infusion on Food Intake and Body Weight

NA Dose (µg/5d)		0 (3)	1000 (2)	2000 (3)	6000 (3)
Intake (kcal/d)	1d	18.4±2.1	12.0, 8.9	3.9±1.5*	0.0±0.0*
	2d	24.0±0.7	20.2, 17.8	16.9±1.1*	0.9±0.7*
	5d	27.2±0.5	25.4, 26.4	22.9±1.6	8.4±6.2
Weight (g)	-1d	84.3±3.3	84.9, 73.9	82.4±1.6	82.4±0.5
	1d	85.4±2.8	83.8, 71.5	78.4±0.9	75.1±1.0
	2d	85.7±2.9	84.4, 71.2	78.3±1.3	70.1±1.0
	5d	90.0±3.4	86.1, 75.2	79.9±1.1	61.3±4.2*
Change in Body Weight (g)		+5.6±0.3	+1.2±1.3	-2.4±0.7*	-21.1±4.7*

Effect of noradrenaline infusion on food intake and body weight. Values are means ± SE for number of animals shown in parentheses or actual values in groups of only 2 animals. Measurements were made at the times indicated prior to or during the infusion period. Doses of noradrenaline are given as the total amount infused over the 5 d period. * indicates significant effect of NA ($p<0.05$).

Table 17

Effect of Noradrenaline Infusion on Organ Weights and Body Temperature

Infusion	1 DAY			5 DAYS			
NA Dose (µg total)	0	400	1200	0	1000	2000	6000
	(3)	(3)	(3)	(3)	(2)	(3)	(3)
BAT Weight (mg)	304.9 ±26.9	256.0 ±6.6	245.4 ±34.7	341.8 ±44.0	263.4, 236.2	237.2 ±19.6	185.3 ±35.1
WAT Weight (mg)	1071.0 ±174.5	897.7 ±15.8	791.7 ±127.7	1077.0 ±67.3	1050.0, 487.0	620.3* ±28.7	295.3* ±23.7
Heart Weight (mg)	254.6 ±7.1	269.3 ±9.4	301.7 ±24.7	290.7 ±16.4	315.0, 311.0	330.0 ±11.8	305.3 ±18.2
Temperature (°C)	38.47 ±0.18	38.07 ±0.89	38.43 ±0.37	38.27 ±0.34	37.60, 37.30	37.90 ±0.29	35.30 ±1.46

Effect of noradrenaline infusion on organ weights and body temperature. Values are means ± SE or actual values where group size is only 2. NA dose is given as the total amount infused over the 5 d period. Number of animals is given in parentheses. * indicates significant effect of noradrenaline ($p<0.05$). Some data were not analyzed because of small group size. WAT is white adipose tissue. There was no significant effect of NA on BAT weight, heart weight or body temperature.

Table 18

Effect of Noradrenaline Infusion on Thyroid Hormones and Brown Adipose Tissue Protein and Deiodinase Activity

Infusion	1 DAY			5 DAYS			
NA Dose (µg total)	0	400	1200	0	1000	2000	6000
	(3)	(3)	(3)	(3)	(2)	(3)	(3)
T_3 (ng/dl)	49.0 ±5.0	50.7 ±3.3	66.7 ±19.4	53.0 ±5.7	68, 44	43.0 ±28.0	54.3 ±3.7
T_4 (µg/dl)	2.23 ±0.37	1.80 ±0.33	----	2.29 ±0.45	2.45, ±1.87	0.65,* 0.25	1.68 ±0.23
Protein (mg)	21.9 ±1.0	27.6 ±2.0	25.1 ±2.0	22.1 ±2.3	21.6, 18.4	26.2 ±5.6	12.3* ±2.4
Deiodinase SA (pmol/h/mg)	0.49 ±0.03	1.32* ±0.24	0.88 ±0.24	0.85 ±0.22	0.89 ±0.35	1.40 ±0.20	1.62* ±0.03
TA (pmol/h)	10.76 ±0.82	35.52* ±4.5	22.66 ±6.9	19.75 ±7.2	19.11 ±6.5	38.83 ±13.7	19.89 ±3.9

Effect of noradrenaline infusion on thyroid hormones and brown adipose tissue protein and deiodinase activity. Values are means ± SE for numbers of animals in parentheses or actual values where group size is only 2. NA dose is given as total dose over the 5 d period. SA is specific activity and TA is total activity. dl is decilitres (100 ml). * indicates significant effect of noradrenaline ($P<0.05$). Some data were not analyzed because of small group size. There was insufficient serum to analyze T_4 in the 1200 µg NA dose group.

Table 19

Effect of Noradrenaline Infusion on BAT Homogenate Uncoupling Protein

Infusion	1 DAY			5 DAYS			
NA Dose (µg total)	0	400	1200	0	1000	2000	6000
	(3)	(3)	(3)	(3)	(2)	(3)	(3)
UCP (µg/mg protein)	10.68 ±4.2	13.02 ±1.0	5.13 ±1.2	15.15 ±7.7	52.29, 34.90	35.0 ±4.2	4.09 ±0.28
(µg/BAT)	229.8 ±84.6	362.2 ±47.3	126.7 ±28.7	338.1 ±166.5	1129.5, 642.2	958.1 ±319.2	48.8 ±7.1

Effect of noradrenaline infusion on brown adipose tissue homogenate uncoupling protein (UCP). Values are means ± SE for numbers of animals given in parentheses. There was a trend towards an increase in UCP with lower NA doses but because of small group size the differences were not significant.

3B: ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN MEDIATING BAT GROWTH:

Background:

Injection or infusion of noradrenaline produces BAT hypertrophy in rats (Desautels and Himms-Hagen, 1979; Mory et al, 1980, 1984) but not in hamsters (Triandafillou et al, 1984a, see section 3A). However denervation of hamster BAT results in tissue atrophy suggesting that the sympathetic innervation does have some trophic effect (Triandafillou et al, 1984b).

Objectives:

In order to study further the potential role of NA in the sympathetic nerves in the trophic response of BAT to cold, to diet, and to short photoperiod, sympathetic nervous system activity was assessed by measurement of noradrenaline turnover in brown adipose tissue. The purpose was to determine whether a correlation exists between sympathetic activity and tissue growth. In addition, serum T_3 levels were measured to see if there is a relationship between this hormone and BAT growth. Eventually the objective was to determine whether myopathic hamsters would respond in the same way as normal hamsters, but with initial negative results in normal animals plus the high cost of myopathic hamsters, it was decided that the experimental design would utilize mainly normal hamsters.

Methods:

Normal and myopathic hamsters were housed individually and maintained as described in METHODS.

Three separate experiments were performed in which noradrenaline

turnover in BAT was measured. In experiment a, started in October, control hamsters were maintained as described in METHODS. Three other groups of hamsters were exposed to cold (4°C) for 6 wk, for 24 h, or immediately following injection of [^{3}H]NA. The second experiment, b, was started in October of the following year. Control normal and myopathic hamsters were maintained as described above. Two groups of normal hamsters were exposed to cold for 3 d or 2 wk. One group of normal hamsters at 24°C was fed chow plus sunflower seeds (high fat diet) for 6 wk. In addition one group of normal and one group of myopathic hamsters was exposed to short photoperiod (4:20 L:D, lights on at 0800 h) for 6 wk. In experiment c, done between June and September, control hamsters were housed as described. Groups of normal and myopathic hamsters were fed the chow-sunflower seed high fat diet for 1, 3, or 12 weeks. Other groups of normal and myopathic hamsters were adapted to short photoperiod for 1, 3, or 12 weeks. In all experiments food intake and body weight was recorded weekly.

L-[2,5,6-^{3}H]noradrenaline was diluted to 75 µCi/ml in 0.9% NaCl and injected i.p. at 150 µCi/kg at lights on. Hamsters were killed by decapitation exactly 1, 2, 3, or 4 h after injection. Trunk blood was collected for serum T_3 determination. In some experiments body temperature was measured using a rectal probe and digital thermometer. Interscapular, subscapular and cervical brown adipose tissue was removed, cleaned and frozen quickly either on dry ice or in liquid nitrogen for storage of no longer than one month.

Noradrenaline was extracted as described in METHODS and measured using HPLC. Column effluent was counted for radioactivity by liquid

scintillation counting. The regression coefficient ± standard error for the logarithmic decline in specific radioactivity of tissue NA was calculated by linear regression as described in METHODS. The rate constant (k) was calculated from the slope (K) of the line (k=K x 2.303). Noradrenaline turnover rate was the product of the rate constant and the noradrenaline content of the tissue. The standard error of the turnover rate was calculated as described in METHODS.

Results:

Cold Exposure:

Hamsters living at 4°C for 2 or 6 wk ate more than warm adapted controls (table 20), but despite hyperphagia they weighed less (table 21).

Acute cold exposure for 4 or 24 h reduced total content of noradrenaline in BAT (figure 9). At 3 d and 2 wk in the cold the NA content remained lower but the turnover had increased substantially (figure 9). However by the time the hamsters became cold adapted after 6 wk, both noradrenaline content and turnover had returned to control levels.

Brown adipose tissue noradrenaline concentration (ng NA/mg wet weight) was unchanged during 4 h acute cold exposure but reduced at all other times (figure 10), probably initially because secretion exceeded synthesis, then because tissue hypertrophy (figure 10) exceeded restoration of NA content (figure 9).

During the first 3 days in the cold hamsters were hypothermic but body temperature returned to normal after 2 weeks (figure 11). The hamsters rapidly became hyperthyroid, with serum T_3 rising to three

times normal after only 24 h in the cold and remaining high for the duration in the cold (figure 11).

High Fat Diet:

Normal hamsters consuming the high fat diet overate by about 20% during the first 3 weeks on the diet but myopathic hamsters did not (table 20). Final body weight and weight gain were significantly increased by the high fat diet in normal hamsters (table 21, 22). However diet had no effect on weight gain in myopathic hamsters and at all times they weighed less than their normal counterparts (table 21, 22).

Brown adipose tissue weight was increased by fat feeding in normal hamsters only (figure 12).

Total BAT noradrenaline content was unchanged by diet (figure 12). At all times NA content of myopathic BAT was less than normal because of the smaller tissue size in these animals (figure 12). Total noradrenaline turnover was unaffected by high fat diet in either type of animal (table 23). In myopathic hamsters total NA turnover was less than normal because of smaller BAT mass (table 23). Serum T_3 was not altered by the high fat diet in normal hamsters but diet did increase serum T_3 in myopathic animals to normal (figure 13).

Short Photoperiod:

Exposure to short photoperiod for 12 weeks increased body weight gain in normal hamsters (table 22) but had no effect on food intake (table 20). Short photoperiod exposure for 12 weeks decreased body weight gain in myopathic hamsters (table 22) and food intake was significantly lower in myopathic hamsters at 1 and 12 weeks but not at

other timepoints (table 20). BAT weight was increased by short photoperiod exposure at 3 and 12 weeks in normal hamsters and at 12 weeks in myopathic hamsters (figure 14). Myopathic BAT always weighed less than normal (figure 14). Serum T_3 was increased at 1, 3, and 6 weeks in short day-exposed normal hamsters, and at 6 and 12 weeks in short day-exposed myopathic hamsters (figure 13). However serum T_3 in myopathic hamsters never reached a normal level (figure 13). Short photoperiod affected neither noradrenaline content (figure 14) nor turnover in BAT (table 23) in either type of hamster.

Discussion:

Acute cold exposure did stimulate BAT sympathetic activity because during the first 24 h stimulated noradrenaline secretion occurred at a greater rate than synthesis so content declined. After 3 d in the cold both synthesis and secretion of noradrenaline were stimulated and after 14 d stimulation was even greater, with NA content returning to normal. In a fully acclimated hamster however the elevated noradrenaline turnover had returned to a control level.

Thus the major result of these studies is a lack of correlation between sympathetic nervous activity and growth of brown adipose tissue in response to cold acclimation or adaptation to high fat diet or short photoperiod in Syrian hamsters. Brown adipose tissue protein content was not significantly increased until the animal was fully cold acclimated (30-40 d, see 1A). This is in sharp contrast to sympathetic nervous system stimulation, where sympathetic activity was increased at an intermediate stage in the acclimation process (3 d and 2 wk) but had returned to a low control level by 30-40 d of cold exposure.

Feist (1970) reported increased noradrenaline turnover rates in BAT from cold acclimated and acutely cold exposed hamsters using α-methyl-p-tyrosine to block NA synthesis. The results were based on the decline in noradrenaline content during an initial 2 h period when the linearity of the decline was unknown. Calculations based on the noradrenaline content decline between 2 and 8 h after injection show no differences between warm and cold acclimated animals. The 2-6 h period studied for acutely cold exposed hamsters likewise shows no differences. Therefore the results of these studies do agree with those of Feist in that there were decreases in brown adipose tissue noradrenaline content in acutely cold exposed hamsters and that noradrenaline turnover was unchanged in acutely exposed and cold acclimated hamsters.

The hamster must use mechanisms instead of or in addition to secretion of noradrenaline from sympathetic nerves to maintain its brown adipose tissue in a hypertrophied state. It is possible that some other mediator acting alone or in concert with noradrenaline controls growth and adaptive changes in hamster brown adipose tissue. This could be another sympathetic neurotransmitter, neuropeptide, or circulating hormone or growth factor. The pattern of sympathetic stimulation may also be of importance in mediating the appropriate responses of BAT (Minokoshi et al, 1988).

In a recent study Hamilton et al (1986) reported a reduced noradrenaline turnover in BAT from long term fat-fed hamsters despite tissue hypertrophy and concluded that there was dissociation of sympathetic activity from tissue growth. The results of these

experiments agree with that study, for brown adipose tissue weight was increased but NA content was normal, therefore when expressed per milligram of tissue noradrenaline turnover was reduced by high fat diet feeding.

In another study, McElroy and Wade (1986) exposed hamsters to short photoperiod (8:16 L:D) for 3 months and found no change in noradrenaline turnover in BAT or heart despite increases in protein, DNA, cytochrome oxidase, and GDP-binding. Similar results were obtained by Viswanathan et al (1986). The results of this series of experiments agree with these published reports. It is possible that a transient stimulation of sympathetic nervous activity could have occurred before the earliest time point studied. This is unlikely because if such a potent stimulator of BAT as cold requires at least 3 d to increase sympathetic activity, then short photoperiod which requires at least 10 weeks to affect the gonads should be slower in stimulating brown adipose tissue. However until noradrenaline turnover is studied after very brief periods of short photoperiod exposure, this cannot be ruled out.

Hamsters rapidly become hyperthyroid when exposed to cold (present experiments; 1A; 1B; Kopecky et al, 1986a, b; Tomasi and Horwitz, 1987). A portion of the increased T_3 could come from that generated by brown adipose tissue deiodinase, which is markedly stimulated in the cold (1A; Kopecky et al, 1986 a, b). Increased thermogenesis in cold acclimated animals could be due in large part to thyroid-induced heat production in organs other than BAT. The increased concentration of UCP in BAT of cold acclimated hamsters (Trayhurn et al, 1983; Sundin et

al, 1987; Kopecky et al, 1986a) is correlated not with sympathetic activity in BAT but with increased serum T_3 levels (Kopecky et al, 1986a, b; present results). However it is not clear how the elevated deiodinase activity is maintained in the absence of detectable sympathetic stimulation in the cold acclimated state.

High fat diet and short photoperiod do not increase BAT deiodinase activity or change serum T_3 levels (see 1B, 1C; Kopecky et al, 1986a, b). Thus lack of sympathetic stimulation in BAT (Hamilton et al, 1986; Viswanathan et al, 1986; McElroy and Wade, 1986; present experiments) and absence of increase in UCP (Kopecky et al, 1986b) would be in accordance with this lack of change in serum T_3.

Preservation of high thermogenic capacity of brown fat would serve in preparation for hibernation and subsequent arousal, where it is known that BAT activation plays a crucial role in rewarming (Horwitz et al, 1985; Nedergaard and Cannon, 1984).

Myopathic hamster BAT was smaller than normal (also Himms-Hagen and Gwilliam, 1980; Triandafillou et al, 1984a, b; Kopecky et al, 1986b) and as a result had a lower total noradrenaline content but the concentration was normal. The rate constant for tissue NA content decline was normal indicating that tissue noradrenaline half life was not altered. As in normal hamsters, neither diet nor short photoperiod affected noradrenaline turnover in myopathic BAT. What cannot be explained is why the high fat diet increased serum T_3 in myopathic hamsters in this experiment but not in another (1B; Kopecky et al, 1986b).

Failure of myopathic hamster brown adipose tissue to grow in

response to high fat diet or short photoperiod, and blunted response to cold, cannot be attributed to defective sympathetic stimulation of brown adipose tissue hypertrophy.

Table 20

Food Intake of Hamsters During Adaptation to High Fat Diet, Short Photoperiod, or Cold

	Experiment a				Experiments b and c			
	n	NORMAL	n	MYOPATHIC	n	NORMAL	n	MYOPATHIC
Controls								
1 wk					15	31.2±0.3	14	26.7±0.1†
3 wk					14	29.1±1.0	13	29.1±0.2
6 wk	13	27.9±1.0	13	25.7±0.7	14	27.2±0.4		
12 wk					16	29.7±0.3	15	29.6±0.2
High Fat Diet								
1 wk					14	39.0±0.3*	11	29.8±0.5†
3 wk					15	36.8±1.3*	15	26.5±0.3†
6 wk					11	27.0±0.3		
12 wk					14	31.7±0.6	14	26.5±0.3†
Short Photoperiod								
1 wk					16	31.8±0.8	10	29.0±0.3†
3 wk					14	30.7±0.7	13	29.7±0.4
6 wk	16	27.1±0.6	12	26.5±0.3				
12 wk					15	29.0±0.5	16	26.8±0.6†
Cold								
1-4 h	15	N.D.						
24 h	16	N.D.						
3 d					9	N.D.		
2 wk					10	39.8±0.8*		
6 wk	11	38.8±1.3*						

Values are means ± SE in kcal/d for numbers of animals indicated (n). N.D. not determined. * indicates significant effect of treatment and † indicates significant difference between normal and myopathic hamsters.

Table 21

Final Body Weight of Normal and Myopathic Hamsters During Adaptation to High Fat Diet, Short Photoperiod, or Cold

	Experiment a				Experiments b and c			
	NORMAL		MYOPATHIC		NORMAL		MYOPATHIC	
	n		n		n		n	
Controls (chow, 14:10 L:D)								
1 wk					15	97.0±2.0	14	71.4±1.6†
3 wk					14	110.0±2.3	13	86.8±1.5†
6 wk	13	119.0±3.8	13	95.5±2.3†	14	116.8±7.9		
12 wk					16	136.7±2.9	15	102.7±1.4†
High Fat Diet (SFS, 14:10 L:D)								
1 wk					14	101.9±3.0	11	71.6±1.8†
3 wk					15	123.1±2.6	15	81.1±2.0†
6 wk					11	127.2±2.2		
12 wk					14	183.6±3.1*	14	108.1±2.0†
Short Photoperiod (4:20 L:D)								
1 wk					16	99.6±1.9	10	75.4±1.3†
3 wk					14	120.9±3.0	13	81.9±1.2†
6 wk	16	107.7±3.7	12	94.1±1.6†				
12 wk					15	144.6±5.7	16	90.5±1.6†
Cold (4°C, chow, 14:10)								
1-4 h	15	114.0±2.5						
24 h	16	114.5±2.7						
3 d					9	99.3±3.9		
2 wk					10	84.8±2.1*		
6 wk	11	94.7±3.3*						

Values are means ± SE. Body weight is in grams, n is the number of animals, and SFS is sunflower seeds. * indicates significant effect of treatment and † represents significant difference between normal and myopathic hamsters. 14:10 L:D represents lighting schedule with 14 h light, 10 h dark, and 4:20 indicates 4 h light, 20 h dark.

Table 22

Gain in Body Weight of Hamsters Acclimated to High Fat Diet or Short Photoperiod (Experiments a, b, c)

	NORMAL n		MOPATHIC n	
Controls				
1 wk	15	6.6±0.7	14	4.7±0.6
3 wk	14	19.3±0.9¶	13	14.1±0.6
6 wk	13	29.4±1.1¶	13	15.8±1.7†
12 wk	16	49.0±3.4¶	15	31.5±2.7†
High Fat Diet				
1 wk	14	13.2±1.6	11	7.0±0.7
3 wk	15	31.8±4.2¶*	15	14.4±1.4¶†
6 wk	11	38.4±1.2¶*		
12 wk	14	96.8±3.7¶*	14	37.5±2.0¶†
Short Photoperiod				
1 wk	16	6.3±1.0	10	5.2±0.7
3 wk	14	23.7±1.6¶	13	12.3±0.6†
6 wk	16	28.7±3.1¶	12	7.7±2.2†
12 wk	15	60.2±5.2¶*	16	19.6±2.6¶†*

Values are means ± SE expressed in grams for numbers of animals indicated (n). * indicates significant effect of treatment, ¶ indicates effect of time, and † indicates significant difference between normal and myopathic hamsters ($p<0.05$). Results from all three experiments are presented. For explanation of diet and photoperiod see table 20.

Table 23

Noradrenaline Turnover in Brown Adipose Tissue of Hamsters Fed a High Fat Diet or Exposed to Short Photoperiod

	Experiment a		Experiments b and c	
	NORMAL	MYOPATHIC	NORMAL	MYOPATHIC
		Control		
1 wk			314±252.9	162±169.5
3 wk			529±424.0	130±114.8
6 wk	881±334.7	237±97.2†	480±171.2	
12 wk			373±403.9	334±237.3
		High Fat Diet		
1 wk			610±191.4	125± 59.2†
3 wk			455±170.4	347± 92.2
6 wk			608±337.6	
12 wk			819±268.2	480± 93.3†
		Short Photoperiod		
1 wk			626±219.1	183± 80.2
3 wk			156±277.0	287± 58.7
6 wk	726±224.3	297±96.3†		
12 wk			304±302.9	156±107.5

Values are means ± SE in ng/h in pooled BAT depots for numbers of animals given in table 20. There were no significant effects of diet or photoperiod on noradrenaline turnover. † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

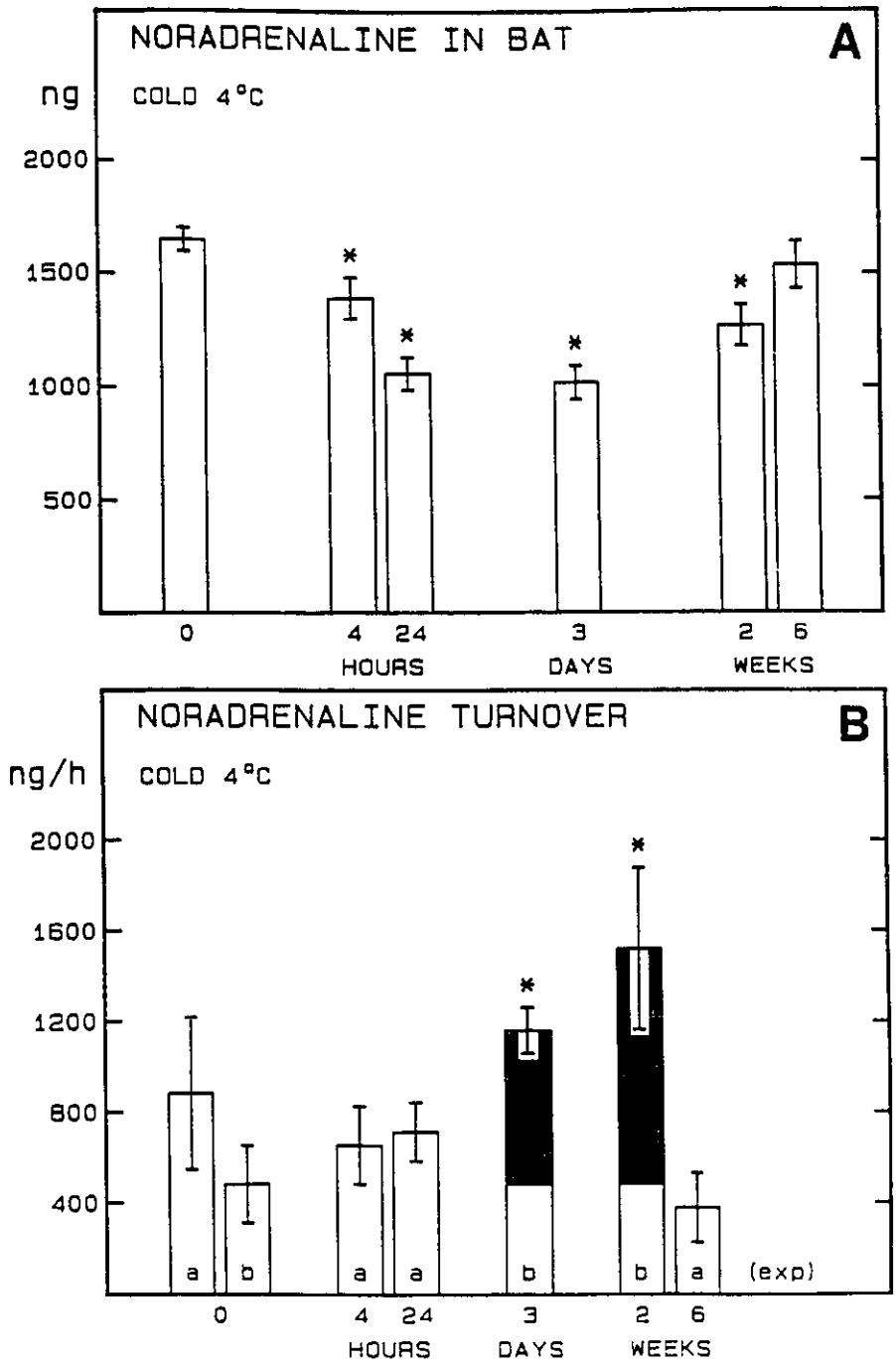


Figure 9. Effect of cold acclimation on noradrenaline content (A) and turnover (B) in pooled brown adipose tissue from normal hamsters. Values are means ± SE for numbers of animals given in table 20. * and black portion of bars indicate significant effect of cold ($p<0.05$). Lower case letters at the bottom of bars in B indicates the experiment (see table 20).

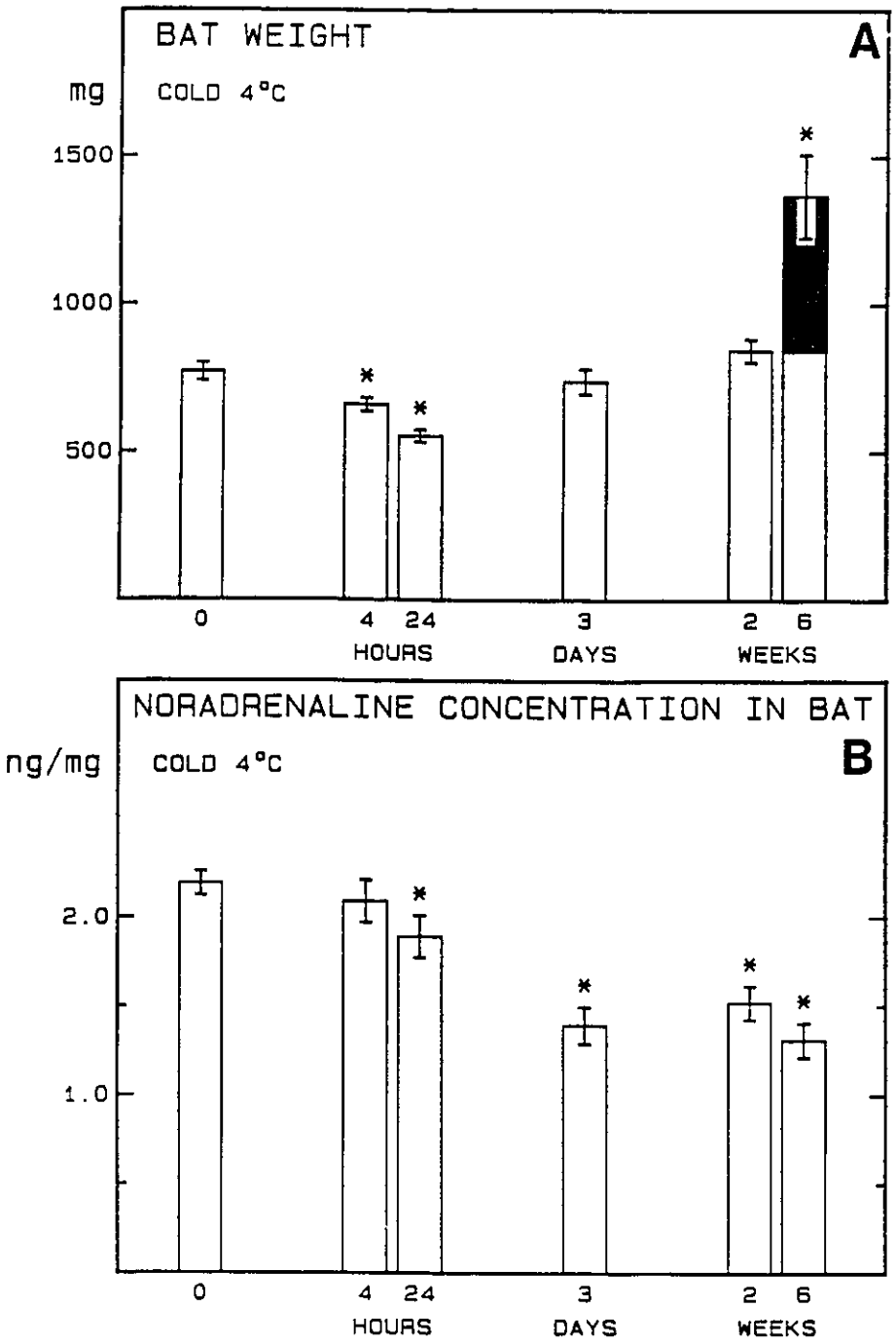


Figure 10. Effect of cold acclimation on brown adipose tissue weight (A) and noradrenaline concentration (B) in normal hamsters. Values are means ± SE for numbers of animals given in table 20. Black portion of bars and * indicate significant effects of cold ($p<0.05$).

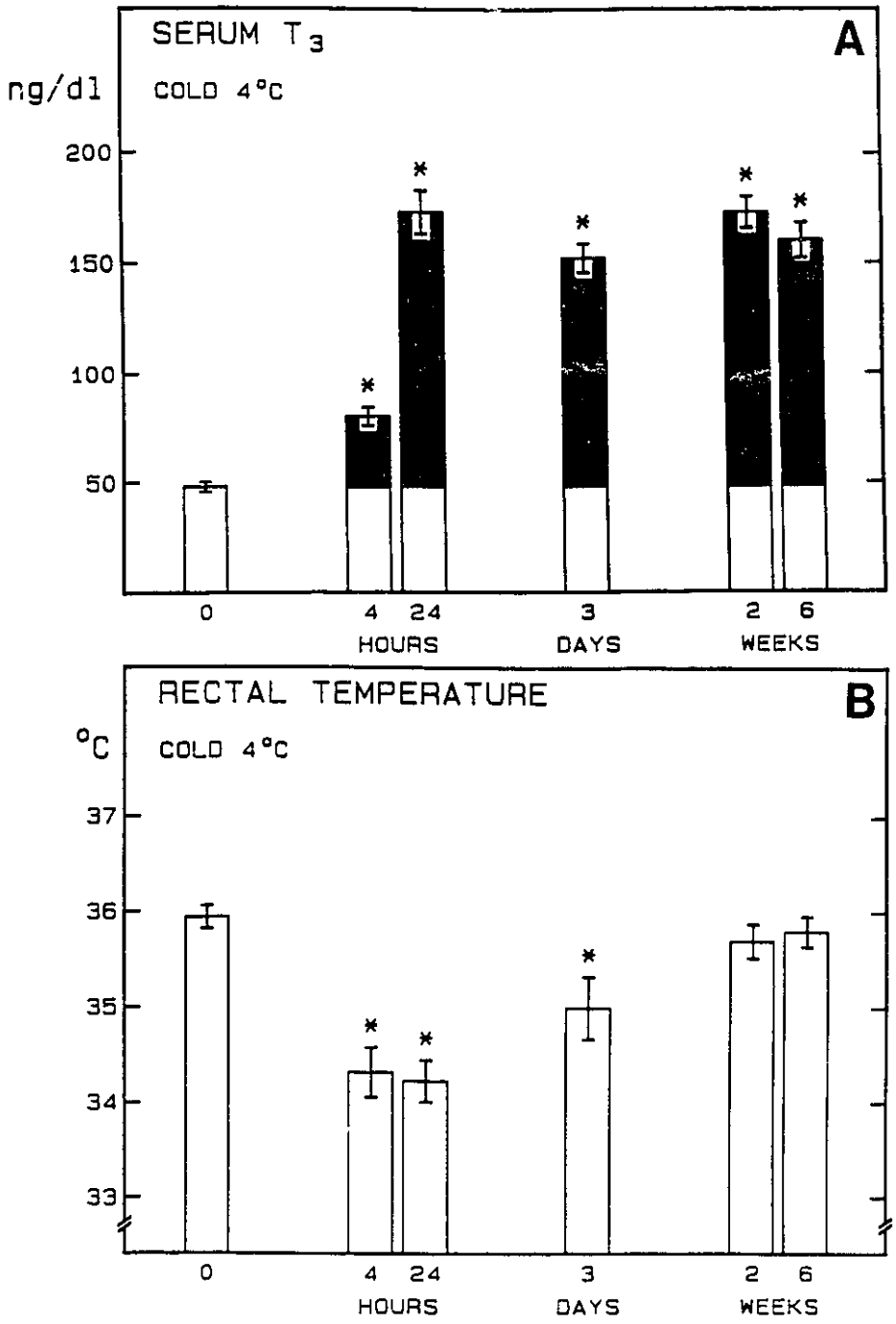


Figure 11. Serum T_3 (A) and body temperature (B) of normal hamsters during cold acclimation. Values are means ± SE for numbers of animals in table 20. Black portion of bars and * indicate significant effects of cold ($p<0.05$).

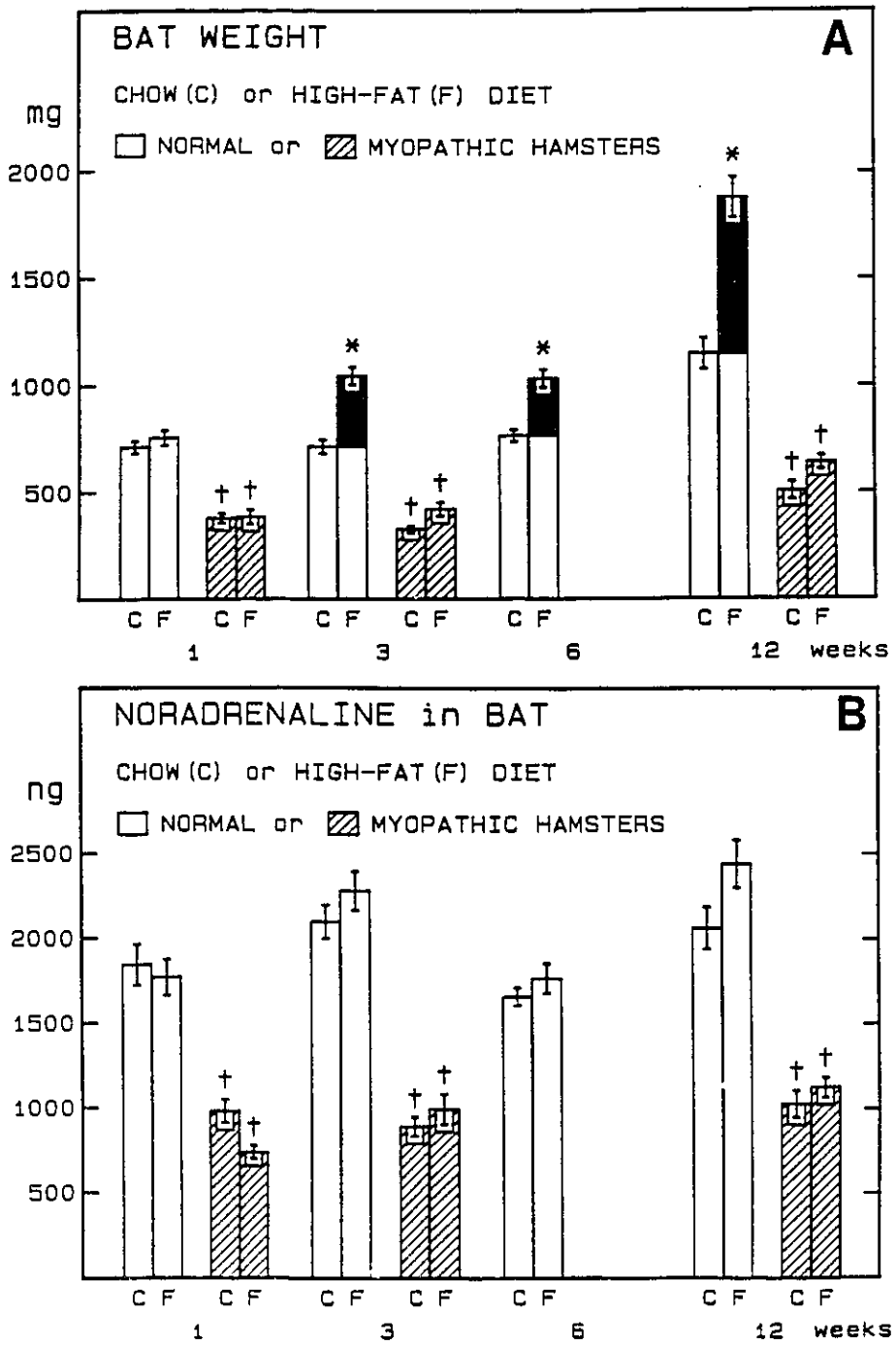


Figure 12. Brown adipose tissue weight (A) and noradrenaline content (B) from normal and myopathic hamsters fed chow or a high fat diet. Values are means ± SE for animal numbers in table 20. Open bars represent normal animals and hatched bars represent myopathic hamsters. Black portions of bars and * indicate significant effect of treatment ($P<0.05$). † indicates significant difference between normal and myopathic hamsters ($p<0.05$). The high fat diet is chow plus sunflower seeds.

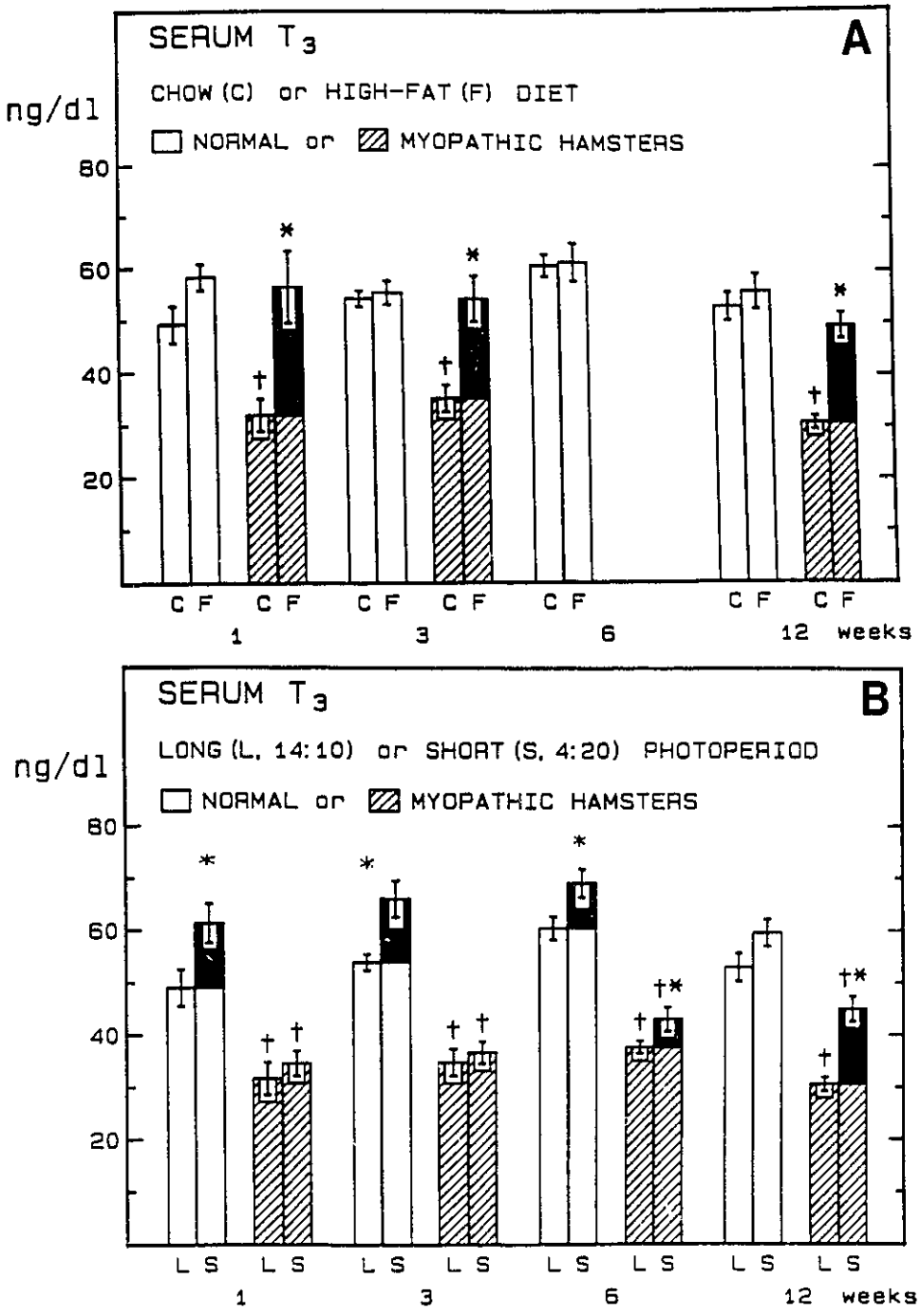


Figure 13. Serum T_3 from normal and myopathic hamsters fed a high fat diet (A) or exposed to short photoperiod (B). Values are means ± SE for numbers of animals given in table 20. For explanation of symbols see figure 12. L is long photoperiod and S is short photoperiod.

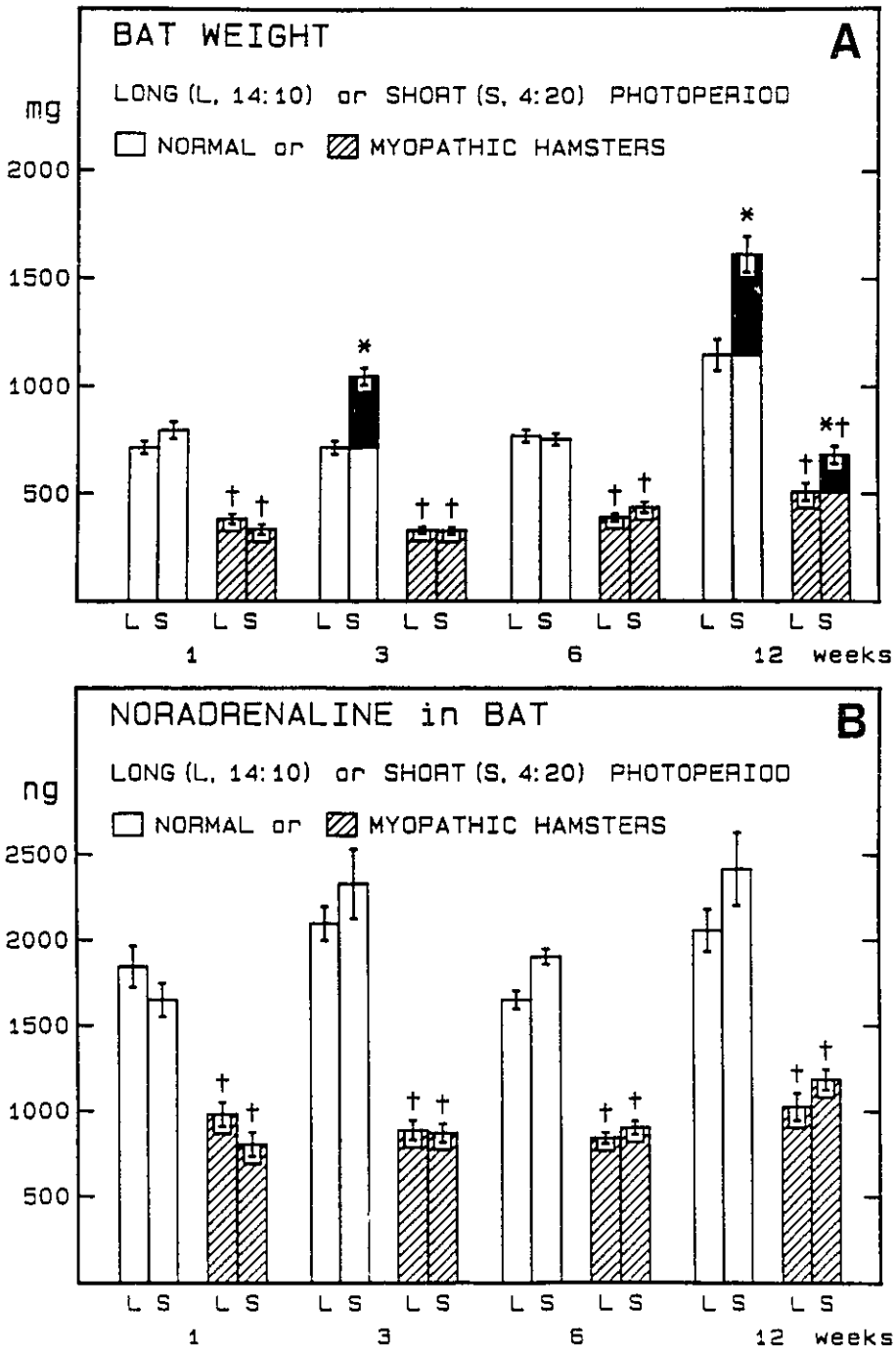


Figure 14. Brown adipose tissue weight (A) and noradrenaline content (B) in normal and myopathic hamsters adapted to short photoperiod. See figure 12 and table 20 for explanation of symbols and information on animals.

3C: EFFECT OF DENERVATION ON STIMULATED BAT:

Background:

Control of brown adipose tissue growth in hamsters does not seem to be mediated by noradrenaline, in that repeated injections or infusion of NA have no effect or even reduce protein content (Triandafillou et al, 1984a; see 3A), and growth can occur in the absence of any change in sympathetic nervous system activity in BAT (see 3B). Moreover, injected or infused NA has little, if any, effect on T5'D activity (see 3A). Paradoxically, deiodinase activity is still high in cold acclimated hamsters when noradrenaline turnover has returned to a normal level (see 1A; 3B). What therefore brings about and maintains the high level of deiodinase activity in BAT of cold acclimated hamsters? It is known that an intact innervation is required for growth to occur (Triandafillou et al, 1984b; Desautels and Dulos, 1988). Thus, a role for the sympathetic nerves in the control of the 5'-deiodinase seems likely.

Objectives:

The objective of this experiment was to determine if an intact innervation to hamster BAT is essential for the increase in deiodinase activity in response to acute cold exposure.

Methods:

Male golden hamsters 6-8 weeks of age were housed individually as described. The animals were anesthetized using a mixture of halothane and oxygen. The interscapular brown adipose tissue was exposed through a dorsal midline incision and either sham operated or unilaterally denervated as described in METHODS. The incision was closed with wound

clips and the animals were allowed to recover for 2 days. After recovery half of the animals were transferred to a 4°C coldroom for 24 h. The other animals remained at 24°C.

The animals were sacrificed and each half BAT pad was removed and treated separately. The half pads were weighed and approximately 30% kept for protein determination and deiodinase assay as described. The remaining 70% was homogenized in perchloric acid for noradrenaline extraction as outlined in METHODS. Tissue NA content was determined using HPLC but the peak was not collected (see METHODS). The data were analyzed using analysis of variance and Duncan's multiple range test post hoc, with a significance level of 0.05.

Results:

Brown adipose tissue protein was not significantly changed within 24 h of denervation or cold exposure (table 24), however both specific and total deiodinase activities were. Cold exposure for 24 h markedly increased both specific and total activity but in innervated BAT only (table 24). Denervation had no effect on deiodinase activity in warm acclimated hamster BAT but it prevented the cold-induced increase in cold exposed hamsters (table 24). Not in all cases was denervation completely successful, as seen by lesser degrees of NA depletion and the large standard errors for the values for NA content in table 24.

There was a positive linear relationship between brown adipose tissue deiodinase activity and noradrenaline content, with a greater correlation in the cold exposed group than in the warm acclimated group (figure 15, r^2=0.8880 vs 0.2461). The slopes of the two lines also differed significantly, with the steeper slope belonging to the line of

the cold exposed group (m=0.1779 vs m=0.0090). Note that incomplete denervations could easily be recognized by the higher NA contents and deiodinase activities.

Discussion:

These results confirm the requirement of an intact sympathetic innervation in mediating the trophic response of hamster brown adipose tissue, using thyroxine 5'-deiodinase activity as an indicator, and demonstrate the probable role of noradrenaline in the cold-induced increase in deiodinase activity. The participation of other neural factors is not, however, excluded.

Table 24

Effects of Denervation and Cold Exposure on Brown Adipose Tissue

	INTACT		DENERVATED	
	24°C	4°C	24°C	4°C
Protein (mg)	10.0±0.8	12.0±1.0	8.9±0.7	11.4±1.0
Deiodinase SA (pmol/h/mg)	2.15±0.25	5.41±0.54*	2.18±0.30	2.57±0.64¶
TA (pmol/h)	21.48±3.48	62.32±5.26*	18.74±2.19	29.58±7.36¶
NA (ng)	492.8±34.0	280.2±19.6*	110.0±37.3¶	117.9±41.0¶

Effect of unilateral denervation and 24 h cold exposure on BAT protein, noradrenaline content, and deiodinase activity. Values are means ± SE for 8 animals in each temperature group. SA is specific activity, TA is total activity and NA is noradrenaline. * indicates significant effect of cold exposure and ¶ indicates significant effect of denervation ($P<0.05$). There were no significant differences in protein content after only 24 h in the cold.

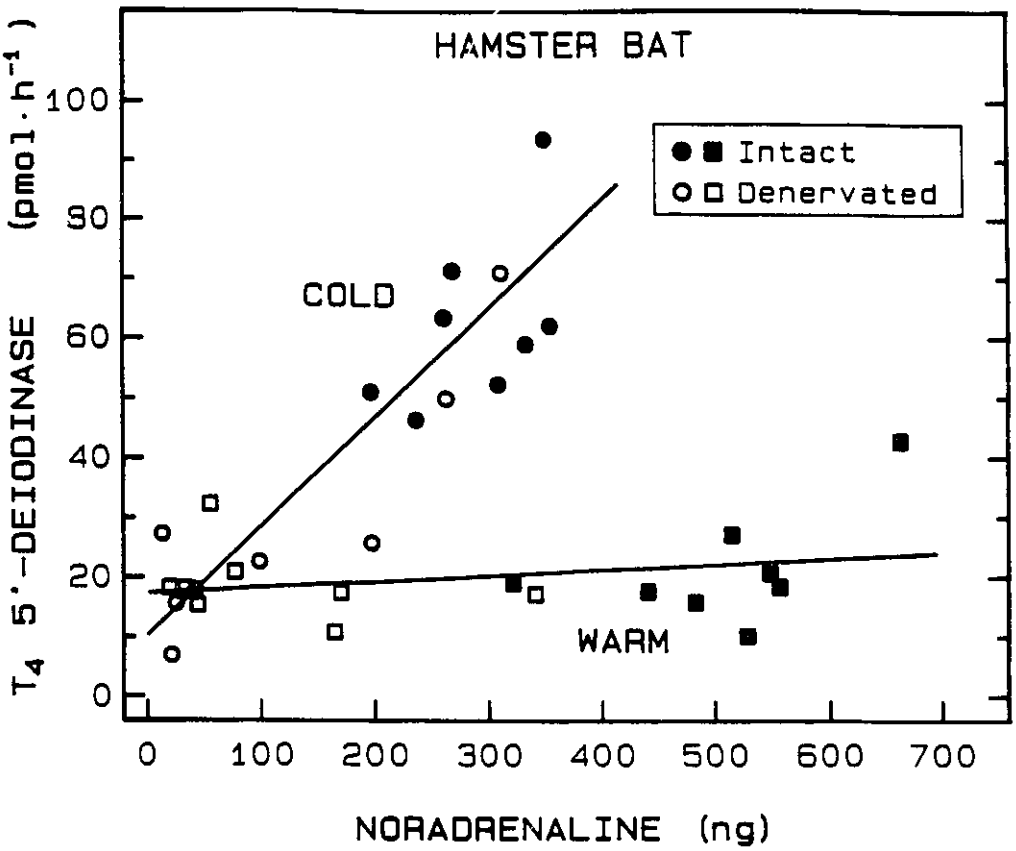


Figure 15. Relationship between BAT deiodinase activity and noradrenaline content. Each point represents values for an individual hamster. The correlation coefficient of the line for cold exposed animals is 0.8880 and the correlation coefficient of the line for the warm acclimated group is 0.2461. Calculations were based on all points.

3D: EFFECTS OF ADRENERGIC AGONISTS IN STIMULATING BAT:

Background:

Experiment 1A demonstrated that cold stimulates hamster BAT T5'D activity and experiment 3B showed that cold at least acutely, increases sympathetic nervous system activity in BAT. This stimulatory effect of cold on deiodinase activity can be prevented by denervation (3C). Therefore it could be concluded that noradrenaline mediates this response. However there was difficulty in demonstrating an effect of either injected or infused NA on BAT T5'D activity (see 3A). Coupled with that is the problematic fact that deiodinase activity remains high in cold acclimated hamster BAT despite a normal sympathetic activity in the tissue (1A, 3B).

Perhaps in the hamster a stronger stimulation of α_1-adrenergic receptors is needed, since it is known that in the rat α_1-receptors predominantly mediate the increase in deiodinase activity (Silva and Larsen, 1983). In both rats and hamsters α_1-adrenoceptor density increases in the cold acclimated state (Raasmaja et al, 1985).

Objectives:

The purpose of this experiment was to study the effects of specific sympathomimetics on hamster BAT activity.

Methods:

Male hamsters 5-6 weeks old were implanted with mini osmotic pumps as described in METHODS, which contained one of the following solutions: vehicle (0.9% NaCl + 0.2% ascorbic acid as a preservative + 1 mM HCl to maintain stable acidic pH), noradrenaline bitartrate, isoproterenol bitartrate, phenylephrine hydrochloride, or isoproterenol

+ phenylephrine. Noradrenaline acts on both α- and β-receptors; isoproterenol is a mixed β agonist and phenylephrine is an α_1 agonist. The pumps delivered 1.05 µl/h and administered a dose of 422 µg/d for 5 d to give a total agonist dose of approximately 2000 µg. This dose was chosen because it appeared to have fewer toxic effects on BAT than the higher dose that was used in experiment 3A. Even though deiodinase activity was not stimulated significantly in experiment 3A by that NA dose, there was a trend towards an increase.

Body weight and food intake were recorded for 2 d prior to pump implantation and every day thereafter until sacrifice. The hamsters were killed by decapitation and trunk blood collected for serum thyroid hormone determination. Brown adipose tissue was removed into isolation medium then cleaned, weighed, and homogenized for determination of protein, deiodinase activity and UCP content (see METHODS). Data were analyzed using analysis of variance followed by Duncan's multiple range test post hoc, using 0.05 as a level of significance.

Results:

Food Intake and Body Weight:

Food intake was significantly depressed in all groups on the day of pump implantation, most likely due to anesthesia and recovery (table 25). The next day all groups were eating normally except those infused with noradrenaline and isoproterenol + phenylephrine, where intake remained lower for one more day (table 25). Three days after surgery all the animals were eating normally. Body weight was significantly reduced only in the noradrenaline infused group on the third day following surgery (table 26). These animals were also eating less than

the others, which would account at least partially, for the weight loss.

White Adipose Tissue:

Gonadal white adipose tissue weight was significantly reduced in those animals receiving noradrenaline and isoproterenol + phenylephrine, indicating mobilization of energy stores (figure 16). It also correlated with the transient decrease in food intake.

Brown Adipose Tissue:

Brown fat protein was not altered by 5 d of agonist or noradrenaline infusion (figure 17) nor was UCP content affected by treatment (table 27). Thyroxine 5'-deiodinase activity however was stimulated by both noradrenaline and the combination of isoproterenol and phenylephrine; neither α nor β agonists alone had any effect (figure 18).

Serum Thyroid Hormones:

Serum T_3 was unaffected by treatment but serum T_4 was significantly decreased in animals infused with noradrenaline, isoproterenol, and isoproterenol + phenylephrine (figure 19). Phenylephrine alone had no effect on T_4.

Discussion:

The principal result of this experiment was that hamster brown adipose tissue deiodinase activity was best stimulated by noradrenaline and by a mixture of α_1- and β-adrenergic agonists which mimic the action of noradrenaline. Stimulation did not appear to be mediated solely through α_1-receptors but one would have to study the effect of the α_1 antagonist prasozin on cold activated deiodinase activity in the

hamster to confirm this.

Foster (1984) has shown in rats that the α_1 agonist phenylephrine, in doses having no effect alone, caused potentiation of isoproterenol-induced increased brown adipose tissue blood flow. This effect appears to be due to potentiation of β-receptor stimulation of cAMP levels and blood flow (Ma and Foster, 1984). Blockade of β-receptors with propranolol reduced the thermic effect of noradrenaline by 84% but α_1 inhibition with corynanthine also reduced the effect of noradrenaline by 48% (Thurlby, 1985). Thus the potentiating effect of α-receptors on β-mediated processes is an important part of the acute response of brown adipose tissue.

Silva and Larsen (1986) reported that although their early studies using α and β antagonists showed no evidence for β-receptor mediated deiodinase activation they have observed a significant stimulation of rat brown fat deiodinase activity, albeit a modest one, with isoproterenol. They suggested that this could result from the large quantities of β catecholamines not normally "seen" by the tissue when noradrenaline is given, or alternatively, the effect was secondary to β-receptor stimulated release of other hormones such as glucagon. Glucagon does have a stimulatory effect on rat brown fat as reflected by increases in protein, DNA, and GDP-binding (Billington et al, 1987) and stimulation of deiodinase activity (Silva and Larsen, 1986). As well, plasma levels are known to rise after acute cold exposure (Seitz et al, 1981). However there is a paucity of information about the role of glucagon on hamster brown adipose tissue. Dryer et al (1971) did report that glucagon had no stimulatory effect on respiration of

isolated hamster brown adipocytes. It is not possible to draw conclusions about the involvement of glucagon in the β-receptor mediated portion of deiodinase stimulation in hamster brown adipose tissue without first measuring levels in cold exposed or agonist infused hamsters, and studying the effects of direct administration on BAT.

Noradrenaline and the mixture of isoproterenol and phenylephrine were effective in mobilizing energy stores as observed by reduction of gonadal white adipose tissue and transient fall in food intake. Despite the obvious actions of these compounds on the animals no significant changes in total BAT protein or UCP were observed after 5 d of infusion. This agrees with previous results (see 3A) where noradrenaline infusion, also for 5 d, had no effect on protein or UCP content. There could be a number of reasons for this. First, the infusion was not carried out for long enough. However Desautels and Dulos (1988) infused noradrenaline for 7 d and found, if anything, BAT atrophy, and Triandafillou et al (1984a) injected hamsters with noradrenaline daily for 2 wk and failed to find a trophic effect on BAT. Second, noradrenaline alone might not be a potent enough stimulator of tissue growth. This is posssible but there is little evidence to suggest that noradrenaline content or sympathetic activity are correlated with BAT hypertrophy in hamsters (see sections 1A, 1B, 1C; 3A, 3B; Triandafillou et al, 1984a; McElroy and Wade, 1986; Hamilton et al, 1986; Viswanathan et al, 1986 ; Sigurdson and Himms-Hagen, 1988; Desautels and Dulos, 1988).

Infusion with mixed or β-adrenergic agonists had no effect on

serum T_3 but did significantly reduce serum T_4. These results differ somewhat from those obtained with cold exposed hamsters (Kopecky et al, 1986a). Cold exposure results in a marked elevation of T_3 in the blood and stimulation of deiodinase activity to approximately three times that in the agonist infused animals (Kopecky et al, 1986a). Perhaps the agonists alone were unable to activate deiodinase sufficiently to produce enough T_3 that would spill over into the circulation, but there was sufficient stimulation to reduce T_4 levels as deiodination was increased. An increase in deiodinase activity can occur as a consequence of a reduction in T_4 level (Silva and Larsen, 1985; Bianco and Silva, 1987). However, in this experiment T_4 was reduced by NA, ISO, and ISO+PHE whereas deiodinase was increased only by NA and ISO+PHE. It therefore seems unlikely that the increase in deiodinase activity observed is secondary to the reduction in T_4 level but more likely due to the effects of the agonists themselves. The mechansim by which this would occur is currently not clear.

One can conclude from this experiment that both α_1- and β-adrenergic receptor mediated events participate in the increase in hamster brown adipose tissue deiodinase activity, but 5 d of agonist infusion were insufficient to produce BAT growth.

Table 25

Effect of Adrenergic Agonist Infusion on Food Intake

	VEHICLE	NA	ISO	PHE	ISO+PHE
DAY -2	21.1±1.2	23.7±1.4	25.9±1.8	24.6±1.3	22.2±2.2
-1	20.0±0.6	20.3±2.7	22.1±2.4	23.1±2.5	21.1±1.3
1	14.5±1.3*	3.5±1.0*	2.8±1.2*	16.8±1.4*	1.2±0.4*
2	26.7±2.4	16.9±1.6*	20.5±3.0	24.9±1.5	17.3±1.3*
3	24.1±2.4	20.3±1.6	30.4±4.4	24.7±1.6	23.4±2.9
4	21.8±3.5	22.8±2.6	23.2±2.0	24.7±2.2	24.9±3.8
5	25.4±1.6	24.0±2.1	27.0±1.5	25.3±1.1	23.2±4.1

Effect of 5 d agonist infusion on food intake. Values are means ± SE expressed in kcal/d for 8 animals /group. The treatment abbreviations are NA noradrenaline, ISO isoproterenol, PHE phenylephrine, ISO+PHE isoproterenol + phenylephrine. The agonist dose was approximately 400 µg/d. * indicates significant effect of treatment ($p<0.05$). Food intake was depressed the least in vehicle and phenylephrine infused hamsters.

Table 26

Effect of Adrenergic Agonist Infusion on Body Weight

	VEHICLE	NA	ISO	PHE	ISO+PHE
DAY -2	85.4±1.9	87.1±2.2	88.9±2.0	87.5±2.3	86.3±2.7
-1	85.0±1.8	86.0±1.8	89.4±1.8	87.2±2.3	86.3±2.7
1	85.1±1.9	86.5±1.7	89.0±1.7	87.2±2.7	86.7±2.8
2	85.5±1.9	81.3±1.5	85.5±2.8	87.2±2.8	81.9±2.5
3	87.9±1.9	81.1±1.5*	89.1±2.5	88.2±2.9	82.7±1.8
4	88.5±2.1	81.4±1.6	91.9±2.4	89.0±2.9	85.1±2.2
5	90.2±2.3	82.1±1.9	92.9±2.3	89.7±2.8	85.4±2.6

Effect of 5 d agonist infusion on body weight. Values are means ± SE in grams for 8 animals/group. For abbreviations and symbols see table 25.

Table 27

Effect of Adrenergic Agonist Infusion on BAT Homogenate Uncoupling Protein

TREATMENT	UCP (µg/mg protein)	UCP (µg/BAT)
Vehicle	10.88±2.4 (7)	242.7±52.6
NA	11.77±2.3 (7)	353.3±48.5
ISO	12.40±1.4 (8)	369.9±49.4
PHE	11.90±2.0 (7)	307.2±60.0
ISO+ PHE	10.61±3.5 (7)	236.5±63.1

Effect of 5 d adrenergic agonist infusion on BAT homogenate uncoupling protein (UCP) content. Values are means ± SE for numbers of animals given in parentheses. For abbreviations see table 25. There were no significant effects of treatment on either concentration or total content of UCP.

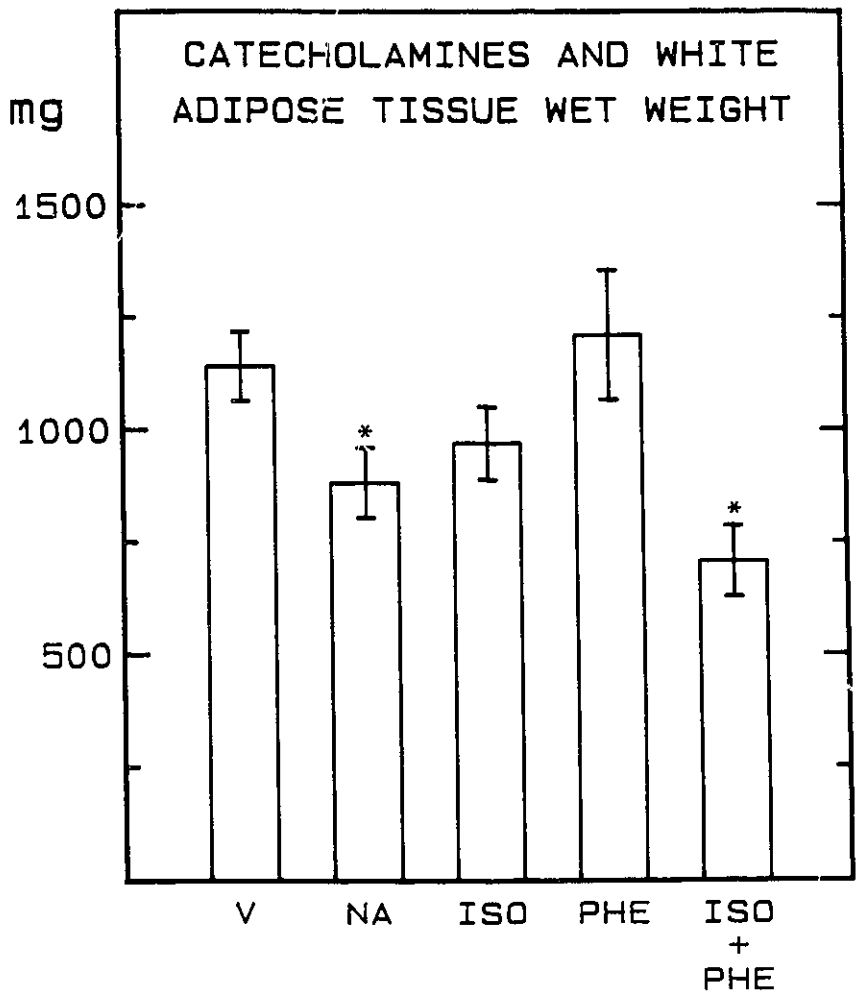


Figure 16. White adipose tissue weight in hamsters infused with adrenergic agonists. Values are means ± SE for 8 animals/group. * indicates significant effect of treatment ($p<0.05$). For abbreviations see table 25.

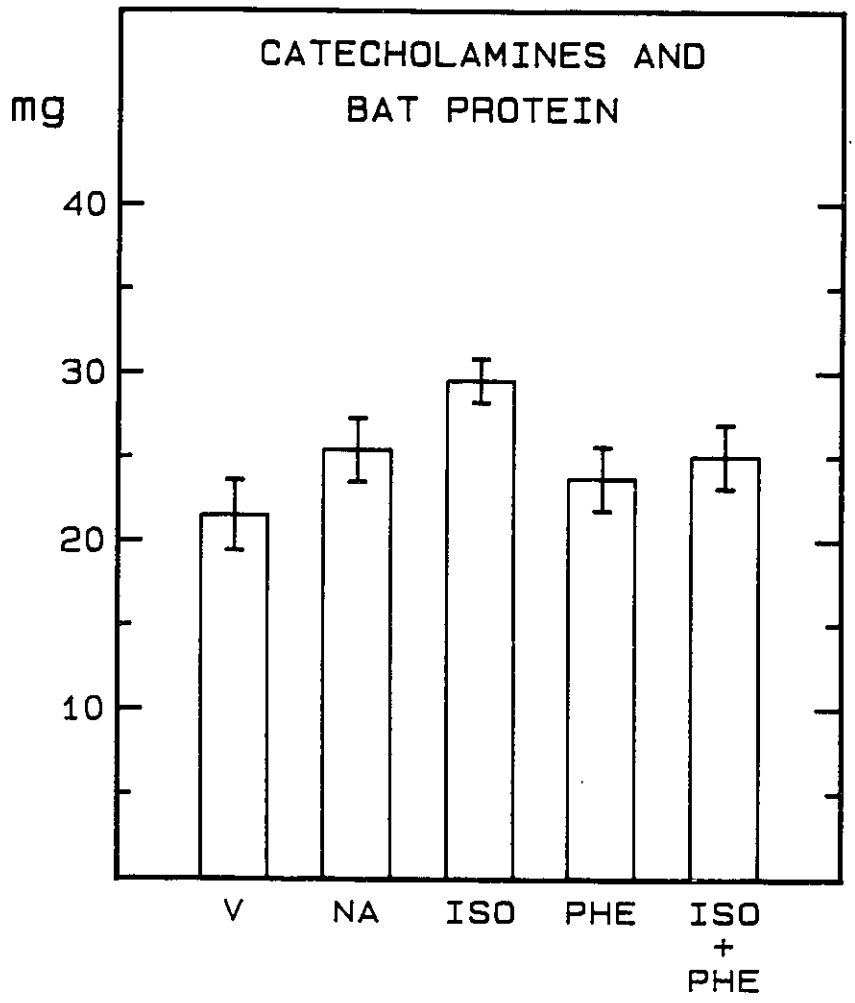


Figure 17. Brown adipose tissue protein content in agonist infused hamsters. Values are means ± SE for 8 animals/group. There were no significant differences between vehicle and agonist infused groups. For abbreviations see table 25.

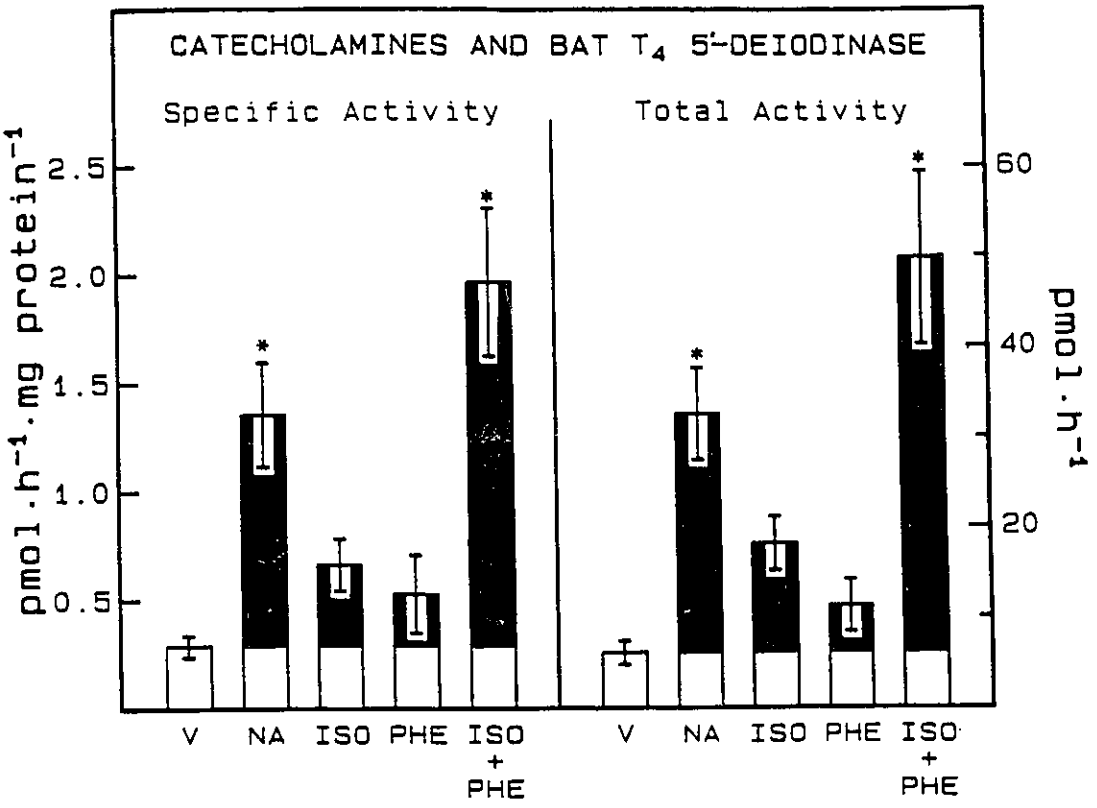


Figure 18. Effect of adrenergic agonist infusion on BAT specific deiodinase activity (left panel) and total activity (right panel). * and black portion of bars represent significant effect of treatment ($p<0.05$). Values are means ± SE for 8 animals/group. For abbreviations see table 25.

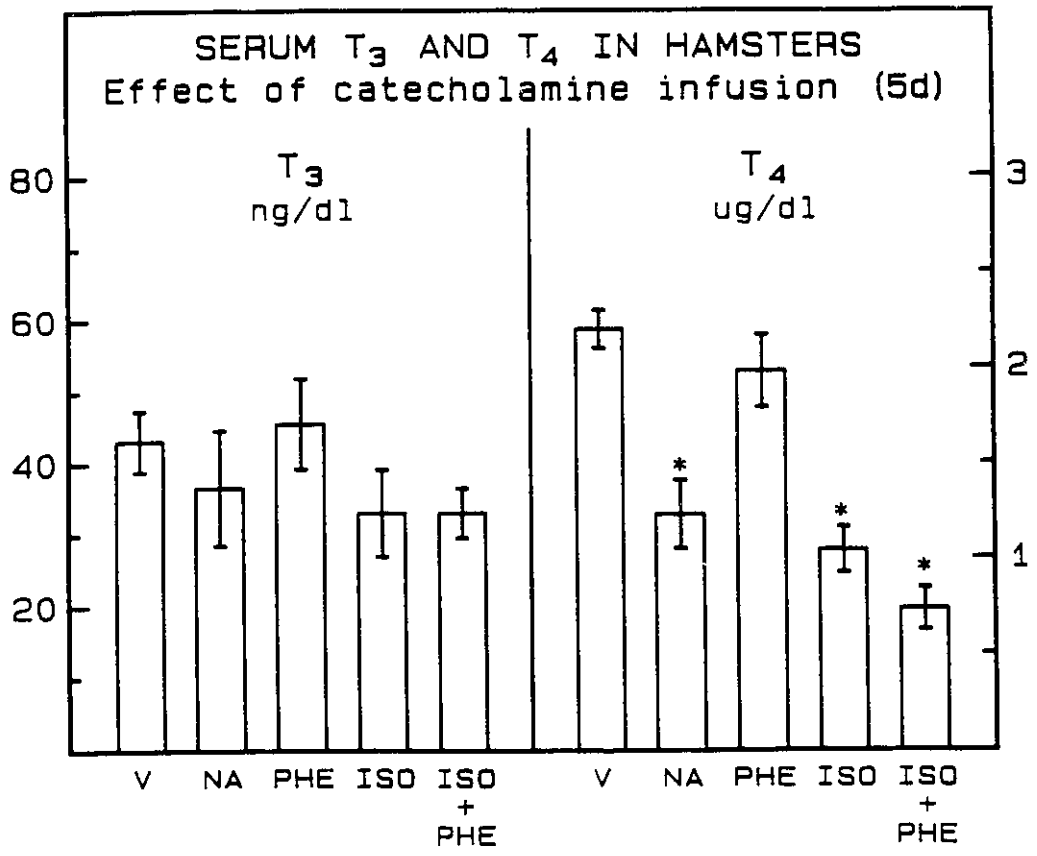


Figure 19. Serum thyroid hormones in agonist infused hamsters. Values are means ± SE for 8 animals/group. Serum T_3 (left panel) was unchanged by agonist treatment. Serum T_4 (right panel) was decreased significantly where indicated by * ($p<0.05$). For abbreviations see table 25.

3E: NEUROPEPTIDES IN BAT-IMMUNOHISTOCHEMICAL STUDIES:

Background:

Attempts to induce hamster brown adipose tissue growth using noradrenaline (Triandafillou et al, 1984a; Desautels and Dulos, 1988; 3A) or adrenergic agonists (3D) have so far failed, yet an intact innervation to the tissue is required (Triandafillou et al, 1984a; Desautels and Dulos, 1988). This has led to the suggestion that factors in addition to or instead of noradrenaline mediate trophic responses (Triandafillou et al, 1984a; Hamilton et al, 1986; Sigurdson and Himms-Hagen, 1988). Such factors could be co-localized with and released from sympathetic nerves (see Hökfelt et al, 1987; Westlind et al, 1984).

Objectives:

The objectives of these studies were to confirm the presence of sympathetic innervation in hamster brown adipose tissue by immunohistochemical means, and to determine if other neuropeptides were present and possibly co-localized in the sympathetic nerves. A second objective was to establish whether cold acclimation or myopathy altered either the level or distribution of these factors in a histochemically demonstratable manner. Another objective was to confirm the abundance of cytochrome oxidase in BAT mitochondria histochemically, and to see if cold acclimation or myopathy affected the level of enzyme.

Methods:

Male Syrian golden and myopathic hamsters were housed at 24°C as described in METHODS. Some animals were transferred to 4°C for 6 weeks to 3 months. Another group of normal animals was used for a

denervation study where 3 hamsters were sham operated and 4 hamsters were subjected to bilateral surgical denervation of the interscapular brown fat pad as described in METHODS. All operated animals were allowed to recover for 4 days.

Animals were sacrificed by deep anesthesia with pentobarbital followed by saline perfusion through the right ventricle as described. The interscapular BAT was removed, cleaned and fixed in modified Zamboni's fixative for 24 h at 4°C. After fixation the tissue was stored in 10% sucrose in phosphate buffer (see METHODS). In one experiment, animals were sacrificed by decapitation and BAT excised and fixed as described. Cryostat sections of 14 µm thickness were cut, placed on coated slides, and rinsed in phosphate buffered saline (PBS) as described. The sections were then incubated with one of the following antisera overnight at 4°C in a humid environment: tyrosine hydroxylase (TH, 1:50, 1:200, 1:400, different lots of antisera); neuropeptide Y (NPY, 1:400); calcitonin-gene-related-peptide (CGRP, 1:200); nerve growth factor (NGF, 2.5S, 1:400). After washing in PBS the fluorescein-linked secondary antibody (donkey antirabbit immunoglobulin) was added and incubated as described in METHODS. After the final wash the slides were coverslipped using a glycerol:PBS antifade mounting medium. The sections were viewed under a Zeiss fluorescence microscope using the appropriate filters and later stored at -80°C to preserve fluorescence. Some sections were reacted for cytochrome oxidase as described in METHODS. As a positive control for the presence of nerve growth factor, submandibular salivary glands were were removed from male mice (C57BL/6J) obtained as extras from the

University animal colony, fixed and treated as described for BAT. Stained tissue from at least 3 animals was examined. Photomicrographs were taken as described in METHODS.

Results:

Tyrosine Hydroxylase:

Tyrosine hydroxylase catalyzes the rate-limiting step in noradrenaline synthesis from tyrosine. Fibres containing this enzyme are present in both the parenchyma and vasculature of brown adipose tissue from warm and cold acclimated hamsters (plate 1: a, b, c, d, e). There did not appear to be a difference in the fibre density or intensity of fluorescence between normal and myopathic hamsters.

Denervation of brown adipose tissue resulted in a dramatic decrease in the number of TH-staining fibres (plate 2: a, b).

Neuropeptide Y:

NPY-reactive fibres were located only in the brown adipose tissue vasculature and not in the parenchyma (plate 3: a, b). NPY fluorescence was found in both warm and cold acclimated tissue (plate 3: a, b) but appeared to be somewhat reduced in denervated BAT (plate 3: c, d).

Calcitonin-Gene-Related-Peptide:

CGRP-immunoreactive fluorescence was very sparse and observed only occasionally in BAT parenchyma from cold acclimated hamsters (plate 4 a, b). Blood vessels were weakly stained but often the fluorescence was not bright enough to be easily visible in photomicrographs.

Nerve Growth Factor:

NGF was detected only in male mouse salivary gland and did not

appear to be present in brown adipose tissue (plate 5: a, b).

Cytochrome Oxidase:

The presence of cytochrome oxidase in the numerous mitochondria of brown adipose tissue was confirmed histochemically (plate 6: a, b, c, d). In warm acclimated hamsters there appeared to be little difference between normal and myopathic BAT (plate 6: a, b). Cold acclimation caused a rather marked change in the staining pattern, made more easily visible by a reduction in lipid droplet size. Interestingly, only some cells were darkly stained whereas others were not. This was observed in two different experiments, one performed in the winter and one in the summer, although the patchiness was less pronounced in tissue from summer cold acclimated hamsters. A similar pattern was observed in myopathic BAT (plate 6: c, d). Cell size in myopathic hamster BAT appeared to be smaller than normal. This was especially evident in tissue from cold acclimated animals (plate 6: c, d).

Discussion:

These results confirm the presence of sympathetic innervation by immunohistochemical demonstration of the enzyme tyrosine hydroxylase in fibres innervating brown adipose tissue parenchyma and blood vessels. This enzyme is necessary for noradrenaline synthesis from tyrosine and its relative abundance in BAT emphasizes the importance of the sympathetic innervation to this tissue. The pattern of TH-immunoreactivity in hamster BAT is similar to that observed in cold acclimated rat brown adipose tissue (Cannon et al, 1986). Large differences in brown fat TH-immunoreactivity between warm and cold acclimated animals were not observed. It is possible that cold

acclimation increased the activity of the enzyme rather than the amount, or that an increase in the amount of tyrosine hydroxylase was not detectable by this method. Another possibility is that there was transient activation of enzyme activity but this had returned to a basal level once the animal was cold acclimated. This could also account for the pattern of changes in noradrenaline content observed in experiment 3B.

Denervation caused a dramatic decline in observable TH fluorescence, confirming the requirement of an intact innervation for the maintenance of tissue NA (see Alexander and Stevens, 1980; Desautels and Dulos, 1988; 3C).

There was no observable difference in TH fluorescence distribution or intensity between normal and myopathic BAT. Total noradrenaline content is lower because of the reduced tissue size in myopathic hamsters but the concentration and half life of NA in the tissue is normal (Triandafillou et al, 1984b; see 3B). Therefore, the noradrenergic innervation to BAT appears to be normal in these animals.

Neuropeptide Y-positive immunostaining was found in fibres innervating only the blood vessels of hamster BAT. These results agree with similar observations made in rat brown adipose tissue (Cannon et al, 1986; Norman et al, 1988). This functional distribution of nerve populations and exclusive occurrence of NPY in vascular nerves points to a possible role of this peptide in control of blood flow in BAT. Neuropeptide Y is a potent vasoconstrictor of arteries and arterioles (O'Donohue et al, 1985; Lundberg et al, 1982). NPY is often co-localized with NA and may act with it in a cooperative manner to

activate post-synaptic receptors on vascular smooth muscle (see Cannon et al, 1986). Electrical stimulation of intercostal nerves causes an initial vasoconstriction, prior to metabolic activation and subsequent vasodilation (Flaim et al, 1977; Cannon et al, 1986) and increased blood flow. Under conditions of physiological activation it is possible that both types of sympathetic nerves are not activated simultaneously. Instead, the parenchymal nerves may stimulate thermogenesis without the vascular nerves counteracting the increased blood flow with vasoconstriction (see Cannon et al, 1986). However, Nnodim and Lever (1988) suggest that stimulated release of NA from sympathetic nerves causes constriction of BAT arteriovenous anastomoses as a means of increasing tissue perfusion to parenchymal capillary beds.

Calcitonin-gene-related-peptide immunostaining was present but very sparse in BAT parenchyma and was sometimes detectable as weakly fluorescing fibres innervating the blood vessels. CGRP is widely distributed throughout the body as a neurotransmitter or neuromodulator centrally, and is released from perivascular nerve terminals to modulate arteriolar tone (see Zaidi et al, 1987). It could be that in brown adipose tissue the major regulator of vascular tone is NPY rather than CGRP. The factors influencing CGRP release from peripheral nerves are still unclear (Zaidi et al, 1987). It was somewhat surprising that there were any CGRP positive fibres in BAT parenchyma. However, this peptide is also located in sensory ganglia and their primary afferents (Zaidi et al, 1987), which raises the possibility of the existence of some sensory innervation to brown adipose tissue. Norman et al (1988)

have recently reported the existence of CGRP-containing fibres in rat brown adipose tissue and have also demonstrated the presence of substance P-containing fibres in the tissue. Substance P is usually associated with sensory neurons. Fishman and Dark (1987) used a neuroanatomical tracer in subcutaneous white adipose tissue to demonstrate the presence of sensory neural processes, so it is not without precedent to speculate that some sensory innervation to brown adipose tissue might exist. The functional significance of this is not understood but there is recent evidence that sensory nerves can play a role in regulating blood flow and vascular permeability, trophic responses such as wound healing and immunologic processes, in addition to their traditional sensory role (Holzer, 1988).

The nervous system grows by cellular hyperplasia, hypertrophy, and by ramification of processes with formation of specific synaptic connections, mediated in large part by nerve growth factor (see Yanker and Shooter, 1982). In addition, NGF can affect neurotransmitter synthesis and play a major role in maintenance of peripheral and sensory neuron function. There is good correlation between nerve growth factor level and density of sympathetic innervation in many tissues (Korsching and Thoenen, 1983; Shelton and Reichardt, 1984) leading to the concept that production of NGF in target organs determines the density of the sympathetic innervation. However, NGF was detected only in salivary gland and not in normal or myopathic BAT. It is possible that the antiserum was not specific or sensitive enough, or like TH, cold-induced changes in NGF level were transient and not detected at the time the animals were studied. Of course it is equally

likely that NGF is not produced or stored in BAT. Shine and Perez-Polo (1976) screened several hamster tissues for the presence of nerve growth factor, finding it in brain and other neuronal tissues, but they did not look in brown adipose tissue. Harper et al (1980) used a bioassay to locate NGF in various tissues from several species, including the hamster, but did not include brown fat in the tissues surveyed. With the exception of mouse salivary gland, none of the tissues examined in that study contained detectable NGF. It would be worth examining BAT from animals during the early stages of cold acclimation in order to determine if indeed nerve growth factor is involved in directing the development of sympathetic innervation in growing BAT.

The presence of cytochrome oxidase in BAT mitochondria can be demonstrated histochemically. An interesting feature of the cytochrome oxidase distribution was observed, namely the patchiness of staining in tissue from cold acclimated animals. While the entire section was immersed in the reaction mixture only portions of the tissue of roughly cell size were intensely stained. This was observed in both normal and myopathic hamsters, although to a somewhat lesser extent in myopathic animals. It is known that brown adipocytes are electrically coupled through the presence of gap junctions (Revel and Sheridan, 1967; Revel et al, 1971; Schneider-Picard et al, 1984) so it is possible that stimulation by cold activates some cells directly, and these cells initiate membrane depolarization and subsequent thermogenic activation. Therefore the metabolic activity of these cells would be higher, resulting in a more intense staining. However, double staining with TH

antiserum after the cytochrome oxidase reaction failed to show fibre bundles in the vicinity of the intensely staining cells. Therefore, the significance, if any, of the uneven staining is not known. Brown adipocyte size in myopathic hamster BAT was reduced in comparison with normal hamster BAT. This observation agrees with a study by Horwitz and Wickler (1983) in which they report decreased brown adipocyte size in myopathic hamsters.

In conclusion, this series of experiments confirms the presence of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis. Of the neuropeptides tested however, none were co-localized in BAT parenchymal sympathetic nerves. This does not exclude the possibility that changes did occur but not at the time the animals were studied. It is also possible that other neuropeptides not studied are involved in mediating BAT trophic responses. A more extensive study employing a wider range of neuropeptide and neurotransmitter antisera should be undertaken to explore this. Recently, Norman et al (1988) reported the absence of somatostatin, bombesin, neurotensin, enkephalin, and vasoactive-intestinal polypeptide from rat brown adipose tissue. However, these animals presumably had been kept at room temperature. Therefore, there is the possibility that cold may activate one or more of these peptides, and this should be investigated using a time course of cold exposure. Nnodim and Lever (1988) have observed the presence of unlabelled nerve terminals in rat brown adipose tissue after treatment with both the false neurotransmitter 5-hydroxydopamine and 6-hydroxydopamine, which destroys noradrenergic nerve terminals. They postulate that these terminals could represent

an additional non-noradrenergic nerve supply to brown adipose tissue.

The presence of abundant cytochrome oxidase in BAT mitochondria was demonstrated histochemically. Cold acclimation caused an unusual patchy distribution of staining that is currently of unknown significance.

Myopathy did not appear to affect either level or distribution of the neuropeptides studied, TH, or cytochrome oxidase in hamster BAT. Therefore, based on these results, failure of trophic responses in myopathic hamster brown adipose tissue cannot be attributed to a deficiency of sympathetic innervation or co-localized neuropeptide.

Plate 1. Tyrosine hydroxylase immunoreactivity in hamster brown adipose tissue. Positive staining fibres were present in parenchyma of both warm (a) and cold (b) acclimated BAT, and in fibres innervating blood vessels (c, warm acclimated; d, cold acclimated; e, detail of vessel from cold acclimated BAT). a and b X325; c and d X163; e X200.

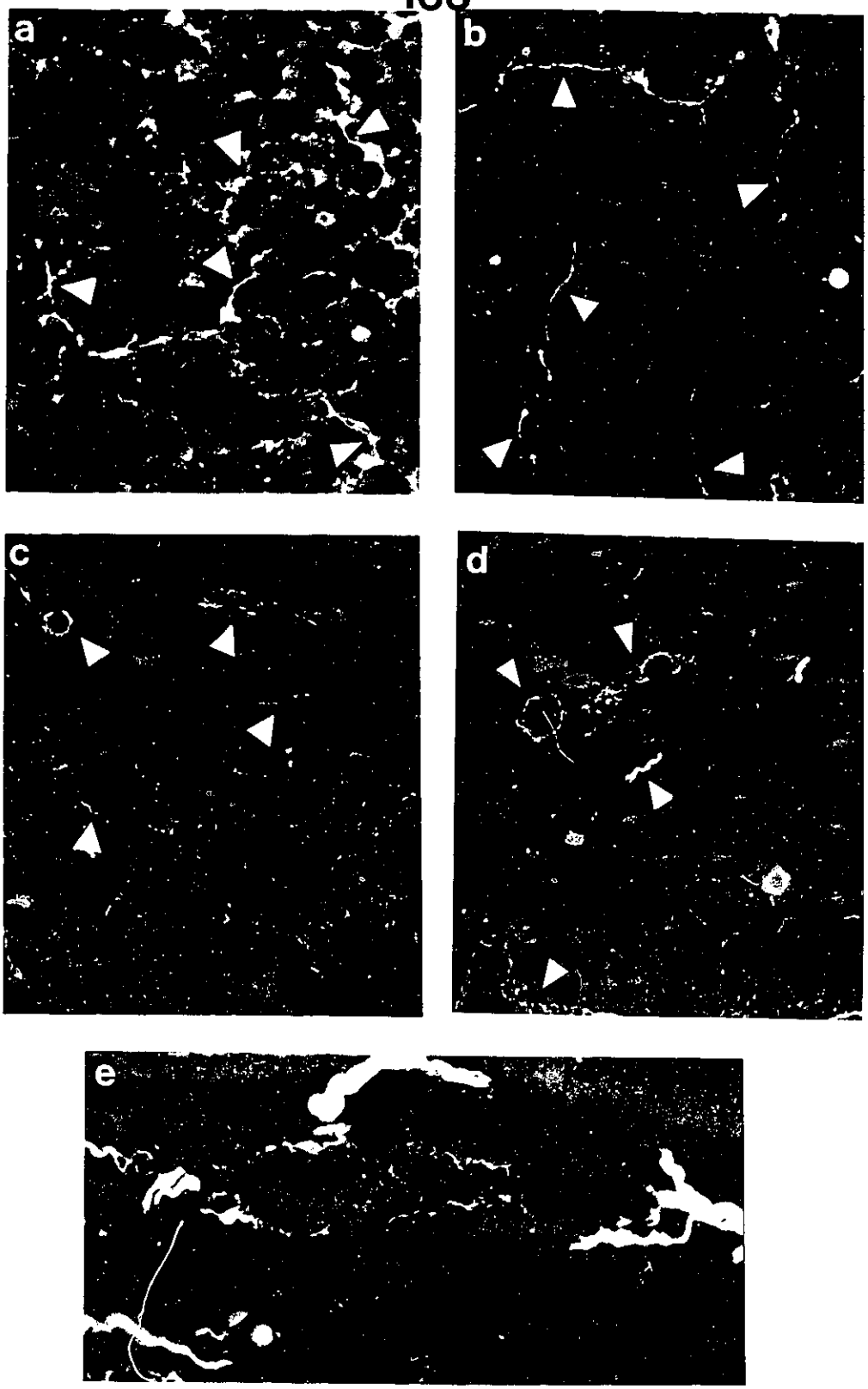

a
b
c
d
e

Plate 2. Tyrosine hydroxylase immunoreactivity in innervated (a) and denervated (b) hamster brown adipose tissue. Note the reduction in number of positive staining fibres in the denervated tissue (b) compared to the sham-operated control (a). X325.

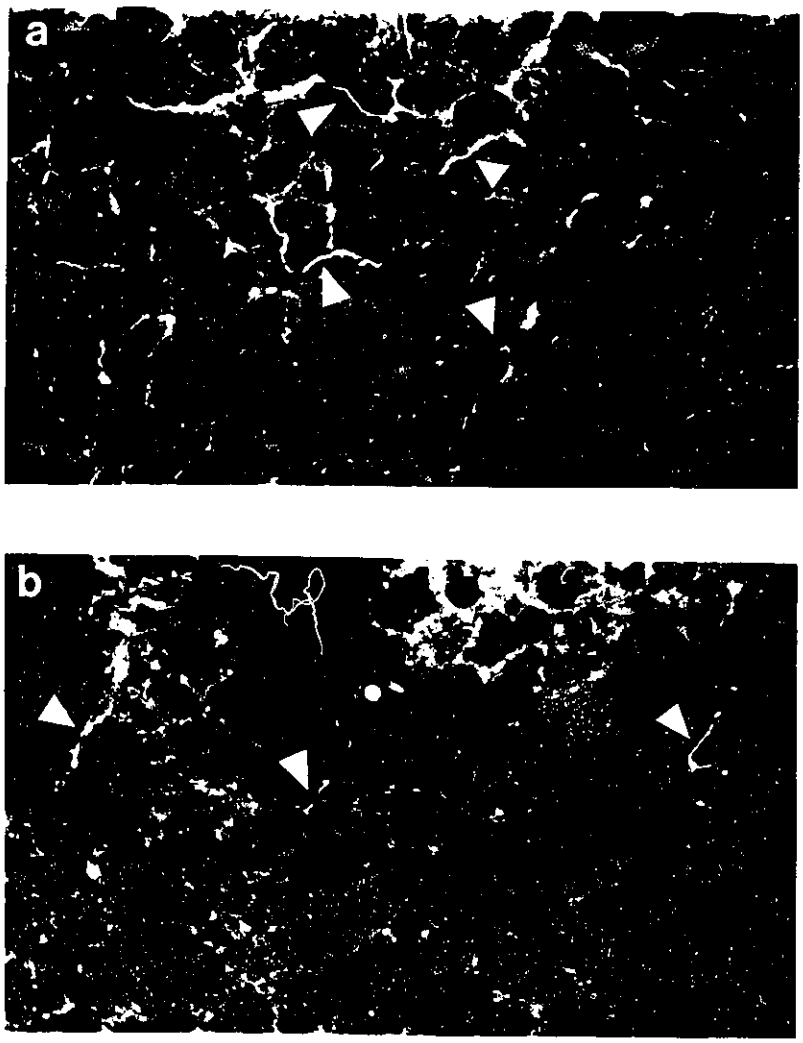

a
b

Plate 3. Neuropeptide Y-positive immunostaining in hamster brown adipose tissue. Immunofluorescence was found only around the vasculature in tissue from both warm acclimated (a, c) and cold acclimated (b) BAT. Denervation (d) appeared to reduce intensity of staining. a, X325; b. c, d, X163.

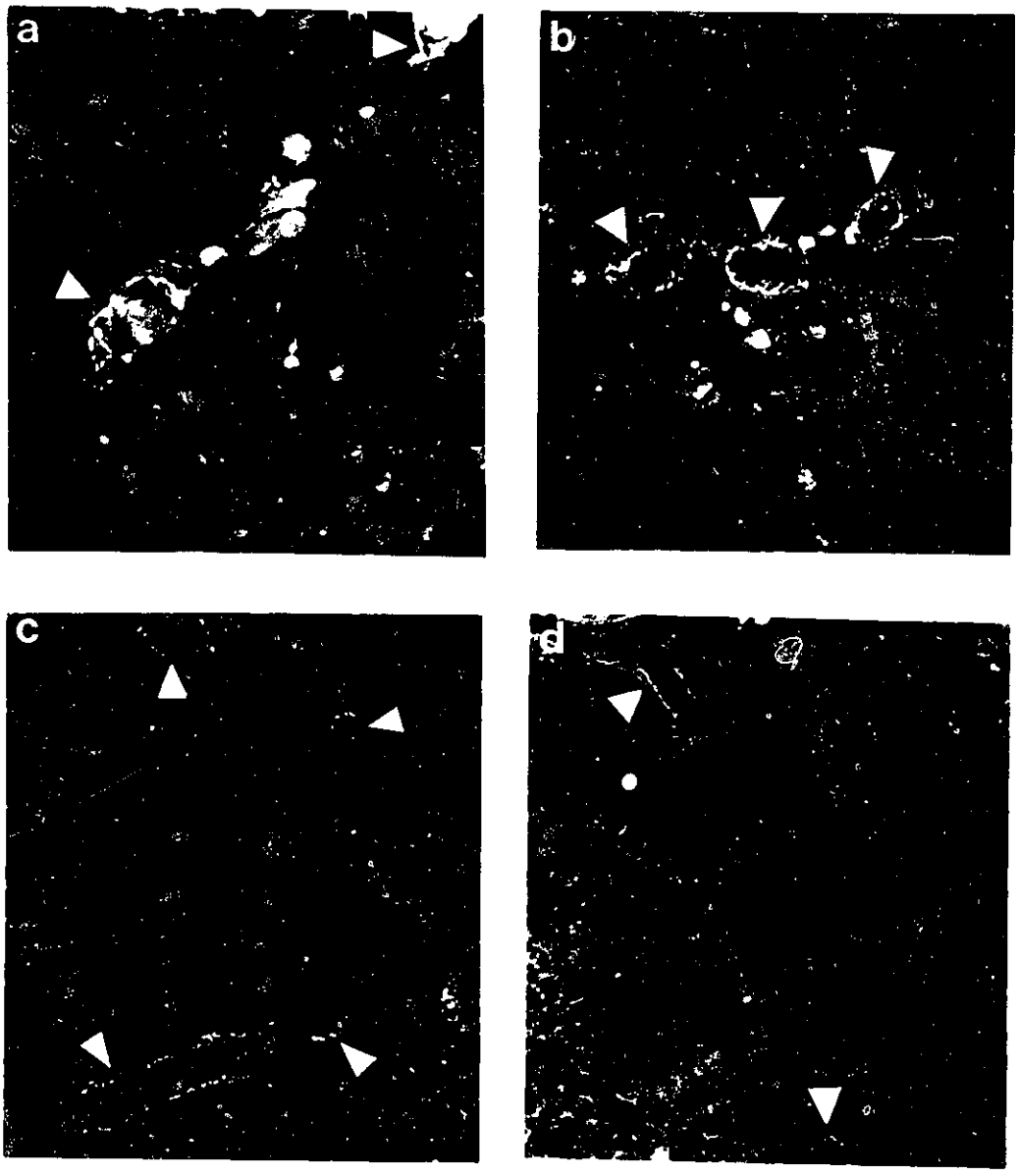
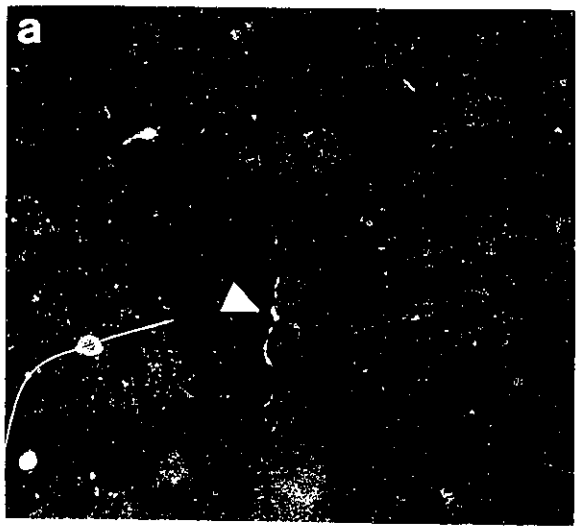

a
b
c
d

Plate 4. Calcitonin-gene-related-peptide immunostaining of hamster brown adipose tissue. Positive immunofluorescence was detected sparsely in BAT parenchyma (a, b). Some staining was observed in the vascular innervation but it was very faint and therefore not shown. X325.


a


b

Plate 5. Nerve growth factor immunoreactivity in male mouse salivary gland (a) and hamster brown adipose tissue (b). The presence of NGF was detected only in salivary gland, not in BAT. X163.

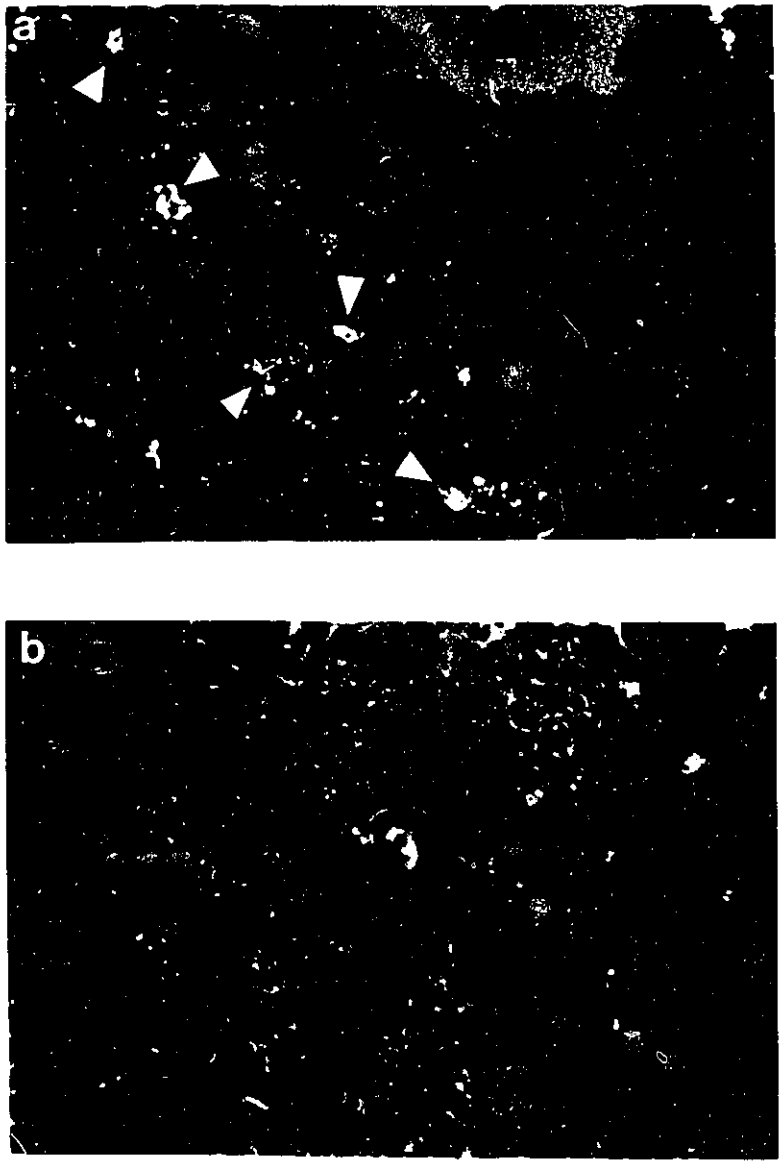
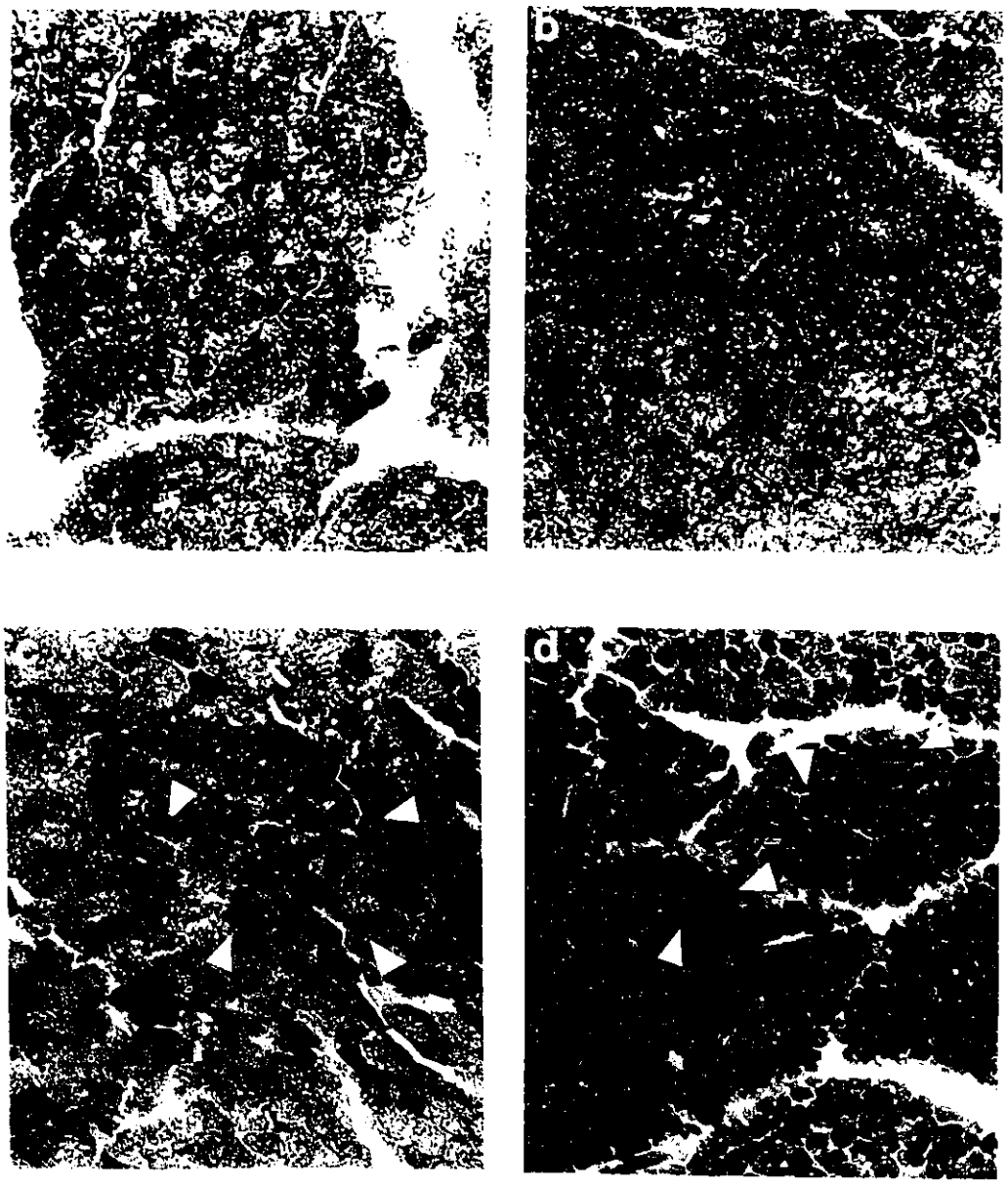

a
b

Plate 6. Cytochrome oxidase reactivity in hamster brown adipose tissue mitochondria. The presence of cytochrome oxidase was demonstrated in both warm (a, b) and cold acclimated (c, d) BAT. Normal (a, c) and myopathic (b, d) tissue appeared similar except that cell size seemed smaller in myopathic hamster BAT (c, normal; d. myopathic). Cold acclimation reduced lipid droplet size (a, warm; c, cold; b, warm; d, cold) and resulted in a patchy distribution of staining that was somewhat similar between normal and myopathic tissue (c, normal; d. myopathic). X163.


b
d

3F: ROLE OF T_3 AS A MEDIATOR OF BAT GROWTH AND FUNCTION:

Background:

Hamster brown adipose tissue hypertrophy occurs and is maintained to the largest extent in cold acclimated animals (Himms-Hagen and Gwilliam, 1980; Kopecky et al, 1986a; see 1A) yet sympathetic activity has returned to a low level in BAT of the cold acclimated hamster (Sigurdson and Himms-Hagen, 1988; see 3B). In fact, noradrenaline itself does not appear to mediate BAT growth in hamsters (see 3A, 3D; Triandafillou et al, 1984a; McElroy and Wade, 1986; Hamilton et al, 1986; Desautels and Dulos, 1988; Sigurdson and Himms-Hagen, 1988) yet does have some influence on T5'D activity (3A, 3D). Cold acclimated normal hamsters are hyperthyroid, hypermetabolic, and have an increased amount of thermogenically active brown adipose tissue (Chaffee et al, 1964; Tashima, 1964; Pohl, 1965; Vybiral and Jansky, 1974; Kazdova et al, 1975; Pospisilova and Jansky, 1976; Rabi and Cassuto, 1976; Trayhurn et al, 1983; Kopecky et al, 1986a; Tomasi and Horwitz, 1987; Sundin et al, 1987; Sigurdson and Himms-Hagen, 1988). Myopathic hamsters on the other hand, are hypothyroid and have a reduced response to cold (Himms-Hagen and Gwilliam, 1980; Wickler and Horwitz, 1984; Triandafillou et al, 1984b; Sigurdson and Himms-Hagen, 1988).

It has been noted in experiment 3B that in fully cold acclimated hamsters the increased concentration of uncoupling protein in BAT mitochondria is best correlated with increased local T_3 production by thyroxine 5'-deiodinase and with elevated serum T_3 levels rather than with sympathetic nervous activity. Bianco and Silva (1987) have suggested that the optimum response of UCP to cold in rats requires a

high BAT T_3 concentration, generated by increased tissue deiodinase activity.

Objectives:

The aims of this study were to determine if T_3 could mimic cold acclimation in its effects on BAT and whether T_3 could improve growth and thermogenic function of myopathic BAT.

Methods:

Normal and cardiomyopathic hamsters aged 5-6 weeks were housed individually as described in METHODS. Triiodothyronine (T_3) sodium salt (Sigma) was dissolved in 0.05 N NaOH + 0.1% BSA and stored at 4°C for no longer than 1 week. For injection the T_3 solution was diluted 10 fold in 10% hamster serum in 0.9% NaCl and injected s.c. at either 3 or 12.5 µg T_3 (free acid)/100g BW daily for 7 days. The injections were made shortly after lights on (approx. 0900 h). Diluted hamster serum served as the vehicle.

In the first of three experiments, groups of 5 or 6 hamsters were injected as described and food intake and body weight recorded daily. On d 7, after the last injection the animals were killed by decapitation. Blood was collected and frozen for subsequent thyroid hormone assay, and body temperature was measured. Interscapular brown adipose tissue was removed into isolation medium then cleaned, weighed, and homogenized. Aliquots were taken for protein, deiodinase, and UCP assays. The remainder was used for isolation of mitochondria for GDP-binding (see METHODS).

In the second experiment, 4 hamsters per group were injected with T_3 daily for 7 d. On the last day, after injection, the hamsters were

anesthetized with sodium pentobarbital (Somnitol, 80 mg/kg) and mimimal oxygen consumption (basal metabolic rate) was measured as described in METHODS. The body temperature of the animals was maintained at 36.7±0.5°C. Measurements were made for 20-40 minutes, until a stable rate of oxygen consumption was achieved. After completion of recording the animals were sacrificed and the blood saved for thyroid hormone determination.

In the third experiment, 5 hamsters per group were injected daily as described and food intake and body weight were measured. Twenty-four hours after the last injection, the hamsters were anesthetized and oxygen consumption was recorded. The hamsters were then allowed to recover for a further 7 d, during which body weight and food intake were again measured. On the 15th d minimal oxygen consumption was measured and afterward the animals sacrificed. Blood was collected for thyroid hormone determination.

Data were analyzed by 2 way analysis of variance and Duncan's test post hoc. Food intakes and body weights were analyzed using repeated measures analysis of variance followed by Tukey's test. The level of statistical significance was 0.05.

Results:

Experiment 1.

Body Weight and Food Intake:

T_3 treatment significantly decreased body weight in normal hamsters but the decline in weight of the myopathic hamsters was not significant (table 28). Myopathic hamsters were always lighter than normal (table 28). Food intake was increased by injection with T_3

during the latter portion of the treatment period (table 29). Myopathic hamsters ate less than normal for the first 4 d but intake returned to normal on days 5, 6, and 7 (table 29).

Body Temperature:

T_3 injections had no effect on body temperature in either animal type (table 28).

Brown Adipose Tissue:

BAT weight was increased by both doses of T_3 in normal and myopathic hamsters, although myopathic brown fat still weighed less than normal (table 30). Protein content was increased by the higher T_3 dose (12.5 µg/100g) in normal hamsters (table 30). There was less protein in myopathic BAT and it was unaffected by T_3 treatment (table 30). Injections had no significant effect on UCP concentration although total content was increased due to an overall increase in BAT protein of normal hamsters receiving the higher T_3 dose (table 30).

Specific deiodinase activity was markedly increased by the higher T_3 dose in normal hamsters (figure 20). The lower dose increased specific activity but this did not reach statistical significance. Myopathic hamster BAT deiodinase activity was not increased by either dose of T_3 (figure 20). Total deiodinase activity was increased by T_3 as a result of tissue protein increases as well as the increase in specific activity in normal but not myopathic hamsters (figure 20).

Serum Thyroid Hormones:

Administration of exogenous T_3 markedly elevated serum levels in both animal types although the lower dose was less effective in myopathic hamsters (figure 21). Serum T_4 was depressed by both doses

of T_3 in both normal and myopathic hamsters (figure 21). Myopathic hamsters receiving only vehicle were hypothyroid compared to normal vehicle treated hamsters with respect to both T_3 and T_4 (figure 21).

Experiments 2 and 3:

Body Weight and Food Intake:

For the first 7 d (during injections) food intake and body weight followed the same pattern as that in experiment 1. However on d 8 all animals exhibited an unexplained drop in intake from which they largely recovered by the next day. This change was unlikely to be due to cessation of handling once injections stopped, as the animals were still being handled during weighing and feeding. These data were judged to be unanalyzable because no good explanation for the sudden change in behavior could be found.

Minimal Oxygen Consumption:

Both doses of T_3 increased minimal oxygen consumption (basal metabolic rate) in normal (figure 22) and myopathic hamsters (figure 23). This persisted for 24 h after the last injection but had disappeared by 7 days post injection (figures 22 and 23). It is worthy of note that the minimal metabolic rate of myopathic hamsters is virtually identical to normal hamsters and that T_3 stimulated metabolic rate to the same extent in both animal types (figures 22 and 23).

Thyroid Hormones:

Seven days after cessation of injections serum T_3 dropped dramatically to below the control value in normal hamsters (table 31). Withdrawal of treatment also led to a decline in serum T_3 in myopathic hamsters but there was no overshoot to below control values (table 31).

Serum T_4 rose back to control levels once T_3 injections were stopped in both normal and myopathic hamsters (table 31).

Discussion:

The major result of these experiments is that T_3 can mimic some aspects of cold acclimation on hamster brown adipose tissue, namely increased growth and deiodinase activity. Total brown adipose tissue protein content including UCP was increased 26% in normal hamsters but T_3 did not specifically increase the amount of UCP or stimulate thermogenic activity. It is not clear however, whether this might be a result of increased deiodinase activity and T_3 generation in BAT or a direct effect of administered T_3.

The animals in these experiments were rendered hyperthyroid by T_3 administration which resulted in a feedback suppression of thyroid hormone secretion. It could be that the increase in hamster BAT deiodinase activity is secondary to the decreased T_4 levels in the blood as a result of treatment. Brown adipose tissue T5'D activity in rats is stimulated by hypothyroidism (low T_4), a characteristic of the type II enzyme (Leonard et al, 1983; Silva and Larsen, 1983).

It is known that thyroid hormones are required for the full thermogenic response of (rat) brown adipose tissue to cold (Bianco and Silva, 1987a, b), yet no specific increase in UCP was found as a result of T_3 treatment in hamsters. Perhaps it is unnecessary for the hamster to increase the UCP concentration in its BAT mitochondria under these circumstances. In the absence of other factors which would participate in the response to cold, T_3 alone may be insufficient to produce a specific increase in uncoupling protein.

Minimal oxygen consumption (basal metabolic rate) was increased by approximately 45-50% in T_3 treated hamsters. This value is close to the 59% increase observed in cold acclimated hamsters (see section 2) and demonstrates that thyroid hormone-induced thermogenesis can account for a major portion of the increase in basal metabolic rate of cold acclimated hamsters.

If thyroid hormone induced thermogenesis is a major source of heat in the T_3-treated hamster, then BAT thermogenesis would not be stimulated. This appears to be the case as GDP-binding was not increased by T_3 treatment. Thyroid hormones could participate in mediating BAT growth in preparation for hibernation, and the hamster might shift a portion of its cold-induced thermogenesis from BAT to other organs via locally produced T_3. This would help maintain brown adipose tissue in a hypertrophied state with a high thermogenic potential but not fully thermogenically active until required for arousal from hibernation. During that time there is massive activation of thermogenesis (Horwitz et al, 1985) and utilization of BAT lipid stores (Nedergaard and Cannon, 1984). The hamster well may confine its major use of BAT to episodes of arousal from hibernation, and use it along with other means of cold-induced thermogenesis during the period of acclimation prior to hibernation and during normothermic intervals between hibernation bouts.

Another major finding of these experiments was that T_3 administration failed to stimulate thyroxine 5'-deiodinase activity or BAT growth in myopathic hamsters. These animals are known to be resistant to certain actions of T_4 on the heart (Hegyvary et al, 1979)

and maybe in other organs as well including brown adipose tissue. If this is so, then they may not sense the reduced level of T_4 and cannot respond by increasing deiodinase activity in BAT. Failure of BAT to grow under these circumstances may be secondary to the reduced local production of T_3 by the deiodinase.

Myopathic hamsters have a normal body temperature and minimal oxygen consumption despite half the normal level of T_3 in their blood. This was somewhat unexpected since MOC is primarily controlled by thyroid hormones (Guernsey and Edelman, 1983; Van Hardeveld, 1986). Therefore, other mechanisms must be involved in raising their metabolic rate. One such factor that could contribute to obligatory thermogenesis in these animals is energy expended in protein synthesis. Skeletal muscle protein turnover is accelerated in myopathic hamsters (Li, 1980; Nicholls et al, 1980). Thus, it is possible that the heat generated by the increased rate of muscle protein synthesis contributes to the overall increase in metabolic rate.

In conclusion, T_3 can produce growth of normal hamster BAT but alone it is not sufficient to induce a specific increase in UCP. This suggests that other factors also participate in mediating BAT growth. Since thyroid-induced thermogenesis can account for a large fraction of the cold-induced increase in basal metabolic rate, it can be postulated that the cold acclimated hamster has shifted a portion of thermogenesis from BAT to other organs, but maintains the tissue in a state of readiness for use in arousal from hibernation.

BAT from myopathic hamsters, on the other hand, fails to respond to T_3, perhaps as a result of insensitivity to altered T_4 levels.

Myopathic hamsters have a normal body temperature and minimal oxygen consumption despite low blood T_3 levels, which suggests that other heat producing mechanisms participate in raising metabolic rate.

Table 28

Experiment 1. Body Weight and Temperature in T_3 Injected Hamsters

	NORMAL			MYOPATHIC		
	VEH (5)	LOW (5)	HIGH (5)	VEH (5)	LOW (6)	HIGH (5)
7 Day Weight Gain (g)	5.7 ±1.1	1.4* ±0.7	1.8* ±1.1	0.04† ±0.7	-1.9† ±0.8	-2.4† ±0.7
Final Weight (g)	107.8 ±3.2	97.5* ±2.9	102.5 ±3.0	82.5† ±2.0	81.5† ±4.4	76.9† ±1.3
Temp (°C)	38.38 ±0.11	38.76 ±0.17	38.66 ±0.26	38.12 ±0.15	38.43 ±0.22	38.24 ±0.10

Body weight and temperature in normal and myopathic hamsters injected with T_3 for 7 d. Values are means ± SE for number of animals given in parentheses. VEH is diluted hamster serum vehicle, LOW is 3 µg T_3/100 g BW, and HIGH is 12.5 µg T_3/100 g BW. Weight gain is the difference between d 7 and d 1 of injection and final weight is that at sacrifice. Temp is rectal temperature measured immediately after death. * indicates significant effect of treatment and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

Table 29

Experiment 1. Effect of T_3 Injections on Food Intake in Normal and Myopathic Hamsters

MAIN EFFECT

ANIMAL (21)	NORMAL	29.3±0.7	F=9.97	p=0.0041
	MYOPATHIC	26.2±0.8†		
T_3 (14)	VEHICLE	25.8±0.4	F=5.24	p=0.0126
	LOW	27.7±0.9*		
	HIGH	29.6±1.2*		

INTERACTIONS

ANIMAL X TIME

	NORMAL (15)	MYOPATHIC (16)
DAY 1	28.4±1.1	22.5±0.7†
2	27.4±1.2	22.7±0.8†
3	30.1±1.3	24.4±0.8†
4	29.2±1.5	26.0±0.7†
5	30.8±1.5	28.4±0.5
6	29.5±1.3	28.8±0.9
7	29.7±1.4	30.3±1.1

T_3 X TIME

	VEHICLE (10)	LOW (11)	HIGH (10)	
DAY 1	26.1±1.6	24.8±1.3	25.1±1.5	F=4.78
2	25.9±1.3	23.3±1.2	25.9±1.8	p=0.0001
3	25.7±1.8	26.4±1.1	29.5±1.6	
4	25.0±1.1	27.8±1.3	29.8±1.7	
5	27.5±1.3	29.3±0.7	31.9±1.8*	
6	25.0±0.5	30.2±0.8*	32.1±1.3*	
7	25.2±1.3	31.0±0.6*	33.7±1.4*	

Effect of T_3 injections on food intake in normal and myopathic hamsters. Values are means ± SE for number of animals in parentheses. See table 28 for details of doses. Table is broken down into main effects and significant interactions. * indicates significant effect of treatment and † indicates significant difference between normal and myopathic hamsters.

Table 30

Experiment 1. Effect of T_3 Injections on Brown Adipose Tissue in Normal and Myopathic Hamsters.

	NORMAL			MYOPATHIC		
	VEH	LOW	HIGH	VEH	LOW	HIGH
Weight (mg)	336.1 ±28.7	498.8 ±14.0	593.2* ±31.8	158.4† ± 8.7	249.9*† ± 9.8	250.3*† ±11.4
Protein (mg)	19.8 ±5.0	22.9 ±6.8	27.0* ±9.7	12.0† ±0.7	15.3† ±0.5	15.8† ±0.6
UCP (µg/mg)	10.7 ±2.8	7.8 ±2.0	12.8 ±1.6	12.1 ±2.0	14.4 ±2.3	15.6 ±1.4
(µg/BAT)	277.3 ±89.0	233.6 ±56.2	517.2* ±55.4	144.0 ±24.1	219.0 ±35.0	249.0† ±28.7
GDP-binding (pmol/mg)	236.2 ±32.4	246.4 ±52.5	296.6 ±36.4	188.4 ±30.2	239.5 ±11.9	252.0 ±20.4

Effect of daily T_3 injections on brown adipose tissue of normal and myopathic hamsters. Values are means ± SE for numbers of animals given in table 28. For abbreviations and symbols see table 28.

Table 31

Experiments 1 and 3. Serum Thyroid Hormones in Normal and Myopathic Hamsters

		DAY 7 OF TREATMENT		7 DAYS POST INJECTION	
		NORMAL			
T_3 (ng/dl)	VEH	90.0± 9.4	(5)	46.6±9.9	(5)
	LOW	550.0±44.5*	(5)	26.6±6.7*¶	(5)
	HIGH	658.8±49.9*	(5)	21.0±7.2*¶	(5)
		MYOPATHIC			
	VEH	29.2± 2.8†	(5)	21.7±3.8†	(5)
	LOW	307.5±31.0*†	(6)	19.0±2.5¶	(5)
	HIGH	617.8±50.1*	(5)	9.0±3.1¶	(5)
		NORMAL			
T_4 (µg/dl)	VEH	2.77±0.25	(5)	2.3±0.6	(5)
	LOW	0.39±0.05*	(5)	2.4±0.3¶	(5)
	HIGH	0.64±0.10*	(5)	2.0±0.6	(5)
		MYOPATHIC			
	VEH	1.88±0.13†	(5)	3.0±0.5	(5)
	LOW	0.27±0.02*	(6)	2.2±0.4¶	(5)
	HIGH	0.30±0.02*	(5)	1.3±0.3	(5)

Serum thyroid hormones in T_3 treated normal and myopathic hamsters. Values are means ± SE for number of animals given in parentheses. Data for day 7 of treatment are the same as in figure 21. For abbreviations and symbols see table 28. ¶ indicates significant difference between d 7 of treatment and 7 d post injection and † indicates significant difference between normal and myopathic hamsters ($p<0.05$).

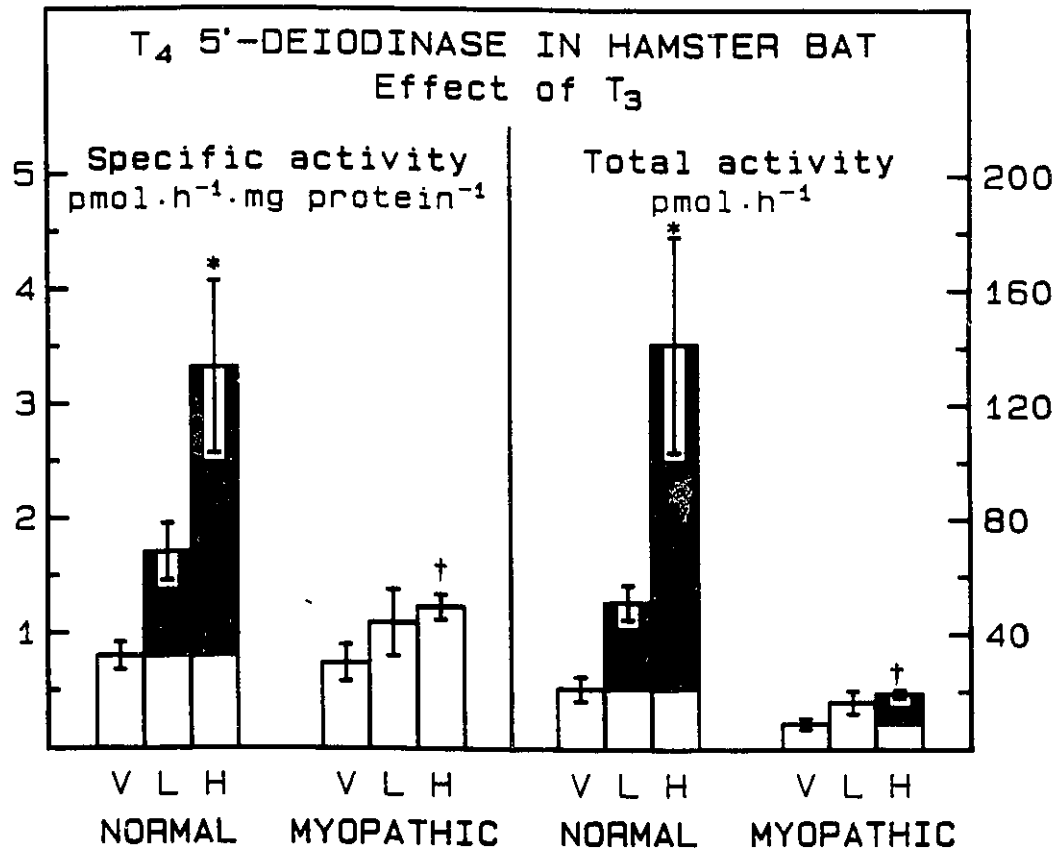


Figure 20. Effect of daily injections of T_3 on BAT T5'D activity in normal and myopathic hamsters (experiment 1). Values are means ± SE for numbers of animals given in table 28. Shaded portion of bars indicates increased activity. * indicates significant effect of T_3 treatment and † indicates significant difference between normal and myopathic hamsters ($p<0.05$). V refers to vehicle, L to low T_3 dose, and H to high T_3 dose (see table 28 for details).

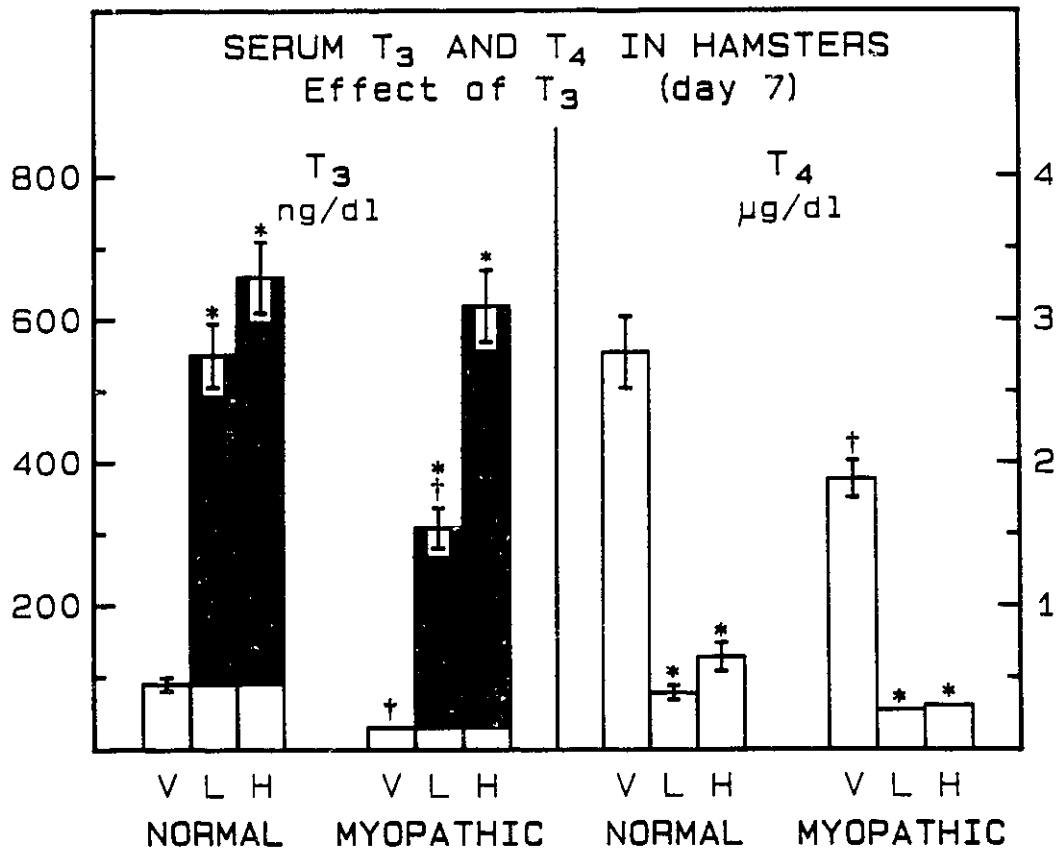


Figure 21. Effect of T_3 treatment on serum thyroid hormones in normal and myopathic hamsters (experiment 1). Values are means ± SE for numbers of animals given in table 28. Shaded portion of bar and * indicate significant effect of T_3 treatment. † indicates significant difference between normal and myopathic hamsters. For abbreviations see table 28 and figure 20.

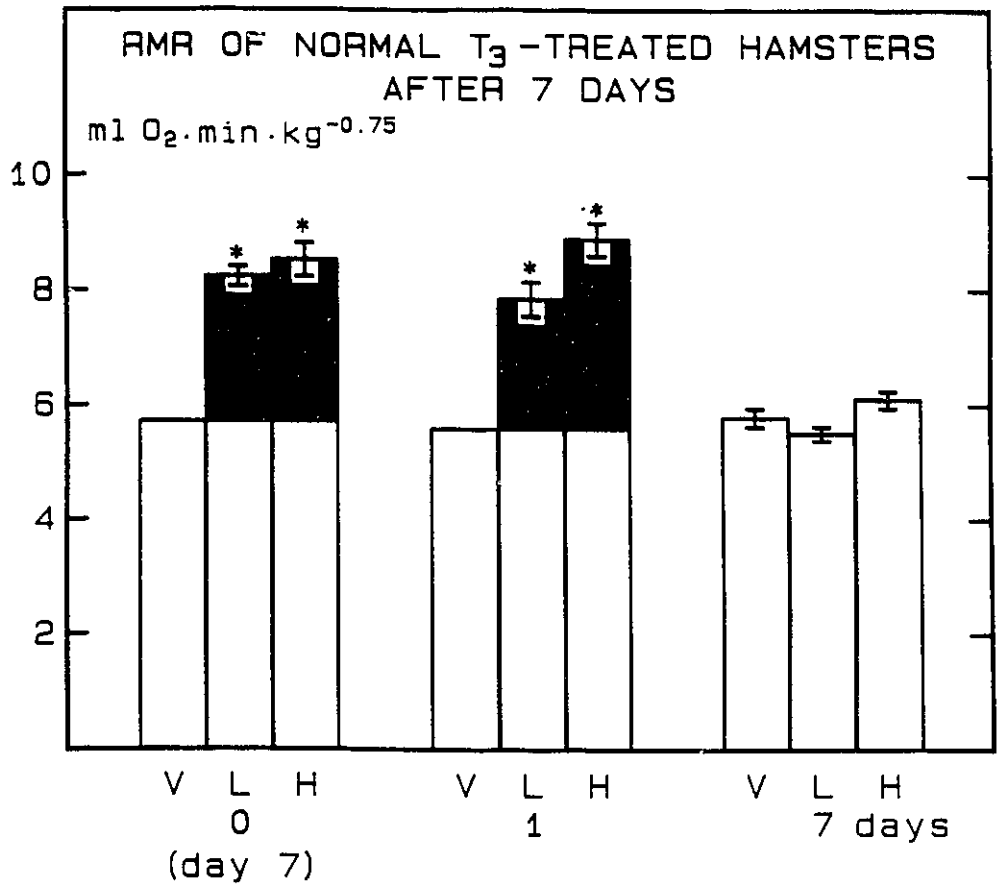


Figure 22. Minimal oxygen consumption (basal metabolic rate, BMR) of T_3 treated normal hamsters during and after treatment (experiments 2 and 3). Values are means ± SE for groups of 5 animals. Shaded portions of bars and * indicate significant effect of treatment. 0 refers to measurement made on last day of injections, 1 refers to 1 d post injection, and 7 days refers to 7 d post injection. For abbreviations see table 28 and figure 20.

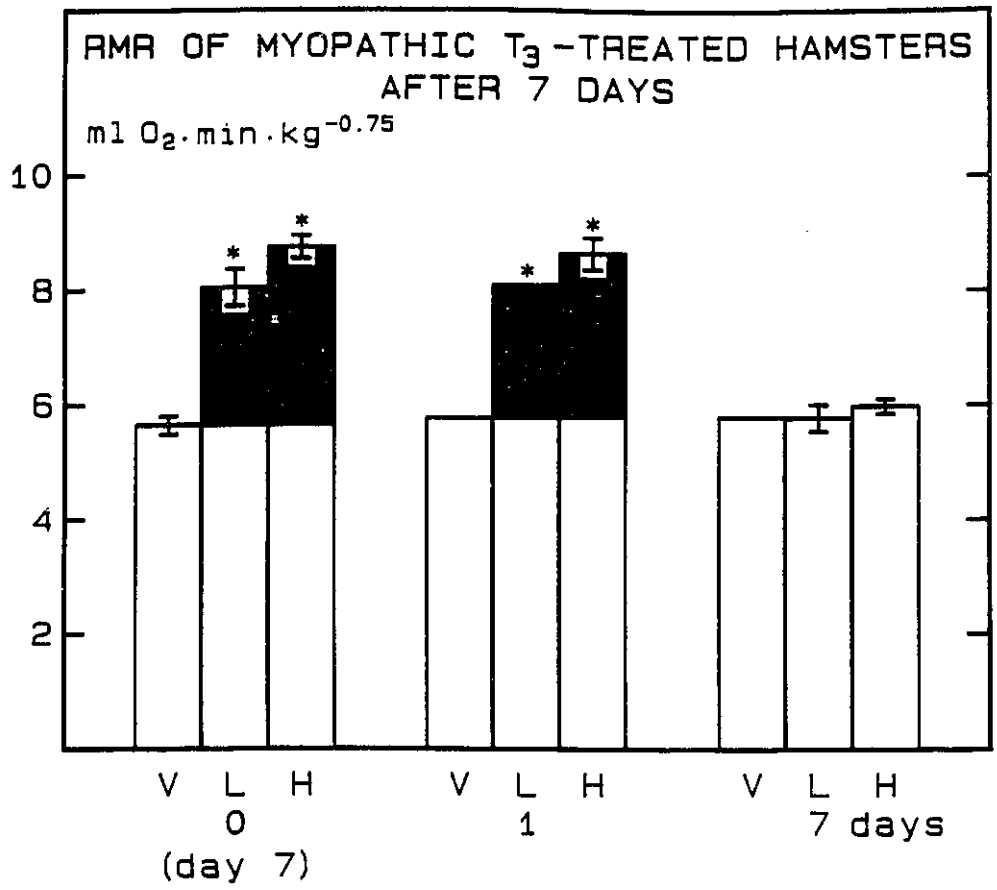


Figure 23. Minimal oxygen consumption (basal metabolic rate, BMR) of T_3 treated myopathic hamsters during and after injections (experiments 2 and 3). Values are means ± SE for 4-6 animals/group. For details see figure 22 and table 28.

4: MYOPATHIC HAMSTERS-SUMMARY OF RESULTS AND DISCUSSION, SECTIONS 1-3:

Responses to Cold:

Myopathic hamsters were cold sensitive in that they had a relatively high mortality rate when transferred to 4°C (1A). However the survivors were able to grow more brown adipose tissue although it remained smaller than normal (1A). Deiodinase activity was increased by cold in myopathic BAT but it was initially less responsive than normal (1A). After cold acclimation however, specific deiodinase activity was normalized but because of the smaller tissue size total activity was less than normal. Hamsters with muscular dystrophy are hypothyroid (1A; 1B; 3B; 3F; Kopecky et al, 1986a, b) in that their serum T_3 level is lower than normal, and although cold exposure caused a marked elevation of serum T_3 it was to a lesser extent than normal (1A; 3B).

Responses to High Fat Diet:

When myopathic hamsters had access to a palatable high fat diet of sunflower seeds, like normal hamsters they generally did not overeat (1B, 3B), but unlike normal animals there was no trophic response of brown adipose tissue to the diet. NA content and half life in BAT were normal and not altered by the diet, as in normal hamsters (3B). Deiodinase activity was unaffected by diet, as was the case in normal hamsters (3B). Serum T_3 was increased by high fat diet feeding in one experiment (3B) but not in another (1B) but the reason for this is not known; there was no T_3 increase in response to diet in normal hamsters. Serum T_4 was decreased at some times during the adaptation to diet in both animal types (1B).

Responses to Short Photoperiod:

Adaptation to a short photoperiod failed to induce brown adipose tissue hypertrophy in myopathic hamsters as it did in normal hamsters (1B), however they were normal in the lack of response of deiodinase activity and noradrenaline turnover to short photoperiod (1C, 3B). Serum T_3 was increased by short day exposure but it did not reach normal values (1B, 3B) and lagged behind the increase observed in normal hamsters (3B). Serum T_4 was increased by short day exposure, the opposite of what occurred in normal hamsters (1B).

Responses to T_3 Administration:

Daily injections of T_3 did not increase myopathic BAT deiodinase activity or growth, in contrast to normal hamsters where both deiodinase activity and protein content were increased (3F). GDP-binding was not affected by T_3 treatment in either normal or myopathic hamsters. Serum thyroid hormone levels were altered in a normal fashion by T_3 treatment, with high T_3 values and low T_4 levels, but vehicle injected myopathic hamsters had lower than normal levels of T_3 (3F). Body temperature and minimal oxygen consumption were normal in vehicle injected myopathic hamsters. Injections of T_3 elevated minimal oxygen consumption to the same extent in myopathic hamsters as in normal animals (3F).

Discussion:

Failure of brown adipose tissue growth in myopathic hamsters fed a high fat diet or adapted to short photoperiod does not appear to be the result of a defective stimulation of BAT growth by the sympathetic nervous system because, in normal hamster BAT, despite tissue growth

neither diet nor photoperiod altered noradrenaline turnover (3B). There also does not appear to be a defective deiodinase enzyme in myopathic hamster BAT because deiodinase activity likewise was unaffected by diet or photoperiod in normal hamsters (1B).

It has been postulated that there is a central defect in the control of brown fat growth in myopathic hamsters which would affect both photoperiod recognition, mediated by melatonin, and recognition of high fat diet, via an unknown mediator (Triandafillou et al, 1984b; 1B). This putative defect would not affect the action of melatonin on the gonads, as short photoperiod causes normal testicular atrophy (Triandafillou et al, 1984b; 1B; Sigurdson and Himms-Hagen, 1988), nor would it interfere with the response to cold (Himms-Hagen and Gwilliam, 1980; Desautels et al, 1986). Whether the proposed central defect is a consequence of myopathy or an independent impairment is currently unknown (see below).

Another possible explanation for the reduced size of myopathic BAT and its failure to grow with diet or photoperiod is that in these animals heat is being produced elsewhere and BAT is actually being suppressed. Thus only a stimulus of sufficient intensity such as cold would be able to overcome this suppression. Where then would this heat come from? Myopathic hamsters are hypothyroid (1A; 1B; 3B; 3F; Kopecky et al, 1986b; Sigurdson and Himms-Hagen, 1988) so thyroid hormone-induced thermogenesis is not likely to play a role here. Myopathic hamsters are very active however, more so than their normal counterparts (unpublished observations; Wagner et al, 1986) and it is possible that heat produced by physical activity, coupled with

increased muscle protein turnover (see 3F) would be sufficient to suppress BAT function. Exercise can indeed suppress brown adipose tissue function and growth even in cold exposed animals (Arnold and Richard, 1987; see Himms-Hagen, 1989) and it is reported to attenuate thermogenic and trophic effects of a high fat diet in rats (Arnold and Richard, 1987). The cause of increased physical activity in myopathic hamsters is unknown but Wagner et al (1986) reported an increase in calcium antagonist receptor number in the brains of myopathic hamsters, and noted that calcium channel activators and blockers can influence behavior. Whether abnormal brain calcium channel activity and impaired BAT trophic responses are in any way connected, or related to muscular dystrophy warrants further investigation.

Under conditions of maximum stimulation (cold exposure) myopathic hamsters can respond and step up thermogenesis, for activation of deiodinase and GDP-binding, and BAT growth do occur (1A; Himms-Hagen and Gwilliam, 1980; Desautels et al, 1986). The hypothyroid state of the animal likely plays a role in its blunted response to cold, for even though serum T_3 levels and deiodinase activity are elevated, they are not normalized (see 1A; Kopecky et al, 1986b). One could speculate that the hypothyroid state results from impaired feedback control of thyroid hormone secretion at the hypothalamic and/or peripheral level, or from resistance to thyroid hormone actions (see 3F). Perhaps resistance to actions of T_4 on heart (Hegvary et al, 1979) could be a generalized phenomenon such that there is failure to recognize or respond appropriately to a thyroid hormone signal. This could explain the lack of deiodinase stimulation in response to exogenous T_3; there

appeared to be failure to recognize the depressed serum T_4 and respond with increased BAT deiodinase activity and tissue growth (see 3F).

Despite hypothyroidism, body temperature and minimal oxygen consumption were normal and responded normally to T_3 injections (3F). There could be a number of reasons for this. Firstly, the hypothyroidism may not have been severe enough to be detected as a decreased minimal oxygen consumption. This is rather unlikely as serum T_3 levels in the myopathic hamsters were approximately half that of normal (see 1A; 1B; 3B; 3F) and in humans at least, hypothyroid individuals have basal metabolic rates ranging from -20% to -40% of euthyroid standard values. Therefore, myopathic hamsters should have had a detectably depressed minimal oxygen consumption. This was not observed. Detection was unlikely to have been the problem, for differences in oxygen consumption were recorded even before correcting for differences in metabolic body size. If detection was not the problem, then other mechanisms must be involved which increased minimal oxygen consumption. One of these could be the increased rate of skeletal muscle protein turnover known to occur in myopathic hamsters (Li, 1980; Nicholls *et al*, 1980). Protein turnover produces heat as part of obligatory thermogenesis (see Himms-Hagen, 1983) and this could contribute to raising metabolic rate.

In summary, one could hypothesize that the altered or absent protein product of the muscular dystrophy gene could influence energy balance and thermogenesis, behavior, and membrane integrity if indeed it is expressed in both brain and muscle as suggested by Nudel *et al* (1988). Alternatively, the gene coding for a regulatory factor

involved with diet and photoperiod recognition may be located near the dystrophin gene. It would be altered or missing in muscular dystrophy, resulting in the defective trophic responses of BAT. It also possible that the abnormalities arising from muscular dystrophy and the defects in brown adipose tissue are independent of each other and only an interesting coincidence.

CONCLUSIONS AND GENERAL DISCUSSION:

Hamster brown adipose tissue can be induced to grow by three stimuli: cold, high fat diet, and short photoperiod. The responses to these stimuli appear to follow two distinct patterns.

1. Cold:

Cold-induced growth of BAT in hamsters involves transient activation of sympathetic activity (3B) and increased deiodinase activity (1A; Kopecky et al, 1986a). The increase in T5'D requires an intact innervation (3C) and can be brought about by noradrenaline (3A) and by a combination of α- and β-adrenergic agonists (3D). However, deiodinase activity is not necessarily correlated with increased sympathetic activity as BAT noradrenaline turnover is increased during the early stages of cold acclimation but T5'D activity remains high throughout the period of exposure. There is some correlation between increased deiodinase activity and increased BAT protein content in cold acclimated hamsters (Kopecky et al, 1986a; 1A), and cold acclimation also increases the concentration of mitochondrial UCP (Kopecky et al, 1986a; 1A; Desautels and Dulos, 1988). T5'D activity can be increased by T_3 administration (3F) and since serum T_3 is elevated by cold (1A, 3B; Kopecky et al, 1986a, b; Tomasi and Horwitz, 1987), growth of hamster BAT may be controlled by a combination of T_3 level and sympathetic activity. This may be brought about by alterations in the pattern of sympathetic nerve firing causing differential release of neurotransmitter and/or co-localized neuropeptide (Hökfelt et al, 1987; Westlind et al, 1984), coupled with changes in hormone secretion. As the pattern of sympathetic activity changes in BAT during the course of

of cold acclimation, so too might the pattern of neurotransmitter/-neuropeptide/hormone release.

2. Diet and Photoperiod:

Unlike responses to cold, growth of brown adipose tissue upon stimulation by diet or photoperiod does not involve changes in sympathetic activity (3B), deiodinase activity (1B, 1C; Kopecky et al, 1986b), or serum T_3 (1B, 1C; Kopecky et al, 1986b). Therefore, the mediators of this type of BAT growth do not appear to be noradrenaline or thyroid hormones, but combinations of factors not studied during the course of this research. These might include diet-derived signals such as insulin or glucagon, and melatonin release from the pineal, but the exact mechanisms by which BAT growth is stimulated by diet or photoperiod are not currently known.

GENERAL DISCUSSION:

Although the identification of specific factors involved in mediating growth of hamster brown adipose tissue remains elusive, one can say that acute thermogenic responses and the longer term adaptive growth of brown adipose tissue are regulated differently, and are not necessarily linked. The mediators of BAT growth in response to different stimuli (cold, diet, photoperiod) do not appear to be the same, but at this point it cannot be determined how much overlap there might be in the signals or pathways.

The results of these experiments further emphasize the differences between rats and hamsters in adaptive strategy for survival. The rat is a scavenger with a diet often low in essential nutrients. Therefore it must overeat if possible in order to gain adequate nutrition. It

could avoid obesity by being able to activate BAT to burn off the excess calories as heat, and in doing so also increase its capacity for nonshivering thermogenesis. Stimulation of brown adipose tissue in the rat leads to growth which would enable the animal to survive in colder climates and thus increase its range. The Syrian hamster is a hibernator and so avoids extremes in environmental temperature. Its diet is presumed to consist of mainly grasses and other plant material, plus lipid-rich seeds in the late summer which it avidly gathers and hoards. It becomes obese on a high fat diet and grows more brown adipose tissue in preparation for hibernation (see INTRODUCTION; 1B, 3B). High fat diet-induced obesity and BAT growth are exaggerated by short photoperiod (Wade, 1983; Wade and Bartness, 1983, 1984). Hamsters seem to prefer not to activate nonshivering thermogenesis as evidenced by their behavior in a temperature gradient (see INTRODUCTION, section 1) and by the slowness of their response to cold as seen by initial hypothermia (3B) and relative slowness in increasing GDP-binding (1A). During the prehibernation period the cold acclimated hamster would increase serum T_3 which could contribute to BAT growth and maintenance of thermogenic capacity, but partially shunt thermogenesis away from BAT into a thyroid hormone-mediated increase in basal metabolic rate. Brown adipose tissue in the Syrian hamster would then be reserved for use in rewarming from periodic hibernation bouts (see 1A, 2, 3F; Sigurdson and Himms-Hagen, 1988).

Not enough comparative work has been done to establish whether or not the thermogenic strategy of the Syrian hamster is typical of all hibernators or specific to this particular species.

The thermogenic defects of the myopathic hamster may or may not arise from the primary lesion in this animal model of muscular dystrophy. In myopathic hamsters acclimated to cold, all aspects of BAT trophic responses are attenuated. These include reduced amount of BAT compared to normal cold acclimated hamsters Himms-Hagen and Gwilliam, 1980; 1A, 3B), lower serum T_3 level (Kopecky et al, 1986b; 1A, 3B), lower specific deiodinase activity during the early phase of growth (Kopecky et al, 1986), and a reduced mitochondrial UCP concentration (1A). Hypothyroidism and relative insensitivity to T_4 probably contribute to the blunted response to cold in these animals.

Trophic responses of BAT to stimulatory diets or photoperiods are completely lacking in the myopathic hamster.

Elevated physical activity and/or muscle protein turnover could have a suppressive effect on brown adipose tissue thermogensis in myopathic hamsters.

Future Prospects:

The research described in this thesis has raised as many, if not more questions than it has answered in relation to control of BAT growth in hamsters.

Obvious avenues for further study include investigation of whether cold-induced BAT growth can be mimicked by administration of both NA and T_3 since alone, neither was sufficient. It may also be possible to record whether the firing pattern of the sympathetic nerves changes in response to cold exposure or hormone administration, and if a correlation with trophic responses exists. A more extensive search for co-localized neuropeptides could be undertaken during various stages of

cold acclimation in order to determine whether transient release of some other factor could be involved in initiation of BAT growth.

Since few of the measured parameters of BAT growth and activation were altered by diet or photoperiod, the search for mediators must be broadened. Insulin and glucagon both have stimulatory effects on rat brown adipose tissue (see INTRODUCTION, hormonal control), therefore the effects of these hormones should be studied in the hamster. Hypothalamic lesion studies have been performed in hamsters (see INTRODUCTION, neural control) but the focus has been on overall energy balance rather than on brown adipose tissue. Such types of lesion experiments may serve to help trace neural pathways involved in regulating peripheral trophic responses to both diet and photoperiod.

The defect(s) in control of myopathic hamster brown adipose tissue has not been elucidated as yet, but the suggestion that BAT is suppressed should be investigated further. As a first step, the physical activity level of these animals should be quantified using motion detectors to get a better idea of just how active they are.

To pursue the idea that heat production from other sources is at least partially responsible for suppression of BAT, one could estimate muscle protein turnover by measuring urinary 3-methylhistidine. Muscle protein degredation is the main source of this metabolite (Fitch et al, 1986). If increased physical activity and muscle protein turnover are negatively correlated with BAT growth, then perhaps drugs could be sought to alter behavior and activity in an effort to see if changes in BAT growth would also occur.

It is quite certain that many more experiments could be designed to

help answer the questions raised by the research presented here.

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CURRICULUM VITAE

NAME: Sandra Lynn (McLeod) Sigurdson

ADDRESS: Department of Biochemistry
University of Ottawa, Health Sciences Building
451 Smyth Road, Ottawa Ontario Canada K1H 8M5

TELEPHONE: (613) 737 6632

BIRTHDATE: October 25, 1957

BIRTHPLACE: Winnipeg, Manitoba Canada

CITIZENSHIP: Canadian

EDUCATION: University of Winnipeg, BSc (4 yr) Biology, 1980
University of Ottawa, PhD Biochemistry, expected 1989

SCHOLARSHIPS: University of Winnipeg Board of Regents General Proficiency Scholarship 1979

WORK EXPERIENCE:

1983-present: graduate student in the Biochemistry Department, University of Ottawa, supervised by Dr. Jean Himms-Hagen. Thesis title: Control of growth and function of brown adipose tissue in normal and myopathic hamsters.

1981-1983: technician in the Immunology Department, University of Manitoba. I was involved in production and characterization of immunoregulatory cytokines from human peripheral blood lymphocytes and synovial fluid from arthritis patients.

1980-1981: technician in the Physiology Department, University of Manitoba. I was involved in purification of human fibroblast growth factor.

1979: summer student at University of Winnipeg. I produced and characterized egosterol-deficient mutants of yeast. My supervisor was Dr. R.A. Woods.

DEMONSTRATORSHIPS/TEACHING:

1988: laboratory demonstrator for 3rd year Metabolism
1987: tutor for 3rd year student in Metabolism
1986: laboratory demonstrator for 3rd year Metabolism
1984: laboratory demonstrator for 2nd year Introductory Biochemistry
1980: laboratory demonstrator for 3rd year Cell Biology

PUBLICATIONS:

S. Lynn Sigurdson and J. Himms-Hagen (1988). Role of hyperthyroidism in increased thermogenesis in the cold-acclimated Syrian hamster. Can. J. Physiol. Pharmacol. 66:826-829.

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L. Sigurdson and J. Himms-Hagen (1985). Effects of high fat diet and melatonin on brown adipose tissue (BAT) in the Syrian hamster (Mesocricetus auratus) in different seasons. Canadian Congress of Biology, London Ontario, June 1985 and Living in the Cold - an International Symposium, Fallen Leaf Lake, California, October, 1985.

ASSOCIATION MEMBERSHIPS:

student member of Canadian Biochemical Society
student member of Ottawa Biological and Biochemical Society