COLD-ACCLIMATION IN AN ENDOTHERMIC POIKILOTHERM, THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*); EFFECTS ON THERMOREGULATION AND REPRODUCTION

Ryan Woodley

A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 2000.

ABSTRACT

Naked mole-rats (*Heterocephalus glaber*) are strictly subterranean mammals found predominantly in the horn of North-East Africa. These Bathyergid rodents live in large eusocial colonies in which only a single dominant female breeds continuously throughout the year. Another unusual feature of naked mole-rats is that these hairless mammals are unable to effectively thermoregulate outside the warm confines of their social burrows. In keeping with subterranean mammalian trends, naked mole-rats have lower body temperatures (T_b) and basal metabolic rates (BMR) than above ground inhabitants. However, unlike other rodents, naked mole-rats, despite the employment of endothermy, exhibit pronounced thermolability with no evidence of regulated hypothermia, and are thus operatively poikilotherms.

Both poikilotherms and homeotherms acclimate to chronic cold exposure, although observed responses differ considerably. Generally, acclimation to cold involves more efficient use of energy and/or heat production, such that acclimation is considered to be highly adaptive, enhancing survival by countering the adverse effects of fluctuating temperature.

Given the unusual thermoregulatory mode exhibited by naked mole-rats, I questioned whether cold-acclimation

responses would follow typical mammalian or reptilian patterns. This was investigated by monitoring a) changes in noradrenaline-induced nonshivering thermogenesis (NST) and brown adipose tissue (BAT) thermogenic capacity and activity, b) alterations in the hypothalamic-pituitary-thyroid axis functional morphology, and c) shifts in thermoregulatory profiles. In addition, changes in food consumption and digestibility were also examined, as were the long-term consequences of cold-acclimation on gestation and reproductive outcome. Furthermore, the effects of short-term cold bouts (5 days; 25°C) during the three trimesters of pregnancy, on body composition and interbirth interval were also monitored to determine at what stage of pregnancy naked mole-rats are most sensitive to cold.

The response of the hypothalamic-pituitary-thyroid axis to chronic cold exposure was typically mammalian. Although thyroxine (T_4) levels increased 40% with cold-acclimation (0.39 ± 0.09ng/dl, control; 0.55 ± 0.09ng/dl, cold-acclimated), both T_4 and thyroid stimulating hormone (TSH) concentrations were within the reptilian range, an order of magnitude lower than reported mammalian levels. Adenohypophyseal thyrotrophs exhibited ultrastructural signs of increased secretory activity, with TSH mediated increases in follicular cell height and reduced colloid volume of the thyroid gland also evident.

Cold-acclimation did not elicit an increase in NST capacity with similar ($P>0.05$) peak metabolic responses evident (1.52 ± 0.49mlO_2/g/h, cold-acclimated; 1.73 ± 0.71mlO_2/g/h, control). Biochemical analyses of BAT thermogenic capacity concurred, with GDP binding unaltered with cold-acclimation and similar to that of cold-acclimated mice (4°C). Sustained high thermogenic capacity regardless of thermal history, suggests that naked mole-rats are constantly primed for thermogenesis at the mitochondrial level. Given their poor insulation and high rates of thermal conductance across their uninsulated skin, maximal thermogenic priming is not surprising. Mitochondrial proton conductance, an indicator of BAT thermogenic activity, was however enhanced in the cold, coinciding with the 50% increase in BMR (0.52 ± 0.07mlO_2/g/h, control; 0.78 ± 0.29mlO_2 /g/h, cold-acclimated). This was facilitated by the 40% increase in free T_4 levels. These heat generating mechanisms, coupled with a vascular-mediated decline in minimal thermal conductance (42%), facilitated a left-shift of the thermoneutral zone from 32-34°C to 27-34° C, conferring considerable energy savings.

Food consumption, like that of other small mammals, increased in the cold. Despite lower T_b's in the cold, digestibility was not impaired, resulting in a 45% increase in assimilated energy intake. Increased energy

intake closely matched the augmented BMR and not surprisingly activity was substantially reduced.

Reproductive success was markedly reduced in the cold (4.5-fold; $P<0.0001$). Despite the shift in thermoregulatory profiles, and concomitant augmented temperature differentials (T_b-T_a; 7.8°C, cold-acclimated; 4°C, control), cold-acclimated pregnant females exhibited lower T_b's (1-2°C) than the control females. The lower T_b's contributed to the significant lengthening ($P<0.0001$) of the gestation period (76 ± 5 days; range 67-84) compared to the control females (68 ± 2 days; range 65-74). Whilst no reduction in litter size and pup mortality was observed, average pup mass in the cold (1.70 ± 0.29g) was significantly greater thar that in the warm (1.49 ± 0.27g; $P=0.0227$).

At all stages of pregnancy, females lost body mass during acute cold bouts, the majority (94%) being attributed to fat loss. Lean mass was only drawn upon during the early pregnancy cold bouts. Acute cold exposure during the early stages of pregnancy was also associated with the longest extension of the interbirth interval and the greatest decline in fecundity.

Although naked mole-rats are exquisitely sensitive to cold and exhibit lower T_b's with cold exposure, they showed predominantly typical mammalian cold-acclimatory responses, with increased T_4 concentrations mediating the

increased BMR and greater T_b-T_a differentials. Thermogenic capacity was however not enhanced, with evidence that even under warm, simulated burrow conditions, these hairless rodents are maximally primed for thermogenesis. Despite a decline in thermal conductance and increased endogenous heat production, the lower T_b's of cold-acclimated naked mole-rats impacted considerably upon other physiological functions, resulting in markedly reduced reproductive success and prolonged gestation in successful pregnancies.

Clearly, while living in the thermally stable confines of subterranean, equatorial Africa, changes in ambient conditions above ground pose little threat to the naked mole-rat. However, even cold-acclimatory responses to a 5°C drop in T_a are unable to sustain the preferred T_b, such that breeding is markedly impaired. This profound sensitivity to fluctuations in environmental conditions may explain the restricted distribution of this mammal to the equatorial underground of the horn of Africa. Furthermore, should the long-term conditions below the ground change by as little as 5°C, survival of these naked rodents could be threatened.

DECLARATION

I declare that this thesis is my own, unaided work. This thesis is being submitted for the degree of Doctor of Philosophy in the Faculty of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

Furthermore, I certify that the studies contained in this thesis were approved by the Animal Ethics Committee of the University of the Witwatersrand (AEC Number 95/80/2A and 95/111/4).

RWoodley

(RYAN WOODLEY)

18 day of May , 2000

ACKNOWLEDGEMENTS

My sincere thanks go to Shelley Buffenstein and Joseph Daly for unselfishly giving of their time and funds in the completion of this thesis. Thanks also to Professor Duncan Mitchell for the use of his laboratory and to Professors Cannon and Nedergaard of the Wenner Gren Institute, Stockholm University, for the opportunity to work in their laboratory with their world-class team.

Sincere thanks to the staff of the Central Animal Unit of the University of the Witwatersrand, especially Mr Wilson Shai for his expert and meticulous care of the animals.

I gratefully acknowledge financial support from the Trustees of the D.R. McIntosh Trust for their generous financial assistance over the past 5 years.

I also wish to thank the following people for their assistance with various aspects in the completion of this thesis: Dr Craig Hartford, David Makoa, Margaret Badenhorst, Liz Marcus, Sherrie Rogers, and Alison Mortimer. Thanks also to Helene Ley for all her help with the numerous computer problems. And finally to Belinda and Clara, thanks so much for all your help and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

rats with evenly dispersed secretory granules, and B) cold-acclimated naked mole-rats (25°C) showing peripherally located secretory granules closely associated with the plasmalemma. 10mm scale bar is equivalent to 1μm.

NOMENCLATURE

ADP - adenosine diphosphate

ATP - adenosine triphosphate

AVP - arginine vasopressin

BAT - brown adipose tissue

BMR - basal metabolic rate

cAMP - cyclic adenosine monophosphate

C_m - minimal thermal conductance

EP - early pregnant

GDP - guanosine diphosphate

LH - luteinizing hormone

LP - late pregnant

MP - mid pregnant

NP - non pregnant

NST - nonshivering thermogenesis

PRMR - peak resting metabolic rate

RER - rough endoplasmic reticulum

RMR - resting metabolic rate

T_3 - triiodothyronine

T_4 - thyroxine

T_a - ambient temperature

T_b - body temperature

TNZ - thermoneutral zone

TRH - thyrotropin releasing hormone

TSH - thyroid stimulating hormone

UCP - uncoupling protein

VO_2 - oxygen consumption

PREFACE

The thermal environment has pervasive effects upon metabolism of all living organisms. Across all phylogenetic groups, organisms adjust their physiological responses to overcome the direct effects of prolonged exposure to varying environmental temperatures (Hochachka and Somero, 1984; Cossins and Bowler, 1987; Huey, 1991; Leroi *et al.*, 1994; Kingsolver and Huey, 1998). Although these responses are quite different in poikilotherms and homeotherms, in both thermoregulatory classes they are regarded as highly adaptive (Mangum and Hochachka, 1998; Huey *et al.*, 1999). In response to cold-acclimation, endotherms generally exhibit a left shift of the thermoneutral zone to a lower range of ambient temperatures, this being facilitated by enhanced insulation and/or augmented basal metabolism (Bligh, 1973; Li and Tokura, 1997). The response of poikilotherms to cold-acclimation may involve a decrease in preferred body temperature with concomitant down-regulation of other physiological systems (Huey, 1982; Geiser *et al.*, 1992; Rome *et al.*, 1992; Hulbert, 1993). However, behavioural and morphological responses are generally more important in attenuating the degree of chronic cold-stress in poikilotherms (Carrascal *et al.*, 1992; Kingsbury, 1994; Martin *et al.*, 1995). Regardless of whether an animal is homeothermic or poikilothermic, cold-acclimation may also impact upon other physiological processes such as

gastrointestinal function and reproduction.

Naked mole-rats are intriguing animals in which to address these comparative aspects of thermoregulation. This rodent, like other mammals, employs endothermy (Hislop and Buffenstein, 1994), yet is unable to regulate body temperature, such that it is directly dependent on ambient temperature (Buffenstein and Yahav, 1991a). Thus, like most poikilotherms, naked mole-rats thermoconform. This led me to question how these mammals would respond to prolonged cold, the mechanisms employed, how this would impact upon other physiological functions, and finally, would they exhibit phenotypic plasticity? Addressing these questions requires a multidisciplinary approach, and as such, components of this study include anatomical, biochemical, molecular, endocrinological and physiological assessments.

This thesis is written as a series of independent papers, each addressing distinct components of the study. Part 1 of the thesis (Chapters 1 to 5) gives a general introduction to the study, and includes relevant background literature on naked mole-rat biology and thermoregulatory physiology (Chapters 1 and 2). Chapters 3 and 4 of the literature review specifically address thermal acclimation to cold in vertebrates and its reproductive consequences. The last chapter of this section (Chapter 5) addresses the aims and hypotheses

under investigation. This section of the thesis will not be submitted for publication. The body of the thesis is contained in Part 2 (Chapters 6 to 11) and contains the research papers resulting from this study and addresses different aspects of cold-acclimation in naked mole-rats, both in terms of thermoregulation and reproductive function. Chapter 6 addresses the changes in thermogenic capacity with cold-acclimation by monitoring changes in peak metabolism following noradrenaline-induced nonshivering thermogenesis. The biochemical basis of altered thermogenic capacity is investigated more closely in Chapter 7, by monitoring changes in thermogenic capacity (uncoupling protein concentration and GDP binding) and thermogenic activity (mitochondrial proton conductance) of brown adipose tissue mitochondria. The involvement of the hypothalamic-pituitary-thyroid axis in facilitating some of these thermoregulatory changes with chronic cold exposure is investigated in Chapter 8. Chapter 9 deals with the thermoregulatory shifts of cold-acclimation and associated changes in energy balance and gut function. The effects of cold-acclimation on reproductive and maternal function are addressed in Chapter 10. The concluding chapter, Chapter 11, integrates the physiological and morphological responses of cold-acclimation exhibited by naked mole-rats, the limitations of these functional systems and the implications thereof on maternal function and reproduction.

CHAPTER 1

NAKED MOLE-RAT BIOLOGY

Naked mole-rats are strictly subterranean (chthonic) mammals that exhibit a suite of physiological and morphological features well suited to life underground, that enable the successful exploitation of their subterranean niche. Many of these features defy accepted mammalian dogmas and force one to ask pertinent questions pertaining to both "why" and "how". These hairless rodents are poikilothermic, unable to regulate body temperature (T_b) outside the warm confines of their equatorial underground milieu. In addition, the reproductive burden is imposed upon only one female within the eusocial colony. Both these unusual features are addressed in depth in this study while the rest of this section focuses upon the unique biology of this extraordinary animal.

1.1 Taxonomy

Naked mole-rats (*Heterocephalus glaber*) belong to a strictly subterranean rodent family (Class - Mammalia; Order - Rodentia; Suborder - Hystricomorpha; Family - Bathyergidae; Species - *Heterocephalus glaber*). The Bathyergidae comprise two subfamilies, five genera and twelve species (Smithers, 1983), with an exclusive African history dating back to the early Miocene (Lavocat, 1978; Honeycutt *et al.*, 1991). *Heterocephalus glaber* belongs to the subfamily Georychinae (De Graaf, 1981). This subfamily is characterized by ungrooved incisors, upper incisors with roots originating behind the molars in the pterygoid process of the mandible, unenlarged forefeet with claws

and a small body size (Honeycutt *et al.*, 1991).

1.2 Distribution and Habitat

Naked mole-rats are endemic to north-east Africa. Their distribution is localized to the equatorial arid and semi-arid regions of the horn of east Africa, including Kenya, Ethiopia and Somalia (Kingdom, 1974). Here they live in eusocial colonies which average between 60-100 individuals with as many as 300 animals living together in a single burrow system (Jarvis, 1981; Brett, 1991a).

Leading a strictly subterranean existence, naked mole-rats live in an extensive system of burrows which may be as much as 2.5m below the surface and which may extend over 3km in length (Brett, 1991a). The burrow system consists of a number of interconnected nesting areas and latrines. Nesting areas are occupied by many individuals and are considerably deeper than foraging burrows (Brett, 1991a).

The burrow system provides for a more thermally stable environment than that above ground. Ambient temperature (T_a) within the burrow system, although relatively high (30°C), is stable with a diel and seasonal temperature range of less than 2°C (Gourou, 1970; Bennett *et al.*, 1988). Relative humidity approaches saturation in the burrows, limiting evaporative heat loss, and the closed nature of the burrows prevents adequate air

movement for convective heat exchange to be of any importance. Heat flow is thus restricted to conduction and radiation. Furthermore, limited air movement, especially at depth, together with underground respiration of plant roots, microfauna, and insect larvae contribute to the prevailing hypoxic and hypercapnic conditions (Arieli, 1977; Ar, 1986).

Their strictly subterranean existence provides some measure of protection against predators, mainly mole-snakes (Jarvis and Bennett, 1991). However, all food and water requirements must be met underground. Naked mole-rats are herbivorous and forage "blind" for bulbs and tubers which meet both their nutritional and water requirements. Foraging involves the random extention of their burrow systems through digging, until edible foods are encountered. Digging is facilitated by chisel-like teeth and powerful masseter muscles which may account for up to 25% of the total muscle mass (Sherman *et al.*, 1992). Any food that is encountered during burrow excavation is eaten or harvested and stored. This process is energetically expensive with reports of digging behaviour incurring costs of more than 300% above basal levels (Lovegrove, 1989).

1.3 Eusociality

Naked mole-rats live in eusocial colonies of 60-100 individuals, although colonies can comprise as many as 300

individuals (Jarvis, 1981). These mammals exhibit an intricate social system with a strict division of labour. The caste system of workers and non-workers culminates in a single breeding female and 2-3 males with whom she mates (Jarvis, 1981). There is also an overlap of at least two generations which assists in the co-operative care of the young (Jarvis and Bennett, 1993). The various castes are morphologically distinct with the breeding female disproportionately longer than the rest of the colony members (Buffenstein, 1996) and the dispersomorph castes having comparatively more body fat, distributed primarily in the neck region (Orian *et al.*, 1997). Non-reproductive animals perform tasks related to their body mass. The smaller individuals are involved with cleaning and extending of the burrows and foraging for food, while the larger individuals care for the pups and are also involved with defending the colony (Jarvis, 1981; Faulkes *et al.*, 1990; Lacey and Sherman, 1991). Co-operative living and the division of labour may reduce overall energy expenditure with respect to foraging, mate acquisition, defence and reproduction. The harsh ecological niche inhabited by the naked mole-rat renders this pattern of social behaviour highly advantageous to both the breeding female and the non-reproductive individuals within the colony, thus resulting in a highly conserved gene pool (Jarvis, 1981). This unique feature of eusociality amongst mammals is attributed, at least in part, to the high energetic costs of foraging for food in an arid

environment in which food is sparse and randomly distributed (Lovegrove and Wissel, 1988; Jarvis and Bennett, 1991).

Suppression of reproduction in subordinate females is achieved by an inhibition of ovulation as suggested by the low progesterone levels in non-reproductive females (Faulkes *et al.*, 1989). Inhibition of the hypothalamic secretion of gonadotropin releasing hormone in response to physical domination imposed by the breeding female, has been implicated as the probable cause for luteinizing hormone suppression and failure of ovulation (Faulkes *et al.*, 1989). Removal of the breeding female from the colony results in rapid hormonal changes in subordinate females which are then capable of breeding. Fighting for dominance then ensues until the establishment of a new breeding female.

1.4 Adaptations to a Subterranean Existence

Naked mole-rats have exploited an underground milieu for millennia, with the subsequent evolution of many morphological and physiological adaptations which favour a chthonic existence.

1.4.1 Morphological Adaptations

Naked mole-rats exhibit many morphological adaptations to their subterranean milieu, including their streamlined cylindrical body, short appendages and loosely folded skin

which facilitate rapid and unhindered movement through their burrow system (Ar, 1987; Jarvis and Bennett, 1991). Cryptorchidism and rudimentary ear pinnae may have evolved as protective mechanisms for the underlying structures, preventing the possibility of physical injury from sharp protuberances within the burrows.

The features of their mouth are also well suited to their digging functions. Their lips meet behind the chisel-like incisors, allowing the mouth to remain closed during burrow excavation (Smithers, 1983; Jarvis and Bennett, 1991). Furthermore, the incisors are hinged to prevent soil from wedging between them when excavating burrows (Thomadakis, pers comm). These effective chisels are operated by powerful jaw muscles, which may constitute up to 25 percent of the total muscle mass of the animal (Sherman *et al.*, 1992). The incisors exhibit perpetual growth but are maintained at a more or less constant length due to wearing down during digging.

Their skin lacks an insulatory pelage, which may assist in rapid heat exchange in an environment where heat loss is impeded by environmental constraints on evaporative and convective cooling. The lack of an insulatory pelage is to some extent compensated for by a thicker epidermal layer (Daly and Buffenstein, 1998). However, the skin lacks a functional hypodermis, rather the tissue consists of isolated or grouped adipocytes

forming a discontinuous layer (Daly and Buffenstein, 1998). This together with the loosely folded morphological arrangement, contributes to the poor insulatory properties of the skin. The vascular arrangement in the skin of the naked mole-rat also promotes rapid heat loss across the integument. Large blood vessels are found in the lower dermis which appear to break up and form a web of capillaries in the upper dermal layers (Daly and Buffenstein, 1998). These vascular networks are usually well developed in mammals that lack thick fur, or in the exposed surfaces of hairy mammals. These morphological features promote rapid heat flux, and while beneficial in the hot, humid subterranean environment of the north-east African soils, may contribute substantially to the inability of the naked mole-rat to effectively regulate T_b outside this thermally stable milieu.

1.4.2 Physiological Responses

Physiological responses to a subterranean existence largely reflect the physical constraints imposed by the environment, namely, lack of light, the prohibitive energetic cost of "blind" foraging, poor gas exchange, and limits to heat exchange.

Life in the dark precludes reliance on visual stimuli. Whilst reduced eye size may protect against infection and injury, it affects both optical and neural components of the visual system, making visual perception

and imaging rudimentary. Naked mole-rats are therefore almost completely blind and rely rather on their well developed senses of hearing, touch and smell for navigation through the dark burrows (Hill *et al.*, 1957; Jarvis and Bennett, 1991).

Furthermore, with no light penetrating the burrows, photo-endocrine systems, dependent upon ultraviolet light for hormone synthesis, are in a state of deficiency (Buffenstein *et al.*, 1994). Naked mole-rats appear to be naturally vitamin D deficient without any associated pathology. Indeed, the enzymatic processes involved in vitamin D homeostasis appear to be down-regulated, such that even when exposed to light, vitamin D status is unchanged (Sergeev *et al.*, 1993). Although vitamin D is generally regarded as essential for life, and in particular mineral balance, mole-rats utilize vitamin D independent processes to maintain highly efficient rates of gastrointestinal mineral absorption and renal retention of calcium, magnesium, and phosphorus (Buffenstein and Yahav, 1991b; Skinner *et al.*, 1991; Pitcher and Buffenstein, 1994).

Food localization underground is a random and energetically costly process (Jarvis *et al.*, 1998). Mole-rats maximize their returns by exhibiting high digestive efficiencies with assimilation efficiencies on even the poorest quality diets in excess of 80%, but more commonly

exceeding 90% (Buffenstein and Yahav, 1991c; Bennett and Jarvis, 1995). While this in part reflects enhanced absorptive processes, to a large extent, this is due to symbiotic microfauna in the gastrointestinal tract (Yahav and Buffenstein, 1991a & 1992). These caecal inhabitants use fermentation processes to efficiently digest fibre into short chain fatty acids which are then absorbed across the gastrointestinal tract and utilized as an important energy source by the naked mole-rat. Furthermore, mole-rats practice coprophagy and obtain a large component of their dietary protein by digesting the bacteria and protozoa present in the faecal pellets. Comparatively long retention times in the digestive tract, microbial fermentation processes in the hindgut and employment of coprophagy all contribute to the maximal exploitation of any available food (Buffenstein and Yahav, 1991c). Highly efficient fermentation processes in the caecum of the naked mole-rat are such that the amount of short chain fatty acids produced, increases substantially when the fibre content of the diet increases (Buffenstein and Yahav, 1991c). Naked mole-rats are thus able to exploit poor quality food resources and maximize energetic returns to compensate for the high energetic demands of foraging in a subterranean habitat.

Because the subterranean habitat is shared by many other respiring organisms such as plant roots and microfauna and flora, the gaseous environment below ground

is more hostile than atmospheric conditions above ground. Hypoxia and hypercapnea prevail and are exacerbated by low rates of gas movement by diffusion and convection below ground. Evidence that naked mole-rats have adapted to hypoxic and hypercapnic conditions may be gleaned from the comparatively high haematocrit and high oxygen affinity of their red blood cells (Johanssen *et al.*, 1976), promoting oxygen uptake despite lower diffusion gradients within their lungs. In addition, the lower oxygen requirements, as indicated by low basal metabolic rates, may also be considered adaptive in an environment with limited oxygen availability (Buffenstein and Yahav, 1991a) and may facilitate the maintenance of normal gas partial pressures in both blood and tissues.

A subterranean environment inhibits many of the more common pathways of heat exchange. Generally, conditions underground are such that the relative humidity is high and air movements (such as wind) minimal, thus precluding effective use of evaporative and/or convective cooling. Although external sources of heat gain (i.e. radiation) are minimal, heat exchange in a closed underground burrow system is primarily facilitated by conduction. Limited mechanisms for heat transfer impact substantially on the thermoregulatory physiology of underground inhabitants and may contribute substantially to the unusual thermoregulatory profile of naked mole-rats (discussed in depth in the next section and Chapter 2). Naked mole-rats

have low rates of metabolic heat production, minimizing the amount of heat requiring dissipation. In addition, they also exhibit another unusual feature that may reduce the risk of overheating in their hot, humid equatorial milieu. These animals appear to have an internal heat sink within the caecum, internally attenuating the effects of metabolic heat production. Caecal temperature is lower than core T_b (Yahav and Buffenstein, 1991a; Yahav and Buffenstein, 1992), and it is speculated that microbial fermentation processes in this subterranean rodent absorb rather than liberate heat. Nevertheless, these rodents predominantly rely upon conductive mechanisms for heat exchange, and in this regard, their lack of an insulatory pelage and a functional hypodermis (Daly and Buffenstein, 1998) contributes significantly to the ease with which heat is lost from the animal to the walls of the burrow system.

Thermoregulatory Physiology

Naked mole-rats exhibit an unusual pattern of mammalian thermoregulation in that they thermoconform over a wide range of T_a's. Body temperature at all times appears to be directly dependent on T_a, although the T_b - T_a differential may vary from 1 - 4°C (Buffenstein and Yahav, 1991a; Urison and Buffenstein, 1995).

Despite this direct relationship between T_b and T_a, patterns of oxygen consumption with changing T_a follow two

distinct patterns (Fig. 1.1). Below an T_a of 28°C, the typical poikilothermic pattern of increasing oxygen consumption with increasing T_a is evident. Above this T_a, the common mammalian pattern is evident, with basal metabolic rate (BMR) approximately 66% of that predicted allometrically (McNab, 1979; Withers and Jarvis, 1980; Buffenstein and Yahav, 1991a).

There are many advantages of a reduced BMR in an underground environment. These include reduced energy, oxygen and water requirements, as well as a reduction in carbon dioxide and heat production. Gaseous exchange in the hypoxic and hypercapnic environment is thus substantially reduced.

The lower BMR and concomitant decline in heat production, culminates in lower T_b's and a greater incidence of thermolability (Buffenstein and Yahav, 1991a). The combination of a very limited degree of heat production and poor insulation, result in T_b, in accordance with Fourier's laws, being determined primarily by external conditions. Low BMR is also considered an adaptive feature of a subterranean existence, in that it reduces the rate of heat production, thus reducing the risk of overheating and thermal death in the hot, humid burrows (McNab, 1979).

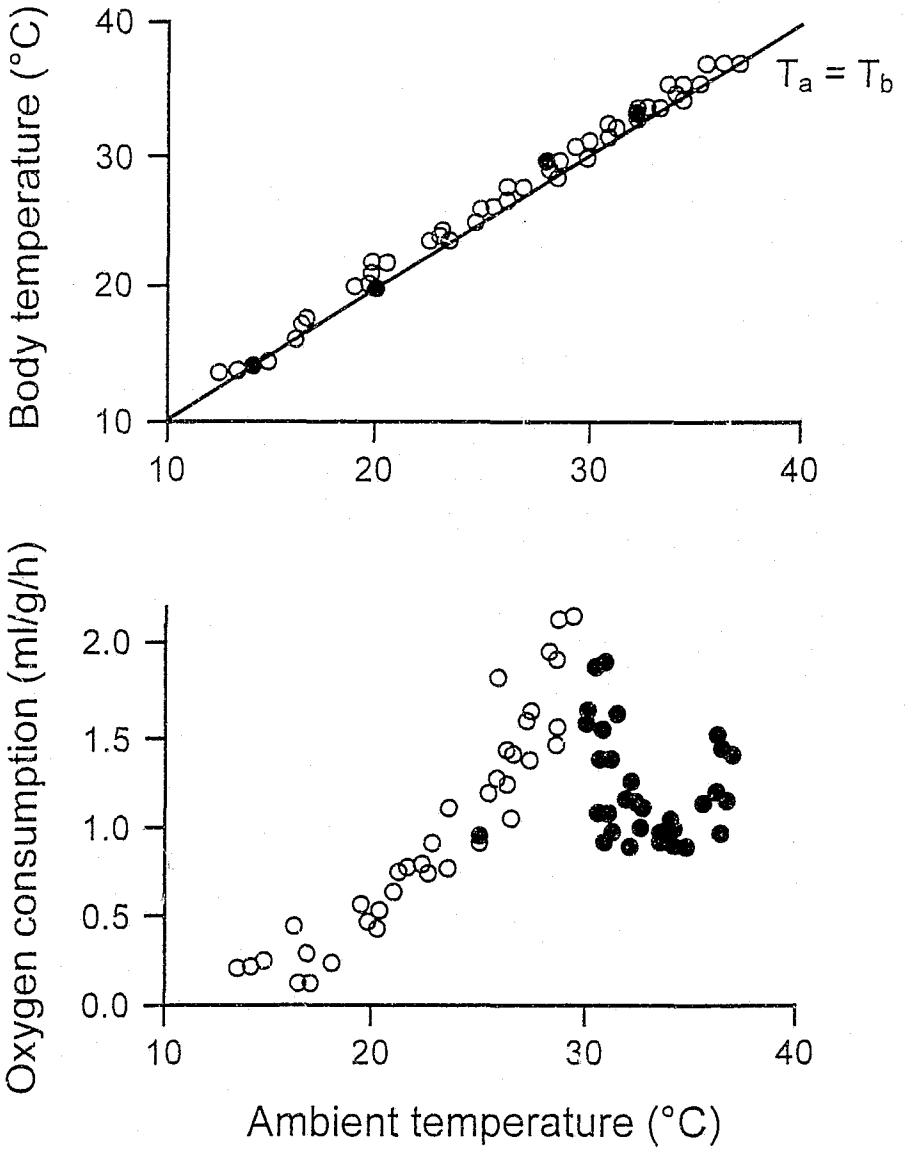


Figure 1.1. Effect of ambient temperature on oxygen consumption and body temperature in individual naked mole-rats (Taken from Buffenstein and Yahav, 1991a). Open circles represent a typical ectothermic /poikilothermic response while closed circles represent an endothermic response to varying ambient temperatures. Where multiple points overlap, scattered circles may appear closed.

Although basal metabolism is low, a large metabolic scope is apparent. Maximal metabolism is observed when digging in wet soils, where metabolic rates may exceed 300% of basal levels (Lovegrove, 1989). This large metabolic scope enables the naked mole-rat to conserve energy during times of rest, while at the same time meeting the high energy demands associated with intensive work periods.

The environmentally appropriate thermoregulatory profile exhibited by naked mole-rats is a direct consequence of their low basal metabolism, as well as the morphological attributes of their skin, facilitating high rates of heat transfer. The pronounced thermolability, and tolerance thereof, coupled with the large metabolic scope, may enable the naked mole-rat to expend considerable amounts of energy when needed, with limited impact on overheating and thermal death in their formidable subterranean habitat.

This subsection on naked mole-rat thermoregulation addresses key features considered highly adaptive to life underground. These and other aspects of the physiological processes involved in naked mole-rat thermoregulation are dealt with in more detail in section 2.3.

1.5 Reproduction in Naked Mole-Rats

Reproduction in the naked mole-rat is continuous throughout the year with a gestation period of 66-76 days and a postpartum oestrus of 8-11 days, such that the interbirth interval ranges between 77-84 days (Jarvis, 1991a). The pregnant female shows a dramatic increment in body mass during pregnancy of 30-50% (Jarvis, 1991a; Buffenstein *et al.*, 1996), although a near doubling of body mass has been reported (Jarvis, 1991a). Body mass returns to non-pregnant values immediately postpartum (Urison and Buffenstein, 1994), indicating that there is very little maternal energy storage during the course of pregnancy, with the vast majority of the mass increment attributed to foetal tissue.

Most subterranean mammals have a mean litter size of three (Nevo, 1979), with a maximum of five (Jarvis, 1969; De Graaf, 1981). The naked mole-rat gives birth to large litters and has the largest variation in litter size amongst the Bathyergidae. Colonies in the wild commonly have litter sizes ranging between 5-15 (Brett, 1991a). In captivity more than 12 pups are often born, with as many as 27 pups being reported for one litter (Jarvis, 1991a). This maximum litter size exceeds that recorded for any other mammal, and in this instance 22 of the 27 pups survived to adulthood (Jarvis, 1991a). The average pup mass at birth is 1.9g, with a range of 1.0-2.4g (Jarvis, 1991a).

Reproduction in the naked mole-rat is energetically costly, with the energy requirements of a single gestational cycle being approximately 1300kJ (Urison and Buffenstein, 1995). The greatest metabolic costs are incurred during lactation, with oxygen consumption 160% of that during the first week of pregnancy (Urison and Buffenstein, 1995). During this reproductive phase the breeding female may lose up to 16% of her body mass (Jarvis, 1991a). Lactation continues for 2-3 weeks postpartum, during the latter stages of which, the breeding female is burdened by the additional costs of concurrent pregnancy (Jarvis, 1991a). From the second week of pregnancy through to parturition, oxygen consumption is elevated and is 1.4-fold greater than that of non-reproductive animals (Urison and Buffenstein, 1995). Oxygen consumption during late pregnancy is independent of both T_a and T_b and appears to have reached a metabolic ceiling (Urison and Buffenstein, 1994). The dissociation of metabolism from both T_a and T_b may confer an energetic advantage to this arid dwelling subterranean mammal, for it may allow the pregnant female to partition a greater portion of her energy budget towards foetal development at a time when these demands are maximal. This adaptive feature may prevent dramatic changes in nutrient requirements, for gut capacity may be constrained in heavily gravid females.

Pregnant females maintain a higher T_b than their non-reproductive counterparts, with the highest temperature differential recorded in the week prior to parturition (2.5 ± 0.6°C; Urison and Buffenstein, 1995). This temperature differential was evident irrespective of whether or not the breeding female was allowed to bask under heat lamps or had to employ endothermy (Urison and Buffenstein, 1995). Body mass increments coupled with increases in percent body fat may contribute to the increased T_b during pregnancy (Buffenstein *et al.*, 1996). Increases in body mass result in a reduction of the surface area to volume ratio, and together with increased insulatory fat deposition, reduce heat flux to the environment. Furthermore, behavioural mechanisms may also contribute to the elevated T_b, for basking is increased during the latter stages of pregnancy, thus reducing the thermal gradient for heat loss to the environment (Buffenstein *et al.*, 1996).

Presumably the elevated temperature optimizes foetal development and is not just a by-product of unregulated events (e.g. decreased surface area to volume ratio or an increase in metabolically active tissue). As such, the energetic costs of maintaining this elevated temperature may be substantially reduced by employing ectothermic means, including basking under heat lamps. Many poikilotherms behaviourally modify T_b during pregnancy. For instance the Indian python although normally ectothermic,

whilst brooding, initiates muscle contractions and as such increases T_b and thus the incubation temperature of the eggs (Hutchison *et al.*, 1966). The selection of an elevated T_b by the naked mole-rat during pregnancy, raises the question of whether gestation length would be altered at lower T_b's as has been reported for other poikilotherms (e.g. the lizard, *Sceloporus jarrovi*; Beuchat, 1988). Despite maintaining an elevated T_b during pregnancy, T_b is not regulated and tracks T_a over the entire temperature range monitored (23-35°C; Urison and Buffenstein, 1994).

The high costs of pregnancy in the naked mole-rat, irrespective of litter size, may explain the selection of eusociality. The strict division of labour within the colony frees the breeding female from energetically costly activities such as foraging, burrow maintenance and defence, allowing her to partition proportionately more of the total energy budget towards energetically costly reproductive processes (Jarvis, 1991a).

Naked mole-rats are highly specialized animals that have evolved a suite of characteristics well suited to the warm and dark confines of their underground equatorial habitat. Many of these features compromise their ability to cope with thermally variable and unpredictable environmental conditions. In this thesis, I questioned whether these animals would, 1) exhibit phenotypic plasticity, 2) acclimate to chronic cold exposure, and 3)

what the impact of prolonged cold exposure would be on other physiological functions, focussing in particular on reproductive success.

CHAPTER 2

THERMOREGULATORY BIOLOGY OF VERTEBRATES

Although temperature is one of the many abiotic and biotic features within an animals' environment, it has pervasive effects, influencing many aspects of an organisms' biology and in particular its rate dependent physiological processes. Few, if any, vertebrates are totally passive with respect to fluctuations in T_a. Rather, regardless of whether an animal is a bradymetabolic ectotherm or a tachymetabolic endotherm, both physiological and behavioural modifications accompany fluctuations in ambient conditions.

2.1 Thermoregulatory Modes

A diverse spectrum of thermal relationships exists in the animal kingdom (Fig. 2.1). At one end of the spectrum are poikilotherms, animals whose T_b is governed by T_a , and these include insects, fish, amphibians and small reptiles (Anon, 1987). At the other extreme are homeotherms whose T_b may be precisely regulated, independent of T_a (e.g. most mammals and birds; Anon, 1987). There is no strict division between these two thermoregulatory antonyms as many animals exhibit considerable plasticity in their mode of thermoregulation and may alternate between precisely regulating T_b, to tolerating pronounced thermolability. The varanid lizard for example, when active, selects and maintains a T_b above ambient, but when inactive, allows T_b to track T_a (Buffenstein and Louw, 1982). Similarly, many small mammals abandon homeothermy and employ adaptive hypothermia. This heterothermy may occur on a daily or a

seasonal basis (Cossins and Bowler, 1987; Geiser, 1988) or may be restricted to periods when food and water are limiting (Buffenstein, 1985; Schmidt-Nielsen, 1990).

Not only are thermoregulatory modes classified according to the precision of T_b maintenance, but they are also based upon the source of body heat. Endotherms are capable of producing heat endogenously, whilst ectotherms rely on heat gain from the environment (Anon, 1987) by basking in the sun (heliothermy) or by lying on warm surfaces (thigmothermy). Behavioural thermoregulation is not exclusive to ectotherms however, as endotherms also exploit behavioural mechanisms to both minimize heat loss (e.g. piloerection, huddling) and maximize heat gain (e.g. sun basking). Only when these mechanisms are inadequate, is heat production increased (Hulbert, 1988).

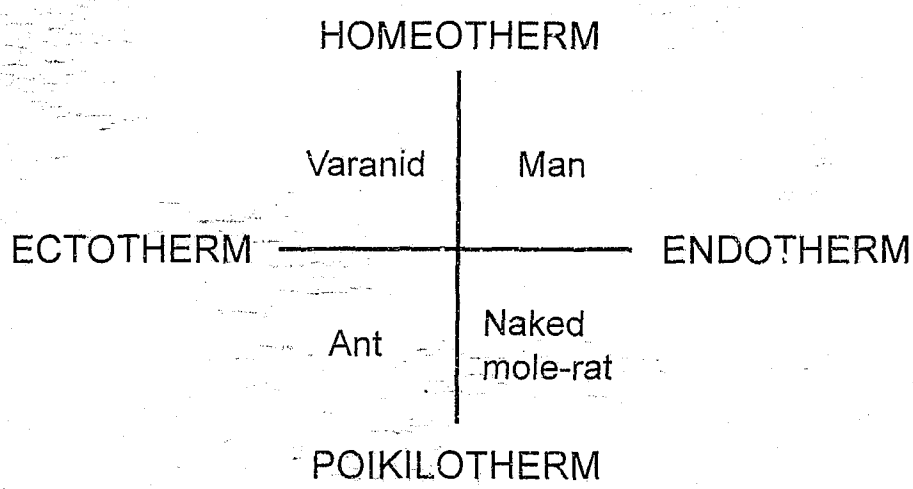


Figure 2.1 Thermoregulatory modes and mechanisms with examples. Taken from Hislop and Buffenstein (1994).

The advantages of homeothermy generally outweigh the added metabolic costs, such that the general evolutionary trend is that higher organisms have become more independent of environmental fluctuations. As such, endotherms have BMR's several times greater than that of similar sized ectotherms with the same T_b (see Hulbert, 1993). In addition, the aerobic scope of endotherms is considerably greater than that of ectotherms (Bennett and Ruben, 1979). Growth rates are commensurate with the higher rates of metabolism, and are similarly greater than in equivalent sized ectotherms (Case, 1978). This facilitates an earlier attainment of sexual maturity. The advantages of homeothermy also include behavioural constancy over a much wider range of T_a's (see Hulbert, 1993). Another possible advantage is that the maintenance of higher T_b's over a wider range of T_a's may result in a faster rate of evolution by influencing the rate of spontaneous mutation (Hulbert, 1980).

Besides these obvious benefits, endotherms are at an ecological disadvantage. Their increased energy requirements necessitate increased food intake and foraging time, with greater risks of predation. Many small mammals are therefore compelled to abandon homeothermy and rather employ facultative hypothermia, albeit for short periods of time when temperatures fall below a critical level (Hudson, 1978; Heldmaier and Steinlechner, 1981; Buffenstein, 1984). Although most mammals are intolerant

of low T_b's with lethal limits in the range of 15-20°C (see Hulbert, 1993), animals that employ torpor and hibernation can and do survive significantly lower T_b's. These bouts of hypothermia exhibited by heterothermic mammals and birds are under autonomic regulation. When arousing from torpor or hibernation, these small animals are capable of raising and maintaining an elevated T_b by endogenous heat generating mechanisms (Lyman *et al.*, 1982; Buffenstein, 1984; Dawson and Marsh, 1989; French, 1993; Geiser, 1994; Malan, 1996; Schmid, 1996). Naked mole-rats do not employ torpor and are unable to significantly raise T_b by endogenous means. As such, this lack of thermogenic potential when subjected to cold conditions, differentiates these unusual mammals from those which employ adaptive hypothermia, and results in their classification as poikilotherms.

2.2 Pathways of Heat Transport

2.2.1 Heat Flow between Body and Environment

Heat flux between an animal and its environment is the sum of the partial fluxes by radiation, conduction, convection and evaporation (Fig. 2.2). The latter two are insignificant in the burrows of naked mole-rats, where air movement is minimal and relative humidity approaches saturation. Total heat flux is influenced primarily by the effective surface area, which in turn is influenced by body size and body posture.

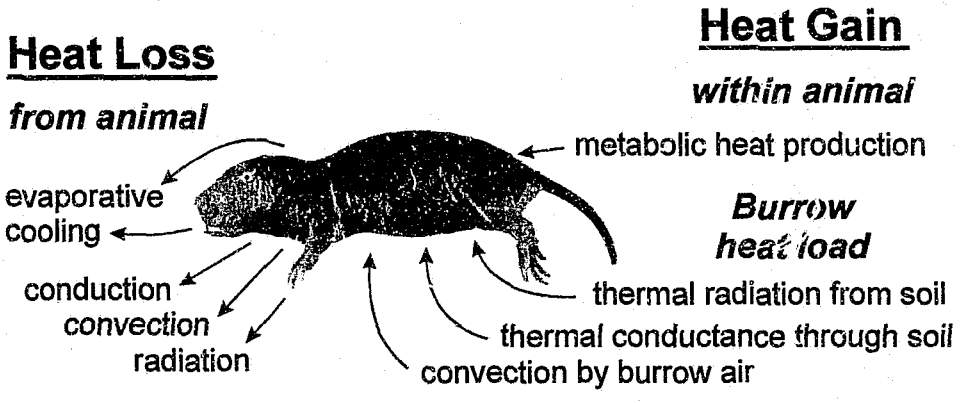


Figure 2.2 Heat flux between the naked mole - rat and its environment.

Conduction is the transfer of heat between two objects down a temperature gradient, by interaction of adjacent molecules without mass movement of the medium. This is a particularly important avenue for heat exchange for animals 1) with large surface area to volume ratios, 2) where a large temperature differential exists between animal and environment, and 3) where there is a large contact surface with the environment (e.g. snakes).

Radiant heat transfer takes place in the absence of direct contact between two objects (Schmidt-Nielsen, 1990). All objects whose temperature is above zero degrees Kelvin emit electromagnetic radiation which travels in a straight line, suffering little absorption by air, until it strikes and is absorbed by another object and is converted to heat at the surface of the absorbing body. The presence of an insulatory pelage results in most of the heat radiated from the body being absorbed by the integument. Heat radiation from the surface of the integument is low due to its low radiant temperature. The lack of an insulatory pelage in naked mole-rats, prevents this means of heat retention.

2.2.2 Heat Flow between the Body Core and Skin Surface

Heat produced within the body is transported to the skin surface primarily by convection via blood circulation. Heat conductivity of body tissues is comparatively poor, thus heat transport via conduction is small in comparison

with the maximum convective transport through the blood. As the epidermis lacks a blood supply, heat is transported exclusively through this layer by conduction.

Similarly, heat gain from the environment via heliothermic and/or thigmothermic behaviour is absorbed by the skin via conduction and transported to the body core by convective transport through the blood, thereby facilitating rapid heating rates. Endotherms are also capable of endogenous heat production by thyroid mediated increases in basal metabolism and brown adipose tissue mediated nonshivering thermogenesis.

2.3 Naked Mole-Rat Thermoregulation

Naked mole-rats defy standard mammalian thermoregulatory dogmas in that they are thermoconformers rather than thermoregulators. High rates of heat flux with the environment may have contributed to the development of poikilothermy in fossorial mammals like the naked mole-rat (Louw, 1993), and to a lesser extent in other Bathyergidae (Bennett and Jarvis, 1993). Endogenous heat is produced (Buffenstein and Yahav, 1991a), with both a functional nonshivering thermogenic response (Hislop and Buffenstein, 1994) and a fever response (Urison *et al.*, 1993) evident. Despite this internal heat production, T_b is not regulated over a wide range of T_a's monitored (12-37°C; Buffenstein and Yahav, 1991a), and is usually maintained approximately 1°C higher than T_a (Fig. 1.1; Buffenstein and Yahav,

1991a). This inability of naked mole-rats to physiologically maintain a constant T_b may be attributed to their low rates of basal metabolic heat production as well as the extremely high rates of heat loss due to their small size and poorly insulated, naked skin.

Behavioural thermoregulation in their natural habitat may however facilitate the maintenance of homeothermy over the narrow T_a range encountered in their burrows (Yahav and Buffenstein, 1991b). Behavioural thermoregulatory mechanisms include thigmothermy in warmer, more shallow burrows in the field (Brett, 1991b), basking under heat lamps in the laboratory, as well as huddling in nests or warmer regions of the burrow (Jarvis, 1991b). Furthermore, the reduction in metabolic costs facilitated by huddling are also highly advantageous in an environment in which food is scarce, randomly distributed and energetically costly to locate (Yahav and Buffenstein, 1991b).

The thermoregulatory pattern of the naked mole-rat has been described as that of an endothermic poikilotherm (Buffenstein and Yahav, 1991a). At T_a's greater than 28°C, a typical endothermic pattern is evident (Fig. 1.1; Buffenstein and Yahav, 1991a). Metabolism is minimal between the T_a range of 31-34°C, however over no T_a range is T_b precisely regulated, hence reference is made to an apparent "thermoneutral zone" (TNZ) (Buffenstein and Yahav, 1991a). Metabolism increases sharply below

thermoneutrality, such that a drop in T_a of only 2-3°C induces a 120% increment above basal levels, which is similar to that seen by other laboratory rodents exposed to far more severe cold stresses of around 5°C (Dicker *et al.*, 1995; Hammond *et al.*, 1996). At T_a's below 28°C, the employment of endothermic mechanisms may be energetically untenable (Buffenstein and Yahav, 1991a). As such, naked mole-rats abandon endothermy at T_a's below *ca.* 28°C and show typical poikilothermic metabolic responses.

Like all fossorial mammals living under hypoxic and hypercapnic conditions, naked mole-rats exhibit low rates of basal metabolism considered well suited to their microhabitat (McNab, 1979; Nevo, 1999). However, even amongst this small group of mammals, the BMR of naked mole-rats is exceptionally low with reported mean BMR's ranging between 0.66mlO_2/g/h (McNab, 1979) and 1.0mlO_2/g/h (Buffenstein and Yahav, 1991a). These inter-laboratory differences may reflect subtle differences in experimental protocol or housing conditions. Nevertheless, even at the higher range of BMR's reported for this species, metabolism is only 66% of that expected allometrically, and is akin to that of reptiles of similar body mass and temperature (Goldman *et al.*, 1999).

Nonshivering Thermogenesis

Brown adipose tissue (BAT) in the naked mole-rat is organised into a bi-lobed structure situated in the

subscapular region. A functional catecholaminergic nerve supply is present, although it appears to be more sparsely distributed than that of typical homeothermic mammals (Daly *et al.*, 1997). The naked mole-rat exhibits the characteristic nonshivering thermogenic response to exogenous administration of noradrenaline, showing significant increases in both metabolic rate (356 ± 199%) and T_b (2.8 ± 0.9°C; Hislop and Buffenstein, 1994). Whilst the naked mole-rat is capable of nonshivering thermogenesis (NST), endothermy is only employed over the very narrow T_a range (Buffenstein and Yahav, 1991a) it normally encounters in its natural habitat (Jarvis and Bennett, 1991). It is possible that below an T_a of 28°C (Buffenstein and Yahav, 1991a), the costs of endothermy for this poorly insulated mammal become prohibitive, with the result that endothermy is abandoned, and rather, their T_b is governed by Fourier's laws of heat transfer and behavioural modifications. I questioned whether the T_a at which the endothermic pattern became evident was influenced by long-term cold exposure and whether this pattern could be altered by cold-acclimation.

CHAPTER 3

MORPHOLOGICAL, PHYSIOLOGICAL, AND BEHAVIOURAL RESPONSES TO COLD EXPOSURE

Across all phylogenetic groups, one finds an impressive ability to adjust physiological, cellular and behavioural responses to overcome the direct effects of varying temperatures. These adaptations allow the organism to cope with changes that disturb its internal thermal environment, and as such, evoke both specific and non-specific responses. Acclimatization is "the functional compensation extending over a period of days to weeks in response to a *complex* of environmental factors, as in seasonal or climatic changes" (Folk, 1974). Acclimation on the other hand, is "the functional compensation over a period of days to weeks in response to a *single* environmental factor", and generally refers to laboratory controlled modifications (Folk, 1974).

Cold affects animals in many ways. Homeotherms have adapted to maintaining their core T_b at a relatively constant level, whilst poikilotherms can endure fluctuating thermal environments with concomitant effects on their T_b and physiology. Cold-acclimation in mammals and birds consists essentially of shifting the TNZ to a lower range of T_a's. This may be achieved by a variety of means, including thicker and more extensive insulation, vascular adaptations to reduce heat loss, and an increased BMR (Bligh, 1973; Mount, 1979). Reptiles are more reliant on less costly behavioural responses to counter cold-stress (Carrascal *et al.*, 1992; Kingsbury, 1994; Martin *et al.*, 1995), but have also been reported to select lower

preferred T_b's with concomitant down-regulation of physiological systems (Huey, 1982; Geiser *et al.*, 1992; Rome *et al.*, 1992; Hulbert, 1993). Similarly, many homeotherms when faced with chronic cold, abandon homeothermy and rather employ controlled heterothermy, by allowing different regions of their body to differentially cool, or by entering either daily torpor or prolonged hibernation.

Energy conservation dictates that behavioural modifications and vasoconstriction are initially employed in response to cold exposure, before energetically costly heat generating mechanisms are initiated. Adaptive changes of the thermoregulatory system include both morphological and functional modifications. Morphological changes (e.g. changes in body shape or insulation) require months to years to develop and are usually preceded by functional modifications which include both changes in the capacity and regulation of effector systems. Such functional modifications require much less time to develop e.g. increased BAT thermogenic capacity with cold exposure. Three weeks is the time normally accepted for cold-acclimation studies (Depocas, 1960), even though certain parameters continue to increase after this time (e.g. Thomsom *et al.*, 1969; Sundin and Cannon, 1980). These cold-acclimation adaptations either reduce heat loss or increase heat production during cold exposure.

3.1 Morphological Responses to Cold Exposure

3.1.1 Body Shape

Although cold-induced increases in thermogenic capacity help counteract increased heat loss during winter months, they necessitate an increase in energy intake at a time when primary production is low and cold temperatures restrict foraging. One way around this involves a reduction in body size, since smaller individuals require less food in total, despite their higher mass-specific metabolism (Heldmaier and Steinlechner, 1981b). Indeed, many small mammals experience a reduction in body mass during cold winter months, with a subsequent reduction in overall energy requirements (Iverson and Turner, 1974; Klaus *et al.*, 1988; Korn, 1989). In addition, the marked increase in BAT thermogenesis has a strong influence on body energy balance. For instance, the cold-acclimated rat, despite a greater food intake, shows smaller gains in body mass than rats at thermoneutrality (Abelenda and Puerta, 1987; Puerta and Abelenda, 1987).

3.1.2 Insulation

The most striking and effective protection against cold is the formation of a thick cover of hair or feathers or of a thick layer of subcutaneous fat. Severe cold exposure, lasting for at least several months, is necessary for a substantial stimulation of hair or feather growth. It is perhaps for this reason that some authors failed to

demonstrate this effect of cold exposure. Nevertheless, increased pelage insulation has been evoked by cold exposure in mice, pigs and cats (see Precht *et al.*, 1973). In addition to subcutaneous fat deposition, cold exposure also results in a change in lipid composition and membrane fluidity. Cold-acclimated individuals have lipids which are comparatively low in saturation, such that a suitable fluidity of cellular and intracellular membranes is maintained. This is necessary for the optimal functioning of membrane bound enzymes at lower temperatures.

Homeothermic maintenance in a small mammal which lacks effective insulation may be impossible due to rapid heat flux to the environment. Naked mole-rats lack an insulatory pelage, thus it is not surprising that this small mammal does not conform to expected mammalian thermoregulatory patterns (Buffenstein and Yahav, 1991a). Furthermore, small mammals due to their large surface area to volume ratios require proportionally more insulation to counter the greater heat flux to the environment. They are however limited in the amount of fat they can store and the thickness of their pelage due to the limitations it imposes on locomotion.

Physiological adaptations to thermal environments are usually accompanied by structural adjustments. The redistribution of blood flow as a means of altering thermal conductance has been well documented in mammals

(Ingram and Legge, 1971) and reptiles (Smith, 1976). Adjustments of tissue insulation brought about by changes in peripheral circulation attenuate heat loss. In the cold-acclimated varanid lizard, *Varanus niloticus*, T_b is frequently maintained slightly higher (± 2°C) than T_a, which may reflect well developed vasomotor control of T_b characteristic of other varanids (Bartholomew and Tucker, 1964; Louw *et al.*, 1976). The regulation of heat loss is manifested as a general decrease in skin temperature, due to a reduction in peripheral blood flow, thereby reducing the temperature gradient between the body surface and the environment.

The existence of arteriovenous anastomoses in many tissues, particularly skin, has been well documented. The general concept of arteriovenous anastomoses opening during heat stress and closing during cold stress is widely accepted (see Hales *et al.*, 1978). Arteriovenous anastomoses are muscular shunts that allow blood to bypass the capillary network. They are particularly abundant in the skin of appendages where they are involved in the regulation of peripheral blood flow and thereby play a significant role in thermoregulation (Wolfenson, 1983).

Not only does the effective retention of heat depend on appropriate circulatory adjustments, but it is also dependent on passive heat flux across the epidermis. Thus changes in epidermal thickness may have an adaptive value

in thermal physiology. Such changes have been demonstrated in rats, where epidermal thickness increased after cold-acclimation (Heroux, 1959).

3.2 Behavioural Responses to Cold Exposure

Behavioural responses to perturbations in temperature are usually much quicker and less energetically costly. These may be divided into two categories: 1) the selection of a less thermally stressful environment, and 2) alterations in the surface area to volume relationship.

Poikilotherms generally do not employ endogenous physiological mechanisms of heat generation, over and above the heat that is naturally produced as a byproduct of metabolism. Rather, these animals rely on finely tuned behavioural thermoregulatory mechanisms e.g. microhabitat selection (Buffenstein and Louw, 1982; Dreisig, 1984) or huddling (White and Lasiewski, 1971), to maintain a constant T_b, and in doing so may become ectothermic homeotherms. This is especially true of large reptiles where behavioural means coupled with their large thermal inertia facilitate relatively precise (within 1°C) regulation of T_b (Bligh, 1973). Rattlesnakes are known to aggregate in large numbers at denning sites during the cold season and may maintain T_b's significantly higher than the den T_a (White and Lasiewski, 1971). Denning aggregation reduces heat loss by increasing the distance through which heat must be conducted and also by a reduction in the

effective surface area per unit mass.

Most lizards use heliothermic and thigmothermic behaviours to gain heat and thus compensate for low environmental temperatures (Patterson, 1991; Carrascal *et al.*, 1992; Martin *et al.*, 1995). In contrast, most amphibians have difficulty increasing T_b behaviourally for any heat gain is offset by the high rates of evaporative water loss and concomitant cooling across their permeable skin (Carey, 1978). They do however employ other behavioural responses including avoidance of extreme temperatures (e.g. temperate montane toads; Carey, 1978) and shifts in activity patterns. For instance, the Andean toad, *Bufo spinulosus*, not only becomes inactive when temperatures are too low, but also burrows to avoid cold (Sinsch, 1989).

Small mammals may construct artificial nests in which to shelter from the cold. For example white-footed mice, *Peromyscus leucopus*, construct larger nests and huddle to reduce heat loss in the cold. Huddling resulted in a 16-33% reduction in daily energy expenditure, whilst the combination of daily torpor, huddling and nest construction resulted in a 74% reduction in energy expenditure (Vogt and Lynch, 1982). For many small mammals, huddling results in lower oxygen consumption rates, reduced food intake, longer survival times when exposed to cold, lower rates of weight loss and higher T_b's

than if alone (see Contreras, 1984). Huddling also reduces the requirement for BAT thermogenesis and results in a reduction of its thermogenic capacity, thereby facilitating an energy saving (Himms-Hagen and Villemure, 1992).

Naked mole-rats employ behavioural thermoregulation by selecting warmer areas within the burrow system (Brett, 1991b), and also by huddling (Withers and Jarvis, 1980; Yahav and Buffenstein, 1991b). This is most pronounced in pregnant females who maintain T_b above that of non-pregnant eccritic temperature (Buffenstein *et al.*, 1996). Huddling behaviour not only decreases the exposed surface area for heat transfer, but also confers considerable savings in energy usage and water loss, so essential in an arid environment where food is sparsely distributed and energetically costly to locate. Huddling therefore also has an important thermoregulatory role, such that in their natural environment naked mole-rats are able to maintain T_b fairly constant, albeit within a very narrow T_a range (Yahav and Buffenstein, 1991b).

3.3 Functional Responses to Cold Exposure

3.3.1 Basal Metabolism

All processes in the body generate heat and are thus inherently thermogenic. Indeed, even basal metabolism can be considered a form of obligatory NST, as it involves

very little muscle activity. Small mammals have a limited capacity to increase insulation in the cold and rely rather on metabolic adjustments to increase thermogenic capacity. Many small mammals therefore show an increase in BMR following cold exposure. The increase in BMR with acclimation, coupled with improved insulation and the concomitant lowering of the lower critical temperature result in a left shift of the onset of thermogenic heat production. At T_a's below the lower critical limit, relative rates of thermogenic heat production with varying ambient temperatures are attenuated. The increased BMR is sustained by increased food consumption which also entails enlargement of the gastrointestinal tract. There is however a limit to the amount of food an animal can efficiently process. Below this limit they must utilize fat stores to make up the energy deficit. In addition, cold exposure also reduces digestibility, especially if T_b declines, such that if food intake is not increased, body reserves must be mobilized (see Stott and Slee, 1985).

Despite the advantages of increasing basal metabolism in the cold, this may be an inefficient mechanism for increasing thermogenesis for metabolism cannot be reduced below this fundamental threshold when there is no immediate heat drain to resist. Consequently many small mammals show no increase in BMR during winter months (e.g. Feist and Feist, 1986; Klaus *et al.*, 1988), but rather rely on other avenues for thermogenesis such as shivering or NST. Even in those species which do increase basal metabolism during winter, they do so only when exposed to

cold (e.g. *Microtus ochrogaster*: Wunder, 1984; *Saccostomus campestris*: Ellison *et al.*, 1992), and do not respond to anticipatory cues such as photoperiod (e.g. Bartness and Wade, 1984). In so doing they prevent an increase in obligatory heat production until it is actually needed.

3.3.2 Shivering Thermogenesis

Shivering is the involuntary, rhythmic contraction of skeletal muscle. Hydrolysis of adenosine triphosphate (ATP), both by myosin ATPase during filament sliding and by the active transport of calcium back into the sarcoplasmic reticulum during muscle relaxation are mostly responsible for the heat generated during shivering. Shivering has been reported in birds, monotremes, marsupials, and placental mammals (Hissa, 1988).

Acute cold exposure not only evokes thermoregulatory reflexes and emergency reactions, but also results in a temporary shift of the shivering threshold to lower T_b's. Chronic cold exposure results in an even greater shift in the shivering threshold, thus allowing for the use of NST at a lower range of T_a's before the less economical shivering mechanism is evoked (e.g. Djungarian hamsters, Bockler and Heldmaier, 1983; guinea pigs, Bruck, 1986). This type of adaptation has been called tolerance adaptation or hypothermic adaptation, since larger variations in T_b are tolerated before the activation of heat generating mechanisms occur, i.e. precision of

temperature regulation is reduced. There is normally no increase in the capacity for shivering thermogenesis in cold-acclimated small mammals (Heldmaier *et al.*, 1985), with the shift in the shivering threshold usually following an increase in NST capacity. If insulation (pelage or subcutaneous fat) increases during cold-acclimation, shivering as well as NST may progressively decrease with prolonged cold exposure.

3.3.3 Nonshivering Thermogenesis

Thermoregulatory NST refers to those processes, other than shivering, which are involved in heat production above basal levels in defence of T_b. In contrast to BMR, NST is an essential component of cold-acclimation in small mammals (Heldmaier *et al.*, 1982), and unlike basal metabolism, is only expressed when necessary to maintain homeothermy in the cold (Jansky, 1973). Thus augmenting NST capacity is more energetically expedient than increasing basal metabolism.

Nonshivering thermogenesis was first demonstrated in cold-acclimated animals, which despite the diminution of shivering were able to maintain elevated levels of heat production to maintain T_b (reviewed by Smith and Hoijer, 1962). The involvement of NST in acute cold exposure was first suggested when non-cold-acclimated rats were given curare to block shivering, but were still able to increase heat production, although not sufficiently so to prevent

hypothermia (Cottle, 1960). The existence of NST is well established now in many small mammals, as is the increase in NST capacity following cold-acclimation (Jansky, 1973; Heldmaier *et al.*, 1989; Horwitz, 1989). A number of NST mechanisms have been proposed in homeotherms, including active sodium pumping across plasma membranes, substrate cycles in specific metabolic pathways, and more recently "hot pipes" in the vasculature (Colquhoun and Clark, 1991), but the best documented mechanism for NST heat production is in BAT. There is also some evidence that skeletal muscle may contribute to NST as well as increase heat production via shivering, although the exact mechanism is unknown.

Small, cold-acclimated placental mammals, when in a cold environment, first rely on NST and only utilize shivering at low T_a's when the magnitude of the NST response is insufficient (Bockler and Heldmaier, 1983; Heldmaier *et al.*, 1989). Nonshivering thermogenesis is generally considered the more efficient of the two, as shivering may disrupt the insulatory pelage, thus reducing its insulatory properties. Shivering also increases convective heat loss and may inhibit locomotion. Nonshivering thermogenesis avoids these potential problems, thus physiological mechanisms exist to ensure that NST is used preferentially (Bruck, 1970). The mechanism of acute NST heat production can be compartmentalized according to its location within the

brown adipocyte.

A) Cytosolic Events. A decrease in core T_b is detected by the hypothalamus which initiates increased sympathetic output to BAT (Perkins *et al.*, 1981). Noradrenaline released from the axon terminals in the vicinity of the brown adipocyte, binds to $ß_3$-adrenergic receptors on the brown adipocyte cell membrane and activates adenylate cyclase which subsequently results in an increase in cytosolic cyclic adenosine monophosphate (cAMP) levels (Himms-Hagen, 1990). cAMP acts as a second messenger and activates protein kinase A, resulting in phosphorylation and the subsequent activation of hormone sensitive lipase. Activated hormone sensitive lipase breaks down endogenous lipid droplets, releasing free fatty acids which serve as substrate for mitochondrial oxidation and provide the signal to uncouple mitochondria, resulting in high rates of substrate oxidation and increased heat production without the phosphorylation of adenosine 5'-diphosphate (ADP) (Klingenberg, 1990). After less than 24 hours the triglyceride content of BAT reaches a minimum (Cameron and Smith, 1964). Thereafter fatty acid uptake (plus *de novo* synthesis) must equal or exceed the rate of breakdown if heat production is to be maintained for prolonged periods. Fatty acid uptake is mediated by lipoprotein lipase which is probably synthesized in the tissue and transported to the capillary endothelium where it has its action (Olivecrona *et al.*, 1977). Brown adipose tissue

lipoprotein lipase is stimulated *in vivo* by the actions of noradrenaline via a ß-adrenergic mechanism which is also responsible for the rapid recruitment of lipoprotein lipase during cold exposure (Carneheim *et al.*, 1984).

B) Mitochondrial Events. In all mitochondria, substrate oxidation results in hydrogen ion (proton) efflux across the inner mitochondrial membrane, and it is in this form (membrane potential difference) that the chemical energy of substrates is temporarily stored. Utilization of ATP by cells generates ADP, the presence of which results in the re-entry of protons into the matrix via the enzyme ATP synthase, with the subsequent regeneration of ATP. Thus substrate oxidation (respiration) generates ATP. Heat generation in such a system only occurs if ADP is continually regenerated by an ATP-utilizing process.

Brown adipose tissue circumvents this process, resulting in the uncoupling of oxidation from ATP synthesis. In stimulated BAT, the mitochondria are uncoupled, rate-limiting respiratory control is bypassed, and respiration occurs at much higher rates (Cannon and Nedergaard, 1989; Horwitz, 1989; Himms-Hagen, 1990). This uncoupling is due to the uncoupling protein thermogenin (UCP), a 32kD protein found as a dimer on the inner mitochondrial membrane (Ricquier and Bouillaud, 1986; Cannon and Nedergaard, 1989; Himms-Hagen, 1990; Klaus *et al.*, 1991). UCP is a proton transporter which is

inactivated by the binding of purine nucleotides (especially guanosine diphosphate, GDP) and activated by free fatty acids (Cannon and Nedergaard, 1985; Ricquier and Bouillaud, 1986; Cannon and Nedergaard, 1989; Himms-Hagen, 1990). In its activated state it allows protons to flow back into the mitochondrial matrix via a nonphosphorylating, heat producing pathway, thus dissipating the proton gradient and bypassing ATP synthase. Since there is no association with ATP synthesis, all the energy from substrate oxidation can be liberated as heat. Nonshivering thermogenic capacity is also closely correlated with the total amount of BAT (see, Chaffee and Roberts, 1971) and the total amount of BAT cytochrome oxidase (Heldmaier and Buchberger, 1985). It is unlikely that cytochrome oxidase activity is rate limiting in BAT (Cannon and Nedergaard, 1983), for the oxidative capacity of BAT mitochondria greatly exceeds their capacity for ATP synthesis due to the low concentration of ATP synthase (Yacoe, 1981; Nicholls *et al.*, 1986). UCP is thus the rate-limiting step in BAT thermogenesis and determines an animals capacity for NST (Cannon and Nedergaard, 1983; Cannon and Nedergaard, 1989; Trayhurn and Milner, 1989).

Regulation of the Total Capacity for Heat Production

Usually prolonged cold exposure is necessary to elicit the full development of NST capacity (Heldmaier and Steinlechner, 1981b; Heldmaier *et al.*, 1982; Foster,

1984). Cold exposure results initially in increased thermogenesis in existing BAT. If this is insufficient, shivering is initiated, but simultaneously, changes occur in existing BAT to increase its total capacity for NST. This increased capacity will obviate the need for shivering and provide increased cold tolerance. Recruitment of BAT involves an increase in cell number (Cameron and Smith, 1964), mitochondria (Roberts and Smith, 1967) and UCP, as well as increased innervation and vascularization (Bukowiecki *et al.*, 1982; Asano *et al.*, 1996). The above is species dependent with the largest dependence on an increase in UCP occurring in rats, mice, and the golden hamster (see, Milner *et al.*, 1989). The guinea pig, and especially the Syrian and Djungarian hamster, show a greater dependence on an increase in mitochondria (Rafael *et al.*, 1985; Himms-Hagen, 1986a & 1986b; Trayhurn and Milner, 1989; Wiesinger *et al.*, 1990). Noradrenaline is implicated in the recruitment process by potentiating cell proliferation of precursor cells and inhibiting apoptosis (Cannon and Nedergaard, 1998; Lindquist and Rehnmark, 1998). The former is a $ß_1$-mediated process, whilst the latter is α_1- and $ß_3$-mediated (Cannon and Nedergaard, 1998). The rate of apoptosis is reduced within 24 hours after cold exposure and is sustained for the duration of the cold exposure (Lindquist and Rehnmark, 1998). Noradrenaline also stimulates increased mitochondriogenesis and cell differentiation (Cannon and Nedergaard, 1998) and is also responsible for the

increased synthesis of UCP (Herron *et al.*, 1990), the latter being mediated by both α- and ß-adrenergic pathways (Jacobson *et al.*, 1986). In addition, noradrenaline also stimulates type II thyroxine 5'-deiodinase which rapidly increases BAT concentrations of triiodothyronine (T_3), bringing nuclear T_3 receptors to near saturation (Bianco and Silva, 1988). Both noradrenaline and T_3-generated signals co-operate to induce full expression of the UCP gene and therefore maximal BAT thermogenic capacity (Bianco and Silva, 1987a & 1987b; Bianco *et al.*, 1988). Lipoprotein lipase activity also increases approximately 80-fold in cold-acclimated rats and mice (Horwitz, 1989), thus increasing the release of free fatty acids from chylomicrons and low density lipoproteins in the circulation, and so increasing the substrate available to BAT cells.

Thermogenesis in BAT contributes approximately one-third of the increase in metabolic rate following acute cold exposure in rats acclimated to thermoneutrality, whilst in cold-acclimated rats, BAT contributes more than two-thirds of the increase in metabolic rate (Foster, 1986). Greater contributions of BAT to thermogenesis have been reported in the Djungarian hamster (Heldmaier and Buchberger, 1985) and guinea pigs (Rafael *et al.*, 1986).

Peak cold-induced metabolism directly reflects thermogenic capacity and may serve as an indicator of

thermogenic endurance (Wickler, 1980; Marsh and Dawson, 1989a & 1989b). Increases in peak metabolic rate and decreases in the cold limit, which is the T_a an animal can tolerate and still maintain T_b, are indications of increased cold resistance. Generally, if peak metabolism increases, cold tolerance increases and the cold limit decreases (see Heldmaier *et al.*, 1985). Several studies have reported increases in peak metabolic rate of between 21-108% in cold-acclimated animals (see Heldmaier *et al.*, 1985; Marsh and Dawson, 1989a & 1989b). For instance, winter-acclimated Djungarian hamsters (Heldmaier *et al.*, 1985) and red-backed voles (Rosenmann *et al.*, 1975) have cold limits approximately 34°C lower than their summer-acclimated counterparts, primarily due to an increase in NST capacity. Cold adaptation does not however increase peak metabolic rate in marsupials, although heat production and cold resistance are increased (Smith and Dawson, 1985; Dawson and Olson, 1988).

3.4 Hormonal Responses to Cold Exposure

Apart from their role in heat production, and to a lesser extent, heat loss, hormones are involved in other responses of homeotherms to cold, including: changes in cellular permeability and transport processes; alterations in central nervous system responsiveness and activity; increases in food intake; regulation of fluid balance; changes in reproductive behaviour, implantation, gestation and lactation.

3.4.1 The Hypothalamo-Hypophyseal System

Stimulation of thermoreceptors in the skin and thermosensitive structures in the anterior hypothalamus result in the secretion of releasing factors from the hypothalamus. The rates of secretion of hormones from the thyroid and adrenal cortex are governed by trophic hormones from the adenohypophysis, which in turn are under the control of the hypothalamic releasing factors.

In addition to the thermoregulatory adjustments of cold-acclimation, changes in the neuroendocrine control of salt and fluid balance also appear to take place during thermal adaptation, and are mediated by the neurohypophyseal release of arginine vasopressin (AVP). Water intake and urine production are reduced by nearly 50% in cold-acclimated guinea pigs (5°C), these changes being mediated by a strong increase in the peripheral release of AVP into the circulation (Zeisberger *et al.*, 1988). In contrast, the thermoadaptive changes in water balance and its neuroendocrine control in the golden hamster are opposite to that which is observed in the guinea pig. In golden hamsters acclimated to 5°C, water intake and urine production are doubled (Roth *et al.*, 1990). The differences in responses shown, may reflect rather, differences in their habitats. As inhabitants of arid environments, hamsters have developed extremely effective mechanisms to maintain fluid balance and may therefore exhibit drinking behaviour and AVP responses

differing from those seen in other species.

3.4.2 Adrenal Cortex

Cold stress results in an activation of the adrenal cortex with the concomitant secretion of glucocorticoids, mostly cortisol and corticosterone which mobilize free fatty acids from white adipose tissue and potentiate the effects of catecholamines (see Wunder, 1979). Together these hormones are responsible for mobilizing the fuels for increased thermogenesis. However, increased glucocorticoids were only present in the cold-exposed rat when in a negative energy balance; once food intake increased and body mass was restored, the glucocorticoid levels decreased (Boulouard, 1963 & 1966)

3.4.3 Adrenal Medulla

Regulation of NST occurs via noradrenaline released from sympathetic nerve endings. Increased secretion of adrenaline from the adrenal medulla is seen only when animals are exposed to severe cold when no further increase in noradrenaline secretion is possible (see Precht *et al.*, 1973). Thus, secretion of adrenaline from the adrenal medulla is seen as a last defensive reaction, set in motion only in emergencies. It is therefore understandable why there is no appreciable deterioration in thermoregulation of the cold-adapted rat under a well tolerated cold stress after removal of the adrenal medulla (see Precht *et al.*, 1973). Hence, release of adrenaline

from the adrenal medulla is more likely to occur in non-cold-adapted animals than in cold-adapted ones.

3.4.4 Thyroid Gland

The thyroid gland is structurally conserved in all vertebrates, however thyroid function and concomitant hormone concentrations are considerably different across the animal kingdom. Differences in thyroid function in reptiles and mammals are even apparent at the same T_a, with thyroid hormone concentrations of mammals an order of magnitude higher than that of similar sized reptiles, thus facilitating the higher activity levels and growth rates characteristic of endotherms (Hulbert and Else, 1981).

Responses of the mammalian thyroid gland to acute cold exposure differ from those of chronic cold exposure (Silva and Larsen, 1986; Fregly, 1989 & 1990). Acute cold exposure (1-2 days) brings about a rapid activation of the thyroid gland via a sequence of events, starting with the hypothalamic release of thyrotropin releasing hormone (TRH), followed by pituitary release of thyroid stimulating hormone (TSH) and thyroid secretion of thyroxine (T_4) and triiodothyronine (T_3) (Fregly, 1989 & 1990). Consequently, concentrations of TSH, T_4, T_3 and reverse T_3 in the blood increase (Vybiral *et al.*, 1985; Kopecky *et al.*, 1986). The response of the thyroid gland to prolonged cold exposure (weeks to months), is however, less clear. Studies of the histology of the thyroid gland

indicate increased secretory activity, as do studies of the loss of radioactive iodine from the gland in animals previously treated with ^{131}I (see Precht *et al.*, 1973). The active thyroid gland is also characterized by an increased number of follicles, increased follicular cell height and a reduction in colloid (Gershon and Nunez, 1988).

In contrast, the activity of the reptilian thyroid gland is directly dependent on T_a (Firth and Turner, 1982). It has been shown for the lizard species, *Sceloporus cyanogenys*, that cold exposure reduces serum T_4 levels, whilst for *Chrysemys picta*, prolonged cold exposure resulted in reduced rates of degradation of thyroid products and reduced thyroid gland activity (see Firth and Turner, 1982). In the varanid lizard, *Varanus niloticus*, higher T_b's result in higher metabolic rates and increased thyroid activity (Buffenstein and Louw, 1982). Concomitantly, they exhibit swifter growth due to increased food intake and superior efficiency of conversion at higher T_b's. The increased assimilation efficiency is probably due to superior efficiency of digestive enzymes and peristaltic activity that results in a more rapid rate of passage of the ingesta (Buffenstein and Louw, 1982). Temperature effects on growth and development of ectotherms can most probably be ascribed to changes in the rate of enzymatically controlled chemical reactions in the tissues (Q_{10} effect).

The nett effect of an increase in thyroid activity is an increased metabolic rate that may facilitate an increase in T_b or its maintenance in a cold environment (Cossins and Bowler, 1987). Thyroid hormones produce an elevation in whole-body heat production (Kennedy *et al.*, 1986), organ metabolism and cellular energetics (Ismail-Beigi and Edelman, 1970). Elevated T_4 levels have also been shown to have an inhibitory effect on gonadotropin secretion (La Rochelle and Freeman, 1974). The low BMR of naked mole-rats (Buffenstein and Yahav, 1991a; Goldman *et al.*, 1999), their long gestation period and low growth rates may all be related to their thyroid status

3.5 Effects of Cold Exposure on Gut Function

Prolonged cold exposure may impact considerably upon gut function (Weiner, 1992; Carey, 1993; Hammond *et al.*, 1994; McDevitt and Speakman, 1994). Ectotherms generally show a decline in food consumption with cold-acclimation, whilst endotherms show increased food intake (Buffenstein and Louw, 1982; Cossins and Bowler, 1987; Haim *et al.*, 1990; McDevitt and Speakman, 1994; McKinon and Alexander, 1999). Gut function may however be rate-limiting, thus determining metabolic ceilings during periods of elevated energy expenditure (e.g. cold-acclimation, pregnancy, and lactation) (Nagy, 1987; Hammond *et al.*, 1994; McDevitt and Speakman, 1994; Hammond and Diamond, 1997). These limitations are usually linked to the ability of the animal to digest and absorb an adequate amount of energy,

as well as by the degree of plasticity in size of the gastrointestinal tract (Haim *et al.*, 1990; Speakman and McQueenie, 1996; Hammond and Diamond, 1997). Gut volume determines the upper limit of food intake and may influence transit time (van Soest, 1982; Yahav and Choshniak, 1990). Physical limitations may be of greater importance in herbivorous animals, such as naked mole-rats, that rely on microbial fermentation to liberate the energy trapped in their high fibre diet. Pregnancy may further impede these physical limitations, especially in naked mole-rats which give birth to large litters with concurrent large increases in body mass (Jarvis, 1991a). Many endotherms increase energy intake in the cold by gastrointestinal hypertrophy, coupled with enhanced nutrient uptake capacity (Karasov and Diamond, 1985; McDevitt and Speakman, 1994; Zhao *et al.*, 1995; Hammond *et al.*, 1996). Similarly, in poikilotherms, intestinal volume, enzyme activity and the rate of absorption of digested food particles may be augmented, even at lower T_b's, to maintain transport capacities and digestive efficiency (Smit, 1967; Karasov *et al.*, 1985; Houpe *et al.*, 1996; McKinon and Alexander, 1999).

Life in the cold poses numerous challenges and organisms are compelled to exhibit considerable plasticity so as to regulate and maintain functional capacities in a more hostile and demanding habitat. Regardless of whether the animal responds by increasing or decreasing heat

production, a cold environment impacts on many morphological, physiological and biochemical systems (e.g. respiration, digestion and endocrinology) that have to work in concert to ensure survival. Since naked mole-rats have inhabited an underground milieu for millennia, I questioned whether they would exhibit plasticity in their responses to chronic cold, and whether the lack of plasticity of any organ system would impact upon their prolonged survival at colder temperatures.

CHAPTER 4

REPRODUCTIVE CONSEQUENCES OF PROLONGED COLD EXPOSURE

Reproductive processes are critical for species survival, however individual survival must take precedence over reproduction. Reproduction must occur in harmony with existing dietary, environmental and social conditions (Bronson, 1985). Cold stress imposes an enormous hurdle in the path of reproductive fecundity, for the additional thermoregulatory costs limit the amount of energy apportioned to reproduction as well as influencing physiological function.

4.1 Energetics of Reproduction in the Cold

Before energy can be allocated towards reproduction, maternal cellular maintenance, locomotor costs of foraging and thermoregulatory demands must be met (Brody, 1945). The major energetic rival of reproduction is thermoregulation which uses 80-90% of the energy from ingested food to maintain thermal homeostasis in an averaged sized mammal at moderate temperatures (Bartholomew, 1977). Once these primary demands have been met, any excess energy can be channelled towards growth, non-foraging behaviour, stored as fat for emergencies, or it can be used to support reproduction. The lower these maternal demands are energetically, the greater the proportion of total energy that can be allocated to the physiological and behavioural costs of reproduction. Thus assimilated energy must be partitioned between a variety of interdependent, yet competing physiological demands. An increase in any one of these demands must be countered

either by an increase in food intake or at the expense of lower priority demands.

Two energy related characteristics of small mammals make them especially susceptible to reproductive inhibition in the cold. Firstly, their large surface area to volume ratio and concomitant high rates of heat transfer, increases their thermoregulatory energetic requirements, even at mildly cold temperatures. Secondly, the energetic costs of the reproductive cycle in small females are extremely high, both in relation to her ability to obtain food, and also with regard to the amount of energy that can be stored as fat (e.g. Merson and Kirkpatrick, 1981 & 1983). It is important to note however that low temperatures *per se* are not of overwhelming importance in the reproduction of small mammals. House mice for example, breed well at -6°C provided they are supplied with adequate food and not made to "work for it" (Barnett, 1973; Bronson and Pryor, 1983; Marsteller and Lynch, 1983). What ultimately regulates reproduction in small mammals in the wild then, is not cold *per se* or even direct food availability, but rather the energetic cost/gain ratio of foraging that is in turn determined by both factors (see, Millar and Gyug, 1981; Wunder, 1984).

The effect of temperature on reproduction is a complex and multi-faceted problem whose many dimensions have never been systematically explored in the laboratory.

A mammals' reaction to thermal challenges is dependent on both its genes and its prior experience. Previous exposure to a thermal challenge predisposes the animal metabolically to function better at that temperature. Whilst the reproductive capabilities of male mammals seems little affected by low temperatures (e.g. Nazian and Piacsek, 1977), in contrast, small female mammals show a variety of specific responses that in concert, can culminate in reduced productivity, even with an *ad lib.* supply of food. These responses include, delayed puberty, cessation of adult oestrus cycles, diminished ovulatory rates and reproductive organ weights, fewer young born per litter, and an increase in the interval between successive litters in mice (Biggers *et al.*, 1958; Barnett, 1965; Marsteller and Lynch, 1983; Perrigo and Bronson, 1985; Marsteller and Lynch, 1987), rats (Chang and Fernandez-Cano, 1959; Piacsek and Nazian, 1981), and hamsters (Reiter, 1968; Schneider and Wade, 1990; Schneider and Wade, 1991). The effects of cold may be primarily due to decreased energy availability, for the effects are exaggerated when food intake is prevented from increasing (Perrigo and Bronson, 1985; Marsteller and Lynch, 1987; Schneider and Wade, 1990 & 1991), and attenuated in animals with large fat stores and energy rich diets (Schneider and Wade, 1990 & 1991). For example, hamsters that are lean before cold exposure become anoestrus sooner than fattened hamsters. Similarly, hamsters on a low energy diet also show fewer oestrus cycles than those on

higher energy diets (see, Wade and Schneider, 1992). Small mammals may employ different physiological strategies in response to low T_a's depending on whether food is readily available or not. For instance, *ad lib.* fed domestic mice reached a limit to the amount of food they could process at about 2°C. Beyond this temperature they utilized fat stores to make up their energy deficit. Ovulatory cycles remained unaffected until -8°C, at which temperature protein reserves were drawn on in addition to fat stores, and survival began to be compromised. Females that were not allowed to increase their food intake in response to low temperatures showed a markedly different pattern of responses. Ovulation ceased by 12°C, well before survival was compromised at 2°C, at which point fat stores were utilized. These females preferentially used protein rather than fat stores to make up the energy deficit experienced between 12°C and 2°C (Manning and Bronson, 1990).

Low temperature probably exerts its effects on reproduction via two pathways. The first involves the same energetic pathway as food insufficiency, whereby luteinizing hormone (LH) and prolactin secretion are depressed by caloric insufficiency, with follicle stimulating hormone less affected. Food restriction inhibits the secretion of gonadotropin releasing hormone from the hypothalamus both by increasing the negative feedback sensitivity to oestradiol, and by steroid independent mechanisms (Howland and Ebrahim, 1973;

Piacsek, 1985; Bronson, 1988; Cagampang *et al.*, 1990 & 1991). The second pathway encompasses the concept of non-specific stress, whereby gonadotropin (mostly LH) secretion is depressed and prolactin secretion is enhanced in association with the enhanced activity of the CRF-ACTH-adrenocortical axis. The latter pathway is probably brought into play when an unacclimatized animal is suddenly confronted by a drop in T_a, or at temperatures so extreme that the animal cannot acclimatize to them (Bronson, 1985). Mechanistically, any cold-induced reduction in body mass or body fat may influence reproduction via steroid metabolism. Fat contains a significant pool of progesterone (Hillbrand and Elsaesser, 1983) and plays an important role in the aromatizing of androgens to oestrogens (Nimrod and Ryan, 1975). A reduction in body mass results in increased conversion of oestradiol to catechol oestrogens (with relatively low biological activity) rather than oestriol (Fishman *et al.*, 1975) which may adversely influence reproduction.

Many small mammals overcome adverse thermal conditions by breeding seasonally when the energetic costs of thermoregulation are less (e.g. shrews, Genoud and Vogel, 1990). Seasonal breeders (e.g. *Peromyscus*) when provided with a favourable and constant environment may breed continuously (Bronson, 1985). Total energy investment during pregnancy involves many components, including the net production of foetal, uterine, placental

and mammary tissue, which are referred to as the production costs of pregnancy (Brody, 1945). Coupled with this are increased maintenance costs associated with the new tissue. For most small mammals the production costs of gestation are far greater than the maintenance costs. For example in the rat, if one excludes maternal fat deposition, the maintenance costs average only 50% of the production costs (Spray, 1950). For the naked mole-rat which has an exceptionally long gestation length, the costs of maintaining the products of conception must be borne for longer, such that external factors (e.g. cold exposure) which impact upon the duration and/or energetic cost of maintenance may have a very significant effect on reproductive fitness.

4.2 Thermoregulation and Pregnancy

Body temperature of most mammals is maintained relatively constant during pregnancy. Deviations both below and above normal T_b may be detrimental to the growth, development and indeed the survival of the foetus. Maternal hyperthermia during early pregnancy is teratogenic and during late pregnancy may be associated with intra-uterine growth retardation (Alexander and Williams, 1971; Fraser and Skelton, 1978). Cold exposure induces maternal peripheral vasoconstriction with concomitant changes in uterine and placental blood flow (Laburn *et al.*, 1992). Hypothermic pregnant dogs for example have reduced uterine blood flow which may reduce foetal heat loss (Assali and Westin,

1962) and thus preclude any dramatic reduction in foetal temperature.

Similarly in poikilothermic viviparous reptiles, excessively high or low temperatures may be detrimental to foetal development and may reduce the viability of the young (Licht and Moberley, 1965; Sexton and Marion, 1974) and result in abnormal morphologies (Fox *et al.*, 1961; Vinegar, 1974). Many poikilotherms, when pregnant, maintain T_b's different to that in the non-pregnant state. There is no clear pattern evident however, with both reduced and elevated T_b's recorded during pregnancy (Gier *et al.*, 1989). The temperature at which reptilian embryos are incubated may influence many aspects of foetal development (Fox *et al.*, 1961; Licht and Moberley, 1965; Sexton and Marion, 1974; Beuchat, 1988). Thus environmental factors which directly impact upon T_b of poikilotherms may have profound effects on the duration and outcome of pregnancy. Live bearing reptiles may therefore employ thermoregulatory mechanisms to buffer the impact of the thermal environment on the embryos (Beuchat, 1988). These viviparous reptiles may thermoregulate more precisely (Beuchat, 1986; Gier *et al.*, 1989; Charland and Gregory, 1990) and also maintain T_b closer to that which is optimal for maternal physiological function and embryonic development. This enables the production of the largest number of viable young (Beuchat, 1986; Beuchat, 1988; Beuchat and Ellner, 1987). The naked mole-rat, also a

poikilotherm, may similarly also be susceptible to thermal influences on gestation.

4.3 Effects of Cold Exposure on Gestation Length

Gestation length is considered to be controlled primarily by genetic regulation. Acute cold exposure of pregnant rats in which rectal temperature was allowed to drop to 16-19°C resulted in a slight lengthening of the gestation period, with the majority of the rats giving birth to normal young (Vidovic, 1952). Courrier and Marois (1953) cooled rats to 16-20°C during the first 11 days of pregnancy with no harm to the embryos, although implantation was delayed, development retarded and parturition postponed. When repeated at a later stage of pregnancy, gestation was halted and uterine haemorrhage occurred. Czajkowsi (1958) induced artificial hibernation in Golden hamsters, which resulted in a lengthening of pregnancy which was proportional to the duration of the hypothermia. Stankiewicz (1974) also noted that cooling mice to 18°C during the first half of pregnancy resulted in delayed implantation and intrauterine mortality.

Although homeothermy renders most mammals independent of temperature, for species which do not maintain a constant T_b, any alterations in T_a may affect gestation. Such is the case with heterothermic bats. Eisentraut (1937) first noted that pregnant *Myotis myotis* were capable of entering torpor, which at a sufficiently low T_a

could become a state of hibernation lasting for several days. Animals were killed at specific stages, with those that had been exposed to the lowest temperatures exhibiting the smallest foetuses. However, since ovulation in a colony of bats bears no relation to the time of copulation (Racey, 1979), the foetuses which Eisentraut was comparing may have been at different stages of development at the start of his experiment. Similarly, Kolb (1950) related a three week delay in the appearance of the first young of a colony of the Lesser Horseshoe bat (*Rhinolophus hipposideros*) to the occurrence of a three week cold spell. Whereas Eisentraut (1937) suggested that the rate of foetal development was slowed in response to lower environmental temperatures, Kolb (1950) suggested that foetal development was actually terminated for the period of cold exposure. Racey (1973) noted that when pregnant Pipistrelle bats (*Pipistrellus pipistrellus*) were maintained at 5°C for 14-17 days, the mean date of birth was prolonged by 5 days, indicating that the rate of foetal development had been significantly retarded. It was also noted that parturition was delayed for a period similar to the duration of torpor.

In contrast to most homeotherms, the temperature at which reptilian embryos are incubated can influence many aspects of development. Gestation length is profoundly sensitive to temperature in poikilotherms. For instance, in the viviparous lizard *Sceloporus jarrovi*, gestation

length can vary nearly two-fold for a change in T_b of as little as 2°C (Beuchat, 1988). Although developmental rates generally increase with increasing incubation temperature in reptiles, it may not be most rapid at the highest temperature compatible with development. Gestation length generally is positively correlated with temperature, occurring more rapidly at higher temperatures (Licht and Moberly, 1965; Sexton and Marion, 1974; Vinegar, 1974; Muth, 1980). However, extreme temperatures that are either too high or too low, may result in reduced viability of the young (Licht and Moberly, 1965; Goode and Russel, 1968; Sexton and Marion, 1974), or abnormal morphologies (Vinegar, 1974; Burger *et al.*, 1987). For live bearing reptiles the impact of the thermal environment can be buffered by the thermoregulatory behaviour of the pregnant female who must maintain a T_b which is both optimal for her physiological functioning as well as for the development of her foetuses. However, numerous studies have shown that the optimal temperature for various physiological functions (e.g. digestion, locomotion) can vary considerably (Huey, 1982), thus the pregnant female must maintain a T_b which is a compromise between these conflicting optima. In numerous species of reptiles, females maintain different T_b's during pregnancy than at other times (Beuchat, 1986), suggesting that the optimal temperature for foetal development is divergent from that which is optimal for the females physiological functions (Ellner and Beuchat, 1984; Beuchat, 1986; Beuchat and

Ellner, 1987).

4.4 Effects of Maternal Cold Exposure on Litter Parameters

In house mice, *Mus musculus*, cold exposure during pregnancy results in litter reduction although pup mass is greater at birth (Barnett *et al.*, 1975). Nestling mortality was also significantly higher in the cold (Barnett et al., 1975). This was largely due to the loss of whole litters, as litter survival post-weaning was no different at the colder temperature. In addition, the incidence of cannibalism was increased at colder temperatures. For instance, Syrian hamsters eat significantly more of their offspring when housed at colder temperatures. The effect of cold on cannibalism was however attenuated in hamsters that were fattened prior to cold exposure and exaggerated in hamsters that were lean prior to cold exposure (Schneider and Wade, 1991). The greater energy expenditure required to maintain thermal homeostasis in the cold, depletes the supply of metabolic fuels available, such that the lactating female may respond by sacrificing some pups to ensure the survival of the rest. Maternal cannibalism of pups is thus a reproductive strategy which allows the mother to balance litter size with energy availability as determined by food supply, body fat stores, and energy required for essential processes such as thermal homeostasis. Similar findings have been reported in poikilotherms (e.g. varanid

lizards), such that those incubated at lower temperatures were significantly larger than those incubated at higher temperatures (Beuchat, 1988).

Although cold may present considerable obstacles in the face of reproductive function, both in terms of energy requirements of homeotherms and also in terms of rates of foetal growth and development of poikilotherms, one of the main questions that remains unanswered is what determines whether the reproductive response to cold is "all or none" or proportional, and furthermore, at what cost is it to the mothers' survival?

CHAPTER 5

AIMS OF THIS STUDY

Naked mole-rats are highly specialized animals that have evolved a suite of characteristics well suited to the warm and dark confines of their chthonic equatorial habitat. Many of these features compromise their ability to cope with thermally variable and unpredictable environmental conditions. I questioned whether these animals would exhibit typical endothermic or ectothermic acclimation responses to chronic cold exposure and what the impact of prolonged cold exposure would be on other physiological functions, focussing in particular on gut function and reproductive success.

The following set of competing hypotheses is therefore proposed and addressed in this thesis, viz:

1) Because naked mole-rats are endotherms, cold-acclimation would follow a typical mammalian pattern. This essentially would involve shifting the TNZ to a lower range of T_a's, this being facilitated by enhanced insulation and/or augmented basal metabolism.

2) Alternatively, because naked mole-rats thermoconform, cold-acclimation would follow a typically ectothermic mode. If this were the case, naked mole-rats would endure lower T_a's with concomitant effects on T_b and other physiological variables.

Several other hypotheses contingent on the outcome of these more fundamental competing hypotheses could also be proposed and these are mentioned in the relevant chapters that explore these topics in depth.

These competing hypotheses were tested by posing the following specific questions:

A) What are the morphological and functional responses of cold-acclimation?

1) Is there a shift in the TNZ?
2) Does BMR change?
3) Is the activity of the hypothalamic-pituitary-thyroid axis altered?
4) Is body composition (fat/lean mass) modified?
5) Is NST capacity enhanced?
6) Is thermogenic capacity and/or activity of BAT mitochondria altered?
7) Is energy intake increased and if so how is this facilitated?

B) What are the effects of cold-acclimation on gestation and reproductive outcome?

1) Are the body composition and T_b changes of pregnancy and lactation altered by either acute or chronic cold exposure?
2) Is reproductive success adversely affected by cold-acclimation?

3) Which stage of the reproductive cycle is most sensitive to the adverse effects of cold?
4) Are interbirth interval and/or gestation length increased with chronic cold exposure?
5) Are litter size and/or pup mass affected by environmental temperature?
6) Is pup mortality rate increased in the cold?

These studies aim to elucidate whether naked mole-rats predominantly conform to typical mammalian or reptilian cold-acclimatory profiles, with a view to understanding the mechanisms employed in these processes and the impact of thermal acclimation.

CHAPTER 6

NORADRENALINE-INDUCED NONSHIVERING THERMOGENESIS IS UNCHANGED WITH CHRONIC COLD EXPOSURE IN A POIKILOTHERMIC MAMMAL, THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*)

6.1 ABSTRACT

The naked mole-rat (*Heterocephalus glaber*), although an endotherm, is unable to effectively regulate body temperature (T_b). I questioned whether thermogenic capacity would change with prolonged (>1yr) exposure to cooler conditions, with the hypothesis being that these unusual rodents would not conform to common mammalian patterns and that nonshivering thermogenic (NST) capacity would not be enhanced by cold exposure. The capacity for NST was assessed following noradrenaline administration (0.8mg/kg, s.c.) to lightly anaesthetized (pentobarbital) animals, and monitoring the concomitant changes in oxygen consumption and T_b. Results concur with the null hypothesis in that prolonged cold exposure did not elicit an increase in NST capacity (1.52mlO_2/g/h, cold-acclimated; 1.73mlO_2/g/h, control, $P>0.05$), with observed NST capacity similar to maximal metabolic rate under various conditions. Rapid heat loss across their uninsulated skin may necessitate continuous maximal stimulation of brown adipose tissue (BAT), and as such, prevent any further increase in thermogenic capacity following cold exposure. These data suggest that the biochemical and molecular control of BAT thermogenesis is maximally primed under all conditions in these poorly insulated mammals.

6.2 INTRODUCTION

Adaptive changes in the capacity for nonshivering thermogenesis (NST) are critical for the survival of small mammals in temperate environments where pronounced seasonal differences in ambient temperature (T_a) prevail. Endogenous heat production via sympathetically mediated NST is enhanced in response to cold, thus compensating for increased heat loss to the environment (Williams, 1968; Hissa and Hirsimaki, 1971). Brown adipose tissue (BAT) is the major site for this sympathetic-induced NST response (Foster and Frydman, 1978 & 1979; Carneheim *et al.*, 1984; Himms-Hagen, 1990). An acute cold stimulus increases thermogenesis in existing BAT, whilst chronic cold exposure markedly increases the capacity for NST via a large recruitment of BAT (Himms-Hagen, 1990). As such, the NST response of non-hibernating rodents following chronic cold exposure is commonly 1.5-fold greater than the maximal induced NST response prior to cold exposure (Wunder and Gettinger, 1996).

Naked mole-rats (*Heterocephalus glaber*) are poikilotherms, unable to regulate body temperature (T_b), despite the employment of endothermic mechanisms (Buffenstein and Yahav, 1991a; Hislop and Buffenstein, 1994). Below apparent "thermoneutrality" (as T_r is never regulated), metabolism increases by *more than 50% over basal levels for every 1°C drop in* T_a till approximately

28°C (Buffenstein and Yahav, 1991a). At this comparatively warm temperature, rates of heat production relative to basal metabolic rate (BMR) are of similar magnitude to those exhibited by other cold-stressed laboratory rodents at considerably lower T_a's of 5°C (Dicker *et al.*, 1995; Hammond *et al.*, 1996). Although naked mole-rats respond to prolonged cold exposure by a left-shift of their metabolism/temperature rate curve (Chapter 9), peak metabolism in that study was of a similar magnitude to that previously noted under various physiological and/or experimental conditions (Buffenstein and Yahav, 1991a; Hislop and Buffenstein, 1994), implying that maximal metabolic rate was unchanged and that endothermic capacity may not be adjustable. I investigated this hypothesis by questioning whether prolonged cold-stress in naked mole-rats would result in increased BAT thermogenic capacity by monitoring changes in oxygen consumption (VO_2) and T_b following noradrenaline intervention. Noradrenaline-induced NST was therefore investigated in animals housed for prolonged periods at either 25°C or 30°C.

6.3 MATERIALS AND METHODS

6.3.1 Animal Care and Maintenance

Male naked mole-rats used in this study were born in captivity from progenitors collected in Kenya (0°38'N/37°40'E) in 1980. They were housed together with their mates in standard laboratory rat cages in which

lengths of perspex tubing were provided for burrowing. Nesting material included sawdust and shredded paper. Animals were fed an *ad lib.* diet of fresh fruit and vegetables as well as a high energy, protein enriched cereal (Pronutro, Becketts, RSA).

Nine adult (36 ± 6g) male naked mole-rats were housed at an T_a of 25 ± 1°C for 17 ± 5 months. A further 6 adult (36 ± 3g) male naked mole-rats housed under standard laboratory conditions (30 ± 1°C) simulating that normally encountered in their natural environment (Jarvis, 1991b) served as the control group.

6.3.2 Experimental Protocol

Nonshivering thermogenic capacity was determined in animals housed for prolonged periods under two different T_a's. Noradrenaline-induced NST was determined at both 28°C and 32°C. These temperatures were specifically chosen as they fall in the range of the apparent "thermoneutral zone" (TNZ) for animals housed at 25°C (27-34°C; Chapter 9) and 30°C (31-34°C; Buffenstein and Yahav, 1991a; Chapter 9) respectively. In order to facilitate direct comparisons and remove any temperature effects, regardless of long-term housing conditions, each animal was randomly tested at both experimental temperatures. Nonshivering thermogenic capacity was determined in lightly anaesthetized animals by measuring the difference between baseline VO_2 and the maximal VO_2 induced by subcutaneous

(s.c.) 0.8mg/kg noradrenaline injection (Heldmaier, 1971; Vybiral and Jansky, 1974). A s.c. injection of the saline vehicle alone (equivalent volume) served as the experimental control. Treatments were randomised with at least one week interval between the different treatments for each animal.

6.3.3 Anaesthesia

Naked mole-rats were housed at the experimental temperature for 1 hour prior to anaesthesia in a constant temperature chamber (Airomatic, Johannesburg, South Africa). They were lightly anaesthetized with sodium pentobarbital (i.p.) 6% m/v (40mg/kg). Although sodium pentobarbital at this dose does slightly depress respiration, it does not affect the magnitude of the VO_2 and T_b changes of the NST response and has been routinely used in NST studies (Vybiral and Jansky, 1974; Ellison and Skinner, 1990).

6.3.4 Temperature Measurements

A calibrated copper-constantan rectal thermocouple was inserted to a depth of 2-3cm into the rectum and the anaesthetized animal placed supine on a wire grid within a sealed perspex metabolic chamber. Chamber T_a was also monitored via a calibrated thermocouple. Both T_b and T_a were recorded via a digital display every 5 min (Model-Bat-12, Physitemp, New York, USA).

6.3.5 Oxygen Consumption Measurements

Oxygen consumption was measured in an open circuit system similar to that described by Depocas and Hart (1957). A positive flow pump (Ametek flow control R-1) maintained an air flow of approximately 60ml/min. Flow rate was measured via the rate of movement of a soap film through a calibrated glass tube of internal diameter (23mm) and volume 200ml. Air was dried *en route* by passing it through Drierite (Saarchem, RSA) prior to entry into an oxygen analyser (Ametek S-3A/ii, Sensor model N37-M). Oxygen consumption was determined from the 12 highest consecutive readings measured over a 6 min period. Body mass was monitored to the nearest 0.1g (Ohaus LS2000, Ohaus Scale Corporation, New York, USA) both before and after VO_2 measurements. All VO_2 measurements were converted to STP and expressed in $mlO_2/g/h$. Furthermore, VO_2 measurements were converted to metabolic heat production using 5.582W/kg as the caloric value for 1ml of oxygen.

6.3.6 Noradrenaline and Saline Treatments

Baseline recordings were continued for approximately 1 hour until T_b had stabilized. The animal was then quickly removed from the chamber and given a s.c. injection of either saline (SABAX, sodium chloride 9%, Johannesburg) or noradrenaline (0.8mg/kg in saline vehicle; Arterenol bitartrate salt, Sigma, St Louis, USA). The same dose of noradrenaline was used for both cold-acclimated and control animals (Hislop and Buffenstein, 1994), as the

dose needed to stimulate maximal VO_2 is not affected by cold-acclimation (Wunder and Gettinger, 1996). The site of injection was always the left hind flank and was swabbed with an alcoholic prep before injecting.

6.3.7 Statistical Analysis

All data are expressed as means and standard deviations. Baseline measurements for both experimental temperatures were compared by unpaired T-tests. Data sets for each experimental temperature (28°C and 32°C) were compared by Ordinary ANOVA with a Tukey-Kramer post hoc multiple comparisons test. Differences between individual data sets measured at both 28°C and 32°C were compared by MANOVA with a Tukey post hoc test. Results were considered significant at $P<0.05$.

6.4 RESULTS

Cold-acclimated animals had higher ($P<0.01$) baseline VO_2 measurements, however noradrenaline-induced VO_2 values were similar ($P>0.05$) to that of control naked mole-rats (Fig. 6.1). Regardless of the experimental T_a, noradrenaline intervention induced significant increases in VO_2 ($P<0.001$; Fig. 6.1). Furthermore, the maximal VO_2 of NST as well as the resultant increase in T_b were not affected by the experimental T_a, and were similar for both cold-acclimated and control animals (Fig. 6.1).

At both experimental T_a's, there was no significant difference ($P>0.05$) in VO_2 or T_b following saline intervention in either group (Fig. 6.1).

6.4.1 Noradrenaline-Induced NST at 32°C

Despite the fact that an T_a of 32°C falls within the TNZ of both control and cold-acclimated animals, baseline VO_2 measurements between the two groups were significantly different. Baseline measurements for cold-acclimated animals (0.55 ± 0.08 $mlO_2/g/h$) were 38% greater than that of control animals (0.40 ± 0.05 $mlO_2/g/h$; $P=0.0011$; Fig. 6.1). Because maximal metabolic rates were similar ($P>0.05$; Fig. 6.1), the percentage increase in VO_2 from baseline values to the maximal levels of NST were 2.1-times greater in control animals (354 ± 244%) compared to cold-acclimated animals (172 ± 79%; $P<0.05$).

6.4.2 Noradrenaline-Induced NST at 28°C

Baseline VO_2 for cold-acclimated animals (0.39 ± 0.06 $mlO_2/g/h$) was 39% greater than that of control animals (0.28 ± 0.03 $mlO_2/g/h$; $P=0.0017$; Fig. 6.1), despite the fact that for the control group this T_a lies approximately 3°C below their TNZ. Because maximal VO_2 levels were similar, the percentage increase from baseline VO_2 to maximal NST levels was 1.7-times greater in control animals (551 ± 260%) compared to cold-acclimated animals (321 ± 115%; $P<0.05$; Fig. 6.1).

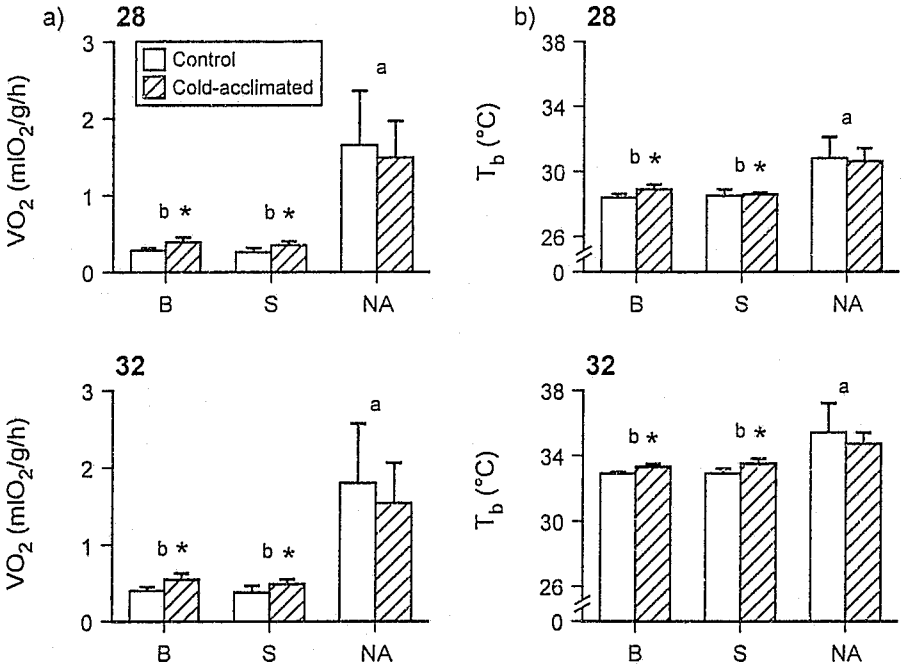


Figure 6.1 Oxygen consumption (a) and body temperature (b) of anaesthetized cold-acclimated and control naked mole-rats, before intervention (baseline, B), and after either saline (S) or noradrenaline (NA) (0.8 mg/kg) injection. Peak NA-induced metabolic rates and T_b's were similar ($P > 0.05$) and significantly greater than both baseline and saline values (a vs b; $P < 0.001$). Cold-acclimated naked mole-rats showed significantly greater metabolic rates and T_b's for baseline and saline measurements (*; $P < 0.01$).

For cold-acclimated animals the percent increase in VO_2 at 32°C (172 ± 79%) was approximately half that at 28°C (321 ± 115%; P=0.0056). For control animals however, the percent increase in VO_2 at 32°C (354 ± 244%), which is similar to that reported by Hislop and Buffenstein (1994), was not significantly different from that at 28°C (551 ± 260%; P=0.2054).

6.4.3 Body Temperature Responses of NST

Experimentally-induced NST resulted in significant but similar increases in T_b from baseline levels in both cold-acclimated and control naked mole-rats (P<0.001; Fig. 6.1). Baseline T_b's of cold-acclimated animals were approximately 0.4-0.5°C greater (P<0.01) than that of control animals regardless of experimental T_a (Fig. 6.1). There was no significant change in T_b following saline intervention (Fig. 6.1).

A strong correlation exists between the rate of metabolic heat production during NST and the rise in T_b (r=0.8236; P<0.0001; n=30; Fig. 6.2).

For cold-acclimated animals the percent increase in VO_2 at 32°C (172 ± 79%) was approximately half that at 28°C (321 ± 115%; P=0.0056). For control animals however, the percent increase in VO_2 at 32°C (354 ± 244%), which is similar to that reported by Hislop and Buffenstein (1994), was not significantly different from that at 28°C (551 ± 260%; P=0.2054).

6.4.3 Body Temperature Responses of NST

Experimentally-induced NST resulted in significant but similar increases in T_b from baseline levels in both cold-acclimated and control naked mole-rats (P<0.001; Fig. 6.1). Baseline T_b's of cold-acclimated animals were approximately 0.4-0.5°C greater (P<0.01) than that of control animals regardless of experimental T_a (Fig. 6.1). There was no significant change in T_b following saline intervention (Fig. 6.1).

A strong correlation exists between the rate of metabolic heat production during NST and the rise in T_b (r=0.8236; P<0.0001; n=30; Fig. 6.2).

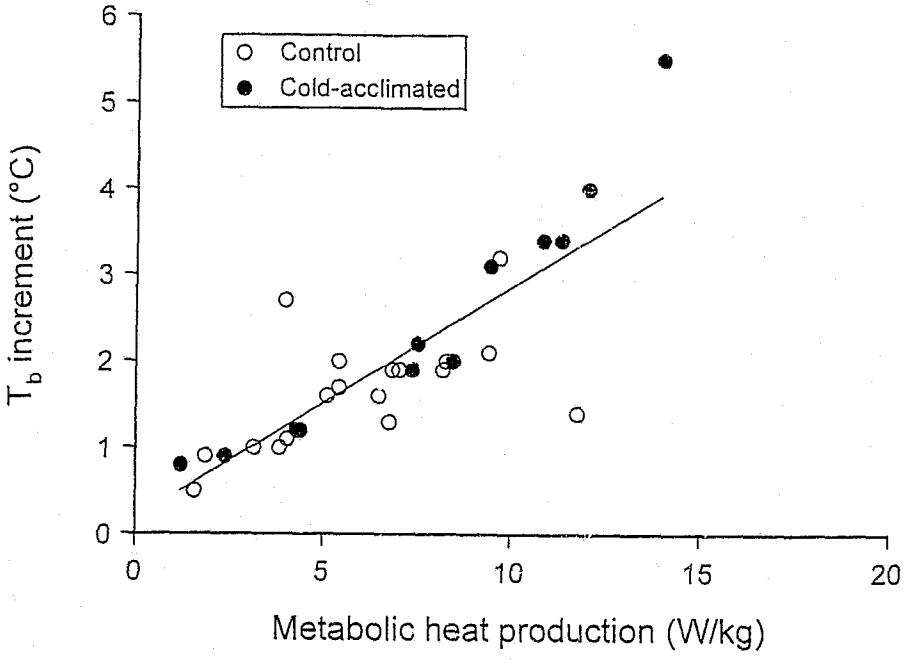


Figure 6.2 The incremental change in body temperature (T_b) in response to noradrenaline intervention is directly correlated to incremental NST induced changes in metabolic heat production. T_b increment (°C) = 0.27 Metabolic heat (W/kg)+ 0.18. (r=0.8236; P<0.0001; n=30)

6.5 DISCUSSION

Chronic cold exposure did not increase NST capacity in the naked mole-rat. This is contrary to the findings for most small mammals, where cold exposure induces BAT hyperplasia (Bukowiecki *et al.*, 1982) and concomitant increases in NST capacity (e.g. Foster and Frydman, 1978 & 1979; Heldmaier and Buchberger, 1985; Rafael *et al.*, 1986; Wunder and Gettinger, 1996). Since baseline metabolic rates were greater in the cold, the percent increase in VO_2 above baseline levels, contrary to typical cold-stress responses, was greater in the control group rather than in the cold-acclimated animals (Fig. 6.1).

Body temperatures measured at the peak of the NST response were similar in both groups (Fig. 6.1). Regardless of thermal history, similar rates of heat production elicit parallel incremental changes in T_b. Even though NST can increase T_b by as much as 5°C (Fig. 6.2), heat retention is short lived. This is attributed to their poorly insulated skin and the high rates of heat loss, such that even the peak T_b increment induced in response to noradrenaline is much lower than that of other small mammals (see, Hislop and Buffenstein, 1994).

Cold-acclimation clearly influenced basal metabolism, as indicated by the 38% increase in VO_2 which may be intimately linked to the 40% increase in free thyroxine

levels of cold-acclimated naked mole-rats (Chapter 8). Similar findings of an increase in BMR with cold exposure have been reported in both homeotherms (Wiesinger *et al.*, 1990; Ellison *et al.*, 1992) and poikilotherms, the latter showing increases in standard metabolic rates at different T_b's (see, Cossins and Bowler, 1987). In mammals and birds such an increase would normally occur in response to extreme cold-stress, thereby facilitating an increase in heat production. While reptiles normally show a decrease in metabolism with acute cold exposure, cold-acclimation does induce an upward shift in metabolism. Increasing basal energy expenditure in the cold, at first glance is not advantageous and would require increased food consumption with concomitant increased foraging costs. However, in endothermic homeotherms, an elevated BMR facilitates a reduction in the lower critical temperature, such that minimal metabolism may be maintained over a wider range of T_a's. The concomitant energy savings may, to some extent, off-set the augmented energy requirements of maintaining this elevated BMR.

Perspectives: *Why does the naked mole-rat not increase its nonshivering thermogenic capacity in response to chronic cold exposure*?

From an evolutionary perspective, one might argue that naked mole-rats have lost the ability to increase the thermogenic capacity of their BAT, for they have exploited

a thermally stable, underground milieu for millennia (Lavocat, 1978). Burrow temperatures, although relatively high (31°C), are very stable with a diel and seasonal temperature range of less than 2°C (Bennett *et al.*, 1988). Naked mole-rats therefore very rarely encounter temperatures outside their apparent TNZ (31-34°C; Buffenstein and Yahav, 1991a). In addition to their thermally buffered habitat, their social lifestyle facilitates the maintenance of homeothermy by behavioural thermoregulation (Yahav and Buffenstein, 1991b). Naked mole-rats huddle in nests, and the concomitant reduction in exposed surface area allows for the maintenance of T_b over the range of T_a's potentially encountered (Yahav and Buffenstein, 1991b). Huddling behaviour in mice has been shown to reduce the requirements for BAT thermogenesis and results in a suppression of thermogenic capacity (Himms-Hagen and Villemure, 1992) and may induce similar effects in naked mole-rats.

It was presumably the exploitation of this thermally stable, subterranean niche that resulted in the evolutionary loss of an insulatory pelage (Daly and Buffenstein, 1998), with the concomitant high potential heat loss. Given the unusual biology of the naked mole-rat, their limited and temperature-independent thermogenic capacity is not that surprising. Even at warm T_a's, equivalent to that found in their burrows, the NST response is substantial, being in excess of a 350%

increase in VO_2 over basal levels. This is far in excess of that which is seen in some laboratory rodents (e.g. Syrian hamsters) that have been acclimated to 5°C (*ca* 200% ; Dicker *et al.*, 1995). The extremely large percent increase in VO_2 seen in naked mole-rats is to some extent due to the very low BMR exhibited by this species, amongst the lowest recorded as a function of body size for all mammals (Buffenstein and Yahav, 1991a; Goldman *et al.*, 1999). From the results of Buffenstein and Yahav (1991a), a drop in T_a of only 2-3°C below thermoneutrality resulted in a more than 120% increase in resting metabolism. Relatively speaking then, the cold-stress endured by the naked mole-rat at temperatures even slightly below thermoneutrality is very high and the resulting increase in metabolism is again indicative of the sustained high thermogenic capacity of their BAT. Because small changes in T_a below thermoneutrality represent such a severe cold-stress for these poikilothermic mammals, their BAT may already be maximally stimulated, and as such, thermogenic capacity would be at a maximum with no further cold-induced increases possible. The findings of this study suggests that the total amount of uncoupling protein in their BAT mitochondria as well as GDP binding (both measures of total thermogenic capacity) are unchanged by long-term cold exposure. These inferences however require further investigation.

Physiological factors other than the thermogenic capacity of their BAT may be limiting factors in the observed thermoregulatory capacity of the naked mole-rat. Limitations of other physiological systems (e.g. gut capacity), may restrict augmentation of BAT thermogenic capacity, and the concomitant elevated food requirements in response to cold. Furthermore, the subterranean niche is a formidable milieu both in terms of acquiring adequate energy intake as well as respiratory gas exchange. In the latter case, the underground gaseous atmosphere is both hypoxic and hypercapnic, impeding rapid gas exchange and limiting the availability of oxygen for aerobic respiratory processes (Buffenstein, 1996). In addition, food location underground involves random digging for patchily distributed roots, bulbs and tubers in dry compact soils and is an extremely costly process, in which the cost-benefits of foraging may not always favour the animal (Jarvis, 1978).

It would be energetically wasteful and extremely costly for the naked mole-rat to increase BAT thermogenic capacity for NST, when the insulatory properties of its skin are such that heat cannot be retained, so that T_b is ultimately determined by heat exchange across the exposed surface area. Indeed, below an T_a of 28°C the naked mole-rat abandons endothermy as the costs involved become prohibitive (Hislop and Buffenstein, 1994). The thermoregulatory shifts observed with cold-acclimation

(Chapter 9), although they do not facilitate T_b maintenance, abrogate any need to increase BAT thermogenic capacity.

In conclusion, naked mole-rats respond to prolonged cold exposure by increasing basal thermogenesis in existing BAT. The inability to augment their BAT thermogenic capacity in response to cold may reflect their evolutionary history in a thermally stable subterranean milieu and also the prohibitive costs of endothermy in their inhospitable habitat. Alternatively, because small changes in T_a below thermoneutrality represent a severe cold-stress for these poikilothermic mammals, their BAT may already be maximally stimulated, and as such, thermogenic capacity is at a maximum with no further increase possible.

CHAPTER 7

BROWN ADIPOSE TISSUE THERMOGENIC CAPACITY IS MAXIMAL IN THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*), WITH NO COLD-INDUCED INCREASE

7.1 ABSTRACT

Cold-acclimation in small mammals normally results in increased thermogenic capacity, facilitated by increases in brown adipose tissue (BAT) and/or its biochemical function. Naked mole-rats (*Heterocephalus glaber*) are unable to effectively regulate body temperature (T_b) and are operatively poikilotherms. I questioned whether these mammals would exhibit cold-acclimation induced changes in BAT thermogenic capacity and activity. This was investigated by monitoring biochemical changes in BAT following prolonged cold exposure (25°C; > 1 yr). Thermogenic capacity of BAT was determined from measurements of mitochondrial GDP binding and uncoupling protein (UCP) concentration. Mitochondrial proton conductance, the central mechanism for thermogenesis in BAT, was estimated from measurements of mitochondrial oxygen consumption and membrane potential. Regardless of ambient conditions, naked mole-rats have large BAT reserves, accounting for 1.5% of total body mass. The metabolic activity of this tissue is however *ca.* 66% of that of cold-acclimated mice (4°C). Cold-acclimation did not result in any significant increase in GDP binding which was similar to that of cold-acclimated mice. The proton conductance pathway across the mitochondrial inner membrane was significantly more active in the cold-acclimated naked mole-rats, with a concomitant elevation in mitochondrial oxygen consumption. Acclimation to 25°C, which represents a severe cold-stress for these poikilothermic mammals, did not result in increased BAT

thermogenic capacity, but thermogenic activity of existing BAT depots was enhanced. This suggests that BAT thermogenic capacity in the naked mole-rat is maximal even at thermoneutrality and is constantly primed for thermogenesis.

7.2 INTRODUCTION

Cold-acclimation in small mammals generally results in an augmented nonshivering thermogenic response, facilitated by the up-regulation of thermogenic "machinery", including increased brown adipose tissue (BAT) mitochondrial mass and an activation of the proton conductance pathway (Nicholls and Locke, 1984; Cannon and Nedergaard, 1985). The proton conductance pathway is regulated by an uncoupling protein (UCP) located in the inner mitochondrial membrane (Heaton *et al.*, 1978), and is inhibited by purine nucleotides, particularly guanosine diphosphate (GDP) (Nicholls, 1976), which competitively binds to UCP (Heaton *et al.*, 1978). Nucleotide-sensitive proton conductance is commonly used as an indicator of functional UCP activity. The concentration of UCP in BAT mitochondria determines the maximum capacity of the mitochondrial proton conductance pathway, whereas the total amount of UCP determines the thermogenic capacity of the *whole* tissue (Cannon and Nedergaard, 1985; Trayhurn and Milner, 1989).

Although acute cold exposure may result in a dissociation between UCP concentration and GDP binding, chronic stimulation

generally results in parallel changes in both these variables (Feil and Rafael, 1994). Thus for prolonged cold exposure, GDP binding also provides an indication of changes in the amount of UCP and its concentration. Also, when thermogenesis is maximally stimulated, GDP binding can reflect the UCP concentration of BAT mitochondria (Trayhurn and Milner, 1989).

Since UCP is the rate-limiting step in BAT thermogenesis and determines an animals capacity for nonshivering thermogenesis (NST) (Cannon and Nedergaard, 1983; Cannon and Nedergaard, 1989), animals which show no cold-induced increase in UCP may be either constantly primed for thermogenesis or may have abandoned the employment of endothermy. One such animal may be the naked mole-rat (*Heterocephalus glaber*). These rodents lack effective insulation and despite their ability to endogenously produce heat, as indicated by noradrenaline-induced NST (Hislop and Buffenstein, 1994; Chapter 6), body temperature (T_b) tracks ambient (T_a) such that they are operatively poikilotherms (Buffenstein and Yahav, 1991a). The relationship between oxygen consumption and T_a follows two distinct patterns. Metabolic rate increases exponentially with increasing T_a up to 28°C. Thereafter a typical endothermic thermoregulatory pattern is evident, with the apparent "thermoneutral zone" (TNZ), as T_b is never regulated, extending between 31-34°C (Buffenstein and Yahav, 1991a). Below the TNZ, metabolism increases by 50% above basal levels for every 1°C drop in T_a up till approximately 28°C, with maximal metabolism reached at T_a's only 3-5°C below

thermoneutrality (Buffenstein and Yahav, 1991a). This slight drop in T_a is therefore a severe cold stress for these poikilothermic mammals, comparable to that of a 20°C decline in T_a for other laboratory rodents (e.g. hamsters, Dicker *et al.*, 1995).

In a previous study (Chapter 6), it was found that prolonged cold exposure (25°C; > 1 yr) did not increase NST capacity, however basal metabolism was substantially (38%) increased. I therefore hypothesized that BAT thermogenic capacity in the naked mole-rat is maximal even when housed at thermoneutrality and that prolonged cold exposure would not further increase BAT thermogenic capacity (GDP binding and UCP concentration), although BAT thermogenic activity (proton conductance) may be enhanced. I therefore assessed BAT thermogenic capacity and activity in both cold-acclimated (25°C; > 1 year) and thermoneutral-acclimated (30°C, control) naked mole-rats.

7.3 MATERIALS AND METHODS

7.3.1 Animal Care and Maintenance

Four non-reproductive pairs of captive-born naked mole-rats (34.6 ± 2.2g) were housed at an T_a of 25°C for a prolonged period (> 1 yr). An equivalent control group (37.6 ± 1.7g) were housed at

30°C, the optimal and commonly used captive housing temperature for these poikilothermic mammals (Jarvis, 1991b). All animals were housed in standard laboratory rat cages with sawdust provided for bedding. Animals were fed an *ad lib.* diet of sweet potato and apple, as well as receiving a protein enriched commercial cereal (Pronutro, Becketts, RSA). Laboratory mice (n = 8; 45.1 ± 0.7g) acclimated to 4°C served as positive controls. They were similarly housed and had an *ad lib.* supply of standard laboratory rat chow and fresh water.

7.3.1 Mitochondrial Isolation

Two animals from each experimental group were weighed and immediately upon removal from their respective housing temperatures killed by cervical dislocation and decapitation. This was done by experienced technicians in accordance with approved animal ethics protocols.

Mitochondrial isolation was done using a modified method of Cannon and Lindberg (1979). The major BAT deposits were quantitatively dissected out and immediately

placed into 2ml of ice-cold 0.25M sucrose (pre-weighed). Average BAT mass was calculated by re-weighing the test-tube. Liver from the 25°C acclimated naked mole-rats served as a negative control. Mitochondria were isolated by differential centrifugation and extraction while placed in a series of different solutions, namely 0.25M sucrose and KCl-Tris (pH 7.1) with a 0.2% bovine serum albumin (BSA) (free fatty acid free) wash included. The final centrifugation was in 0.5ml KCl-Tris with the final mitochondrial pellet being resuspended in a minimal volume of KCl-Tris. Mitochondrial protein was determined in duplicate by the fluorescamine method (Nedergaard *et al.*, 1983) with BSA as the standard. Fluorescence was measured using a Turner Model 430 Spectroflurophotometer (Turner Instruments, Palo alto, CA) with an emission wavelength of 475nm and an excitation wavelength of 390nm. The mitochondrial suspension was then adjusted to a final protein concentration of 20mg/ml in KCl-Tris. Mitochondria for GDP binding, oxygen consumption, and membrane potential were stored on ice for not more than 2hr before use. Those used for the determination of UCP were frozen (-20°C) for later analysis.

7.3.3 Determination of Thermogenic Capacity

1) *GDP Binding*

GDP binding was determined as described by Sundin and Cannon (1980). Mitochondria at a final concentration of 1mg/ml were incubated in a basic medium containing 100mM sucrose, 20mM K-TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], 5µM rotenone and containing 10µM [8,5'- ^{3}H]GDP (specific activity 12.9 Ci/mmol). [^{14}C] Sucrose (specific activity 608 mCi/mmol) was included in order to

calculate the volume of medium trapped on the filter. EDTA (1mM) was present in all incubations to inhibit adenylate kinase activity. Duplicates of each mitochondrial suspension (20mg/ml protein) were prepared by the addition of 25µl of mitochondrial suspension to 400µl of the incubation buffer. 50µl Cold GDP (to give final concentrations of 5µM, 10µM, 30µM and 1mM) was added to the incubation medium to determine a GDP binding curve. Mitochondrial suspensions were incubated for 10min at room temperature and a pH of 7.1, after which samples of 400µl were taken from this cocktail and rapidly filtered by water pump suction through Sartorius (Gottingen, Germany) cellulose nitrate membrane filters (pore size, 0.45µm). The filter was added directly, without washing, to scintillation vials containing 5ml of scintillant (Emulsifier Scintillator Plus™, Packard Bioscience, Netherlands). Filters were left to completely dissolve, resulting in the disintegration of the mitochondria, enabling the samples to be counted for ^{3}H and ^{14}C. Radioactivity was measured using a Intertechnique Liquid Scintillation Counter SL4000 (Plaisir, France). GDP binding was calculated as the excess amount of [^{3}H]GDP found on the filter after correcting for trapped buffer by the use of [^{14}C] sucrose as an extramitochondrial marker.

2) *UCP Concentration*

Mitochondrial UCP concentration was measured by an immunoblotting procedure similar to that described by Herron *et al.*, (1990). The cervical BAT depots were quantitatively dissected out and

mitochondria isolated. Protein concentration was determined as described above, and the mitochondrial suspension diluted in sample buffer to give a 1:4 ratio of protein to sodium dodecyl sulphate (SDS). Mitochondrial proteins (20µg/lane) were separated by SDS-polyacrylamide gel (12%) electrophoresis and transferred to nitrocellulose membranes by Western blotting. 5µl of a molecular marker (Bio-Rad Kaleidoscope Prestained Standards) was also loaded onto the gel. Non-specific binding was quenched overnight with 1% milk solids. The membrane was incubated in a 1:1000 dilution of rabbit anti-rat UCP serum for 1 hour. Thereafter it was incubated in a 1:2000 dilution of the secondary antibody (anti-rabbit IgG Horse Radish Peroxidase linked) for an additional hour. The membrane was then incubated for 2 min using an accelerated luminescence kit (Amersham) and then prepared for development.

7.3.4 Determination of Thermogenic Activity

Proton conductance of the mitochondrial inner membrane was estimated from the relationship of mitochondrial oxygen consumption to membrane potential (i.e. slope of the curve of mitochondrial oxygen consumption against mitochondrial membrane potential for different GDP concentrations,

expressed as arbitrary units). Both membrane potential and oxygen consumption were measured at 33°C, which is similar to the T_b of naked mole-rats when housed at the optimal housing conditions of 30°C (Buffenstein and Yahav, 1991a).

1) Mitochondrial Membrane Potential

Mitochondrial membrane potential was measured in isolated mitochondrial suspensions (20mg protein/ml) using a dual-wavelength Aminco DW-2 UV/VIS Spectrophotometer with a slit width of 30nm and wavelengths 516nm (Mono1) and 495 (Mono2). 11µl of mitochondrial suspension was added to 1.1ml of buffer (see 7.3.5), except for the mouse sample where 5.5µl was added due to the inherent high mitochondrial activity. Substrate used was glycerol-3-phosphate for the BAT mitochondria and succinate for the liver mitochondria (5.5µl). A GDP dose response curve was determined by the serial addition of GDP to give final concentrations ranging from 0mM to 2.002mM GDP. Following this 5.5µl of the following were added in succession: 100mM ADP, 1mM atractylate and 1mM FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone).

2) Mitochondrial Oxygen Consumption

Mitochondrial oxygen consumption was determined polarographically using a Yellow Springs Instrument 4004 Clark Oxygen Probe calibrated with dithionite. Additions were identical to those used for the measurement of membrane potential, except that 1.1µl of 1mM FCCP was added instead of 5.5µl.

7.3.5 Buffers

Sample buffer for SDS-PAGE had the following composition: 62.5mM Tris, 4% SDS, 30% glycerol, and Bromophenol blue, pH 6.8. The buffer used for mitochondrial membrane potential and oxygen consumption had the following composition: 125mM sucrose, 20mM Tris pH 7.2, 2mM $MgCl_2$, 1mM EDTA and 0.1% fatty acid free BSA. The following additions were made to 1.1ml of the buffer: 5.5µl K_2PO_4, 5.5µl rotenone, 5.5µl substrate and 5.5µl rhodamine 123.

7.3.6 Chemicals

[^{14}C]sucrose and [^{3}H]GDP were purchased from Amersham Radiochemicals, England. Bovine serum albumin fraction V (fatty acid free) was from Pente (Miles, Kankakee, IL, USA). Fluorescamine (Fluram) was from Roche (Basel, Switzerland) and sucrose^{AR} was from AnalaR (BDH Chemicals, Poole, U.K.). Rotenone was from S.B. Penick & Co. (New York, NY). GDP was from Sigma Chemicals Co. (St. Louis, MO). All other chemicals were of *pro analysi* grade.

7.3.7 Statistical Analysis

All data in the text are presented as means and SEM. Kruskal-Wallis Nonparametric ANOVA tests were performed for appropriate data sets. Results were considered significant for $P<0.05$.

7.4 RESULTS

7.4.1 Gross Morphological and Quantitative Observations

Unlike laboratory mice where subscapular BAT deposits predominate, there was no well defined major BAT depot in the naked mole-rat. Although the cold-acclimated naked mole-rats had significantly greater ($P<0.01$) BAT per unit body mass compared to cold-acclimated mice, prolonged cold exposure did not induce an augmentation of BAT per unit body mass ($P>0.05$; Table 7.1).

7.4.2 BAT Thermogenic Capacity

1) *GDP Binding*

Naked mole-rat BAT showed similar GDP binding to that of cold-acclimated mice (Fig. 7.1). Cold-acclimation, however, did not increase GDP binding ($P>0.05$; Fig. 7.1).

Table 7.1. Effect of prolonged cold exposure on body mass and brown adipose tissue (BAT) for both cold- and thermoneutral-acclimated naked mole-rats (*Heterocephalus glaber*), as well as for cold-acclimated (4°C) mice.

Parameter	CA NMR (25°C)	TNA NMR (30°C)	CA Mouse (4°C)
Body Mass (g)	34.6 ± 2.2	37.6 ± 1.7	45.1 ± 0.7
BAT Mass (mg)	641 ± 26 **	436 ± 23	278 ± 14 **
BAT Wet Mass, % Body Mass	1.88 ± 0.15 **	1.16 ± 0.07	0.62 ± 0.03 **

Values are means ± SEM for 4 pooled preparations (8 animals) in each group. Differences between groups were evaluated by Kruskal-Wallis Nonparametric ANOVA tests. CA = cold-acclimated; TNA = thermoneutral-acclimated; NMR = naked mole-rat. ** indicates $P<0.01$.

2) *UCP Concentration*

The positive control (mice acclimated to 4°C) showed a strong band at 32kD's, corresponding to UCP. A corresponding weak band was seen for both naked mole-rat groups (Fig. 7.2, although these did not show up well upon photocopying), indicating similar degrees of reactivity and concentration in these two groups. However, the weakness of these bands may reflect a lack of specificity of the rabbit anti-rat UCP antibody to naked mole-rat UCP, cross reactivity with some proteins of higher molecular weight, or incomplete separation of the UCP dimer.

7.4.3 BAT Thermogenic Activity

Cold-acclimation resulted in markedly higher (52%) BAT mitochondrial oxygen consumption in naked mole-rats ($P<0.05$), however these rates were considerably lower than that of cold-acclimated mice ($P<0.01$; Fig. 7.3a).

Mitochondrial proton conductance (measured from the slope of the curve of mitochondrial oxygen consumption against mitochondrial membrane potential, units: $nmolO_2/mg/min/mV$) was also significantly greater in mice than in naked mole-rats ($P<0.01$; Fig. 7.3c). Cold-acclimated naked mole-rats showed a 55% increase in mitochondrial proton conductance (2.32 ± 0.17) compared to that of the thermoneutral-acclimated control group (1.50 ± 0.05; $P<0.01$; Fig. 7.3c).

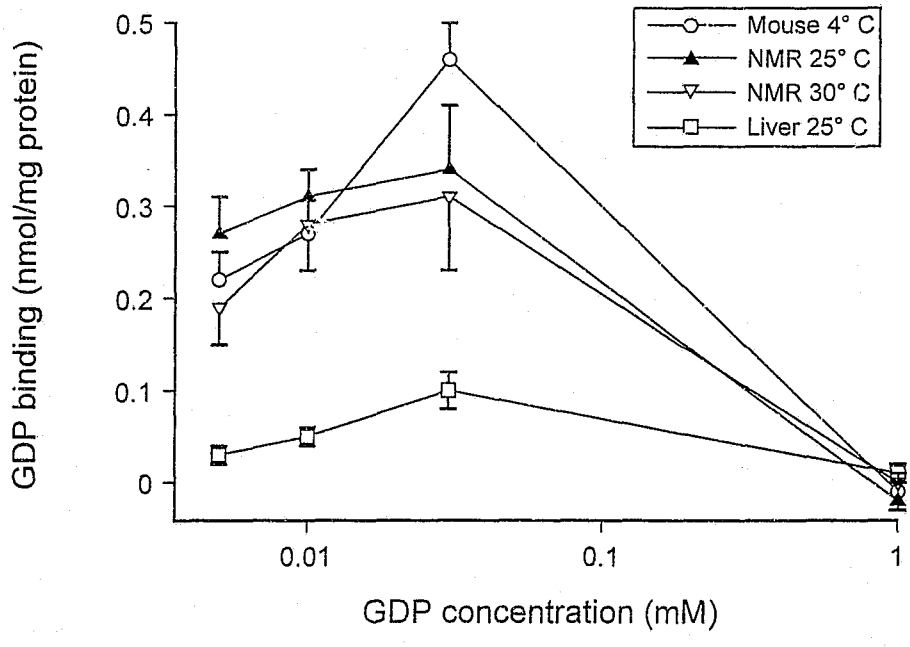


Figure 7.1 Effects of cold-acclimation on brown adipose tissue mitochondrial GDP binding in naked mole-rats. Brown adipose tissue from cold-acclimated mice (4°C), and liver from cold-acclimated naked mole-rats (25°C) served as the positive and negative control, respectively. NMR = naked mole-rat.

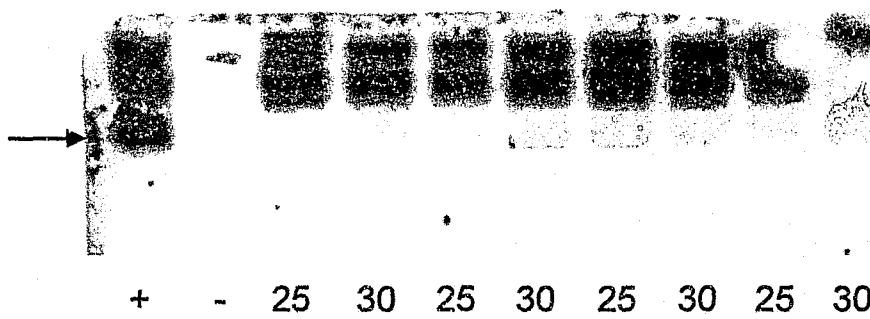


Figure 7.2 Western blot analysis of UCP in BAT from naked mole-rats (*Heterocephalus glaber*). Positive control (+) = cold-acclimated mice (4°C). Negative control (-) = liver from cold-acclimated naked mole-rat. 25 = cold-acclimated naked mole-rats (25°C). 30 = thermoneutral-acclimated control naked mole-rats (30°C). Arrow indicates 32 kD band corresponding to UCP.

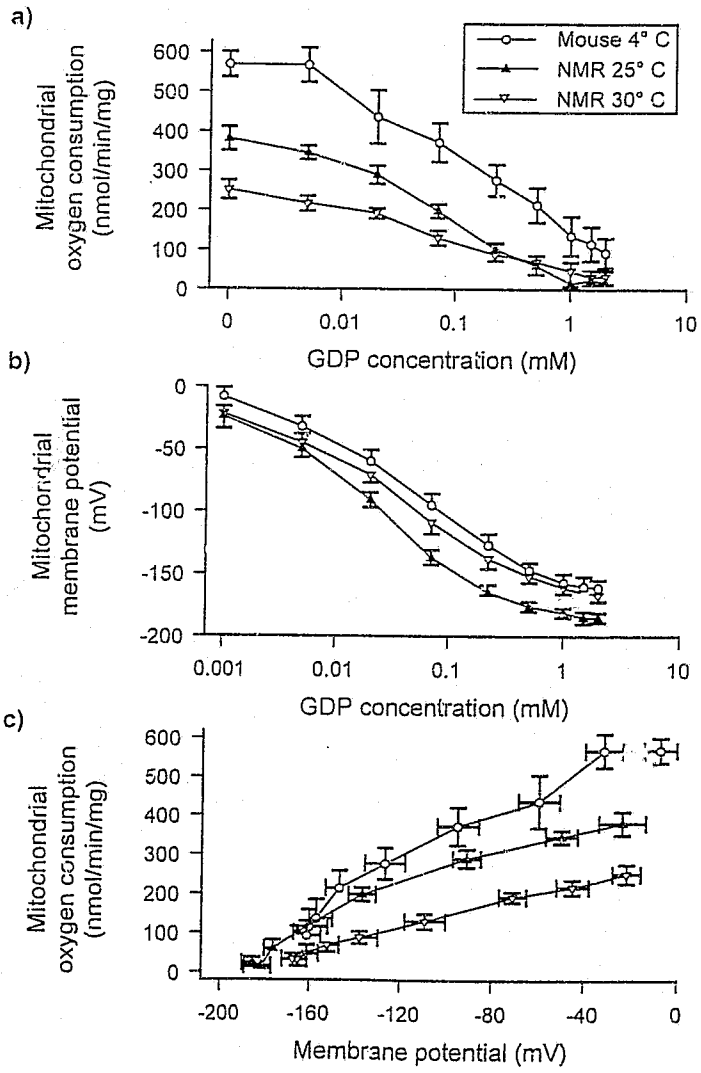


Figure 7.3. Changes in brown adipose tissue (BAT) thermogenic activity following addition of GDP with cold-acclimation in naked mole-rats (*Heterocephalus glaber*) and mice: a) GDP-sensitive mitochondrial oxygen consumption, b) GDP-sensitive mitochondrial membrane potential, and c) GDP-sensitive mitochondrial proton conductance. NMR = naked mole-rat. Data are means and SEM from 8 animals in each group.

7.5 DISCUSSION

In naked mole-rats, BAT mass regardless of acclimation temperature was similar, although cold-acclimated mole-rats showed significantly greater BAT mass per gram body mass compared to mice, whilst that of thermoneutral-acclimated naked mole-rats was similar to that of cold-acclimated mice. Clearly, unlike most small mammals (e.g. mouse, Ashwell *et al.*, 1983; hamster, Sundin *et al.*, 1987; rat, Trayhurn *et al.*, 1987), cold-acclimation does not increase BAT thermogenic capacity in this rodent. However, BAT thermogenic activity as indicated by pronounced increases in mitochondrial oxygen consumption and proton conductance was enhanced.

An organisms ability to acclimate to cold and increase its thermogenic capacity is usually considered to be highly adaptive (Cossins and Bowler, 1987; Mangum and Hochachka, 1998; Huey *et al.*, 1999). Not surprisingly therefore, the most pronounced changes occur in animals encountering extreme temperature fluctuations and prolonged periods of severe cold. In their natural equatorial habitat, living in thermally buffered burrows, naked mole-rats do not encounter extreme temperature fluctuations. Although the observed lack of a cold-acclimatory thermogenic response could be attributed to their evolutionary history inhabiting a thermally stable subterranean milieu for millennia (Lavocat, 1978), even a 2-3°C drop in T_a is considered an extreme cold stress for this animal (see section 6.2). A more

likely explanation for the unchanged thermogenic capacity therefore, is that this capacity is maintained at maximal levels, even when naked mole-rats are housed at thermoneutrality.

Given the poor and ineffective insulation of naked mole-rats (Daly and Buffenstein, 1998), even at temperatures slightly below thermoneutrality, heat loss is considerable and as such these unfurred animals are permanently primed for thermogenesis. The implications of BAT thermogenic capacity constantly kept at a maximum is that it would allow the naked mole-rat to regulate its T_b more efficiently in the context of its naturally warm burrow environment and social lifestyle (Yahav and Buffenstein, 1991b). This response of naked mole-rats is similar to that shown by some hibernators, such as the Richardson's ground squirrel, whose BAT thermogenic capacity is also kept constantly primed for maximal thermogenesis (Milner *et al.*, 1989).

The unchanged thermogenic capacity (as indicated by morphological and biochemical measurements in this study), together with similar noradrenaline-induced peak metabolism in both cold-acclimated and control naked mole-rats (Chapter 6), imply that these animals are constantly primed for thermogenesis at the maximal level these mammals can sustain. Similar peak rates of metabolism with prolonged cold, pregnancy, lactation and experimentally-induced NST activity (Buffenstein and Yahav, 1991a; Hislop and Buffenstein, 1994; Urison and Buffenstein, 1995; Chapters 6 and 9), infer that a metabolic ceiling exists in

naked mole-rats. This may be constrained by other physiological and morphological features, impeding further metabolic adjustments. These findings concur with previous reports of maximal sustainable metabolic rates with chronic cold exposure as well as during pregnancy and lactation (Nagy, 1987; Hammond *et al.*, 1994; McDevitt and Speakman, 1994; Hammond and Diamond, 1997).

Uncoupled BAT mitochondrial oxygen consumption of naked mole-rats, regardless of ambient housing conditions was considerably lower (cold-acclimated, 66%; control, 44%) than that of cold-acclimated laboratory mice (Fig. 7.3a). This lower thermogenic activity reflects their very low basal metabolic rates (*ca.* 66% of that expected allometrically for burrowing mammals; Buffenstein and Yahav, 1991a), low T_b and fossorial lifestyle (McNab, 1979). Uncoupled BAT mitochondrial oxygen consumption, and thus heat production, was significantly greater (52%, $P<0.01$) in the cold-acclimated naked mole-rats compared to the control group (Fig. 7.3a). This finding concurs with the recorded increased (50%, Chapter 9) basal metabolism in cold-acclimated naked mole-rats. The observed increase in BAT mitochondrial thermogenic activity in cold-acclimated naked mole-rats can be attributed entirely to the dissipation of the respiratory gradient by UCP as the addition of GDP completely inhibited mitochondrial respiration. Other cold-acclimated mammals also show considerably higher BAT thermogenic activity, but with concurrent increases in both UCP concentration and GDP

binding (e.g. hamsters; Sundin *et al.*, 1987) augmenting thermogenic capacity.

In conclusion, BAT thermogenic capacity of naked mole-rats is maximal and not enhanced with cold-acclimation, but thermogenic activity is increased with concomitant increased heat production. As such, naked mole-rats show a typical acute response to prolonged cold exposure, rather than the expected cold-acclimatory thermogenic response. These anomalous findings with prolonged cold exposure further support previous speculations that even at warm temperatures, thermogenic capacity is maximally primed to counteract the high rates of heat loss in these hairless, thermally labile mammals. The energetic cost of a sustained increase and utilization of BAT thermogenic capacity is prohibitive and this may be constrained by other physiological and morphological systems. From an evolutionary perspective, as naked mole-rats have inhabited a thermally stable milieu for millennia, although capable of physiologically adjusting to acute cold-stresses, they have not evolved the mechanisms characteristic of cold-acclimation.

binding (e.g. hamsters; Sundin *et al.*, 1987) augmenting thermogenic capacity.

In conclusion, BAT thermogenic capacity of naked mole-rats is maximal and not enhanced with cold-acclimation, but thermogenic activity is increased with concomitant increased heat production. As such, naked mole-rats show a typical acute response to prolonged cold exposure, rather than the expected cold-acclimatory thermogenic response. These anomalous findings with prolonged cold exposure further support previous speculations that even at warm temperatures, thermogenic capacity is maximally primed to counteract the high rates of heat loss in these hairless, thermally labile mammals. The energetic cost of a sustained increase and utilization of BAT thermogenic capacity is prohibitive and this may be constrained by other physiological and morphological systems. From an evolutionary perspective, as naked mole-rats have inhabited a thermally stable milieu for millennia, although capable of physiologically adjusting to acute cold-stresses, they have not evolved the mechanisms characteristic of cold-acclimation.

CHAPTER 8

COLD-INDUCED CHANGES IN THYROID FUNCTION IN A POIKILOTHERMIC MAMMAL, THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*)

8.1 ABSTRACT

Cold-acclimation induces very divergent responses in thyroid function in reptiles and mammals, reflective of their different modes of thermoregulation. Naked mole-rats, unlike other mammals, are unable to effectively employ endothermy and are thus operatively poikilotherms. I therefore investigated changes in thyroid status with chronic cold exposure in the naked mole-rat. At ambient temperatures simulating burrow conditions, free thyroxine (T_4; 0.39 ± 0.09ng/dl) and thyroid stimulating hormone (TSH; 1.12 ± 0.56μU/ml) levels were in the same range as that of reptiles, an order of magnitude lower than that reported for most small mammals. However, the response of naked mole-rats to chronic cold was typically mammalian; free T_4 levels (0.55 ± 0.09ng/dl) and thyroid follicular cell height were significantly greater in the cold. No significant elevation in TSH (1.28 ± 0.83μU/ml) levels was found, although adenohypophyseal thyrotrophs exhibited ultrastructural signs of increased secretory activity. Low thyroid hormone concentrations may contribute substantially to the unusual thermoregulatory mode exhibited by naked mole-rats.

8.2 INTRODUCTION

Across all phylogenetic groups, organisms adjust their physiological responses to overcome the direct effects of prolonged exposure to varying environmental temperatures (Hochachka and Somero, 1984; Leroi *et al.*, 1994; Kingsolver and Huey, 1998). In response to extreme cold, terrestrial vertebrates may employ winter dormancy (Gregory, 1982; Cossins and Bowler, 1987; French, 1993) or defend body temperature (T_b). The precise response may be limited by physiological or morphological constraints impeding physiological adjustments. In response to cold, endotherms defend T_b by behavioural thermoregulation, augmented insulation, thyroid hormone secretion and enhanced heat production (Huey, 1982; Carneheim *et al.*, 1984; Tomasi, 1991; Geiser, 1993). Thyroid hormones play a pivotal role in this regard, and are critical in the central regulation of T_b, stimulating thermogenesis and regulating cellular metabolism (Seitz *et al.*, 1985).

The thyroid gland is structurally conserved in all vertebrates (Norris, 1985). Furthermore, regardless of gross morphological differences across the phyla, follicular structure and function are similar. Responses of the thyroid gland to environmental cues are however considerably different across the animal kingdom (Else and Hulbert, 1987). Although there are exceptions to the rule, homeotherms generally increase thyroid activity at low

temperatures (Denckla and Marcum, 1973; Kennedy *et al.*, 1986; O'Riordan *et al.*, 1988; Gordon, 1993), whereas in poikilotherms, thyroid activity is higher during exposure to warmer conditions (Pandey and Munshi, 1976; Buffenstein and Louw, 1982; Firth and Turner, 1982; Norris, 1985).

Most small mammals respond to cold exposure by augmenting thyroid mediated processes (Denckla and Marcum, 1973; O'Riordan *et al.*, 1988; Gordon, 1993). These increase basal metabolism and heat production (Wunder, 1979; Whitaker *et al.*, 1990; Wunder and Gettinger, 1996). Poikilotherms on the other hand, generally show a decrease in both thyroid activity and metabolism in the cold (Buffenstein and Louw, 1982; Firth and Turner, 1982). There are however reports of reptiles which maintain higher levels of thyroid activity during cool periods (Evans and Hegre, 1938; Millar, 1955; Lynn, 1970; Mason, 1977).

Naked mole-rats exhibit an unusual mode of thermoregulation in that they are both endothermic, capable of employing nonshivering thermogenesis (Hislop and Buffenstein, 1994) and poikilothermic, in that T_c tracks that of ambient temperature (T_a) (Buffenstein and Yahav, 1991a). Their unusual mode of thermoregulation is considered highly suited to a strictly subterranean existence (Buffenstein, 1996). Subterranean rodents live in an environment where both gas and heat exchange are

markedly restricted. In response to these environmental stresses, most subterranean rodents exhibit low metabolic rates and relatively low T_b's (Nevo, 1999). Naked mole-rats have taken this trend to the extreme and exhibit the lowest mass specific metabolic rate for mammals (McNab, 1979; Withers and Jarvis, 1980; Buffenstein and Yahav, 1991a; Goldman *et al.*, 1999). Indeed, metabolic rates of these hairless mammals are similar to that reported for reptiles of similar body mass (Goldman *et al.*, 1999) and not surprisingly, with such low rates of heat production and limited insulation, naked mole-rats are unable to regulate T_b and are effectively poikilothermic (Buffenstein and Yahav, 1991a).

Given the anomalous and unique thermoregulatory mode of naked mole-rats, I questioned whether thyroid function would follow typical mammalian or reptilian trends in response to cold-acclimation. The following set of competing hypotheses was therefore proposed for investigation: 1) thyroid gland activity and hormone secretion are elevated in response to prolonged cold exposure, thus conforming to mammalian norms, or 2) being poikilothermic, the naked mole-rat exhibits decreased thyroid activity in response to prolonged cold exposure. In testing this set of competing hypotheses I measured T_c, free thyroxine (T_4) and thyroid stimulating hormone (TSH) concentrations, as well as the distribution and ultrastructure of pituitary thyrotrophs, and thyroid gland

histomorphology in two groups of naked mole-rats housed for at least 18 months at either 25°C or 30°C.

8.3 MATERIALS AND METHODS

8.3.1 Animal Care and Maintenance

Non-breeding pairs of naked mole-rats were housed in standard laboratory rat cages; only the males being utilized for this study. Perspex tubing was provided in which to burrow and sawdust served as bedding. Animals were fed an *ad lib.* diet of apple and sweet potato as well as receiving a high energy, protein rich and vitamin supplemented cereal (Pronutro, Becketts, RSA).

8.3.2 Thermal Acclimation

Naked mole-rats were housed at an T_a of 25 ± 1°C for a period exceeding 18 months. A control group were maintained at their preferred housing temperature (30 ± 1°C) which is identical to that experienced in their natural habitat (Jarvis, 1991b). At both housing temperatures relative humidity was maintained above 60%. The lower T_a (25°C) was selected as this represents a significant cold stress for these poorly insulated mammals.

8.3.3 Body Temperature Measurements

A calibrated copper-constantan thermocouple was inserted into the rectum to a depth of approximately 2cm and the T_b

recorded to within 0.1°C via a digital display (Model-Bat-12, Physitemp, New York, USA).

8.3.4 Thyroid Function Assessment

Thyroid function was assessed by both histological and endocrinological methods. Animals were anaesthetized by halothane (Fluothane, Zeneca, RSA) inhalation and killed by cardiac exsanguination.

Thyroid Hormone Analysis

Blood from 9 cold-acclimated naked mole-rats and 6 control animals was collected in heparinized syringes and immediately centrifuged at 3000rpm for 5 min. The plasma fraction was stored at -70°C for later analysis of TSH and free T_4 levels by standard radio-isotopic assays (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

Histological Investigations

The thyroid glands of 3 naked mole-rats from each group were carefully dissected out, fixed in 10% buffered formalin, and routinely processed for light microscopy. Serial sections (5μm) were cut in the transverse plane and stained with haematoxylin and eosin. A Flexible Image Processing System (FIPS; Council for Scientific and Industrial Research, RSA) linked to a Leitz Laborlux II light microscope was used for the determination of follicular cell height. For each lobe, a 400X magnification area was selected for the most active and

least active follicles and the follicular cell height of 8 well nucleated cells, with distinct cell borders, counted in all four compass planes. This procedure was followed for every tenth section.

Immunocytochemistry

The pituitary glands of 6 naked mole-rats from each group were removed, fixed in Bouins fixative, routinely processed and embedded in paraffin wax. Serial sections of each pituitary were cut at 5μm through the entire gland. Sections from each animal were selected from five levels of the gland, from the ventral to dorsal surface, and immunostained for TSH cells (thyrotrophs) using a modification of the streptavidin-biotin technique. Sections were dewaxed in xylene and treated with 3% hydrogen peroxide in methanol for 20 minutes. They were then incubated in 10% rabbit serum for 30 minutes, followed by incubation with rabbit anti-rat TSH (UCB Pharmaceuticals) at a concentration of 1:4000 in phosphate buffered saline (PBS) for 1 hour. Following rinsing in PBS, sections were incubated with a biotinylated goat anti-rabbit IgG (1:200; Amersham, Weil) for 40 minutes. After further rinsing in PBS, sections were incubated with a streptavidin-biotin peroxidase complex (1:300; Amersham, Weil) for 20 minutes. All incubations were conducted on a hot plate at 37°C. The peroxidase complex was revealed with 3,3' diaminobenzidene hydrochloride (DAB, Merck, Johannesburg). Immunocytochemistry controls for each batch

of sections stained were as follows: a) the primary and secondary antisera were replaced with horse serum or buffer, and b) absorption control: increasing concentrations of TSH were absorbed with a 1:8000 dilution of anti-TSH.

Electron Microscopy

The pituitary glands from 3 naked mole-rats from each group were fixed in 2.5% glutaraldehyde-PBS solution. The tissue was post-fixed in 1% osmium tetroxide, dehydrated in increasing concentrations of alcohol and cleared in propylene oxide. The tissue was then infiltrated and embedded in epon-araldite resin and polymerised at 60°C for 48 hours. The thin-semithin serial sectioning technique was used to cut 0.1µm sections followed by 1µm sections. Resin was removed in the 1µm sections by placing them into sodium ethoxide. These sections were then stained with the streptavidin-biotin technique to demonstrate TSH cells. Gold sections were mounted on grids and routinely stained with uranyl acetate and lead citrate. TSH cells that were immunostained on semi-thin sections were then matched on the gold sections and viewed with a JEOL 100S transmission electron microscope at 80kV.

8.3.5 Statistical Analysis

Data are presented as means and standard deviations. Appropriate data sets were analysed using unpaired T-tests. Results were considered significant for $P<0.05$.

8.4 RESULTS

8.4.1 Body Temperature

Cold-acclimated animals had significantly lower T_b's (31.1 ± 1.7°C) than the control group (33.1 ± 0.6°C; P=0.0281), but the temperature differential (T_b - T_a) was much greater for the cold-acclimated animals (6.1°C) compared to that of the control group (3.1°C).

8.4.2 Endocrine Status

The cross-reactivity and specificity of the TSH assay for naked mole-rat TSH is not known, hence TSH-like levels are eluded to. Concentrations of TSH varied considerably within each group and were not significantly different between the two groups (Table 8.1). Cold-acclimated animals had 40% higher free T_4 levels (0.55 ± 0.09 ng/dl; n=9) than the control group (0.39 ± 0.09 ng/dl; n=6; P=0.0056; Table 8.1).

8.4.3 Thyroid Gland Histology

Follicular cell height was significantly greater in the cold-acclimated animals for both the active (P<0.001) and inactive areas (P<0.01) of both lobes (Table 8.1). The follicular cells of the cold-acclimated animals were often binucleated, the gland being distinguished by dense areas of follicular cells with very little colloid (Plates 8.1 and 8.2).

Table 8.1. Cold-induced changes in thyroid stimulating hormone (TSH), free thyroxine (T_4) and thyroid morphology in naked mole-rats. FCH = follicular cell height.

	Cold-Acclimated (25°C)	Control (30°C)	Percent Change	P Value
FCH (μm) (inactive)	8.523± 1.504	6.805± 1.238	25	<0.0001
FCH (μm) (active)	14.627± 3.344	9.476± 2.065	54	<0.0001
TSH (μU/ml)	1.28±0.83	1.12±0.56	14	0.6842
Free T_4 (ng/dl)	0.55±0.09	0.39±0.09	41	0.0056

Plate 8.1. Thyroid gland histology of A) thermoneutral-acclimated (30°C) control naked mole-rats, and B) cold-acclimated (25°C) naked mole-rats showing increased follicular cell height and reduced colloid volume with cold-acclimation. Sections were stained with haematoxylin and eosin. 10mm scale bar is equivalent to 20μm.

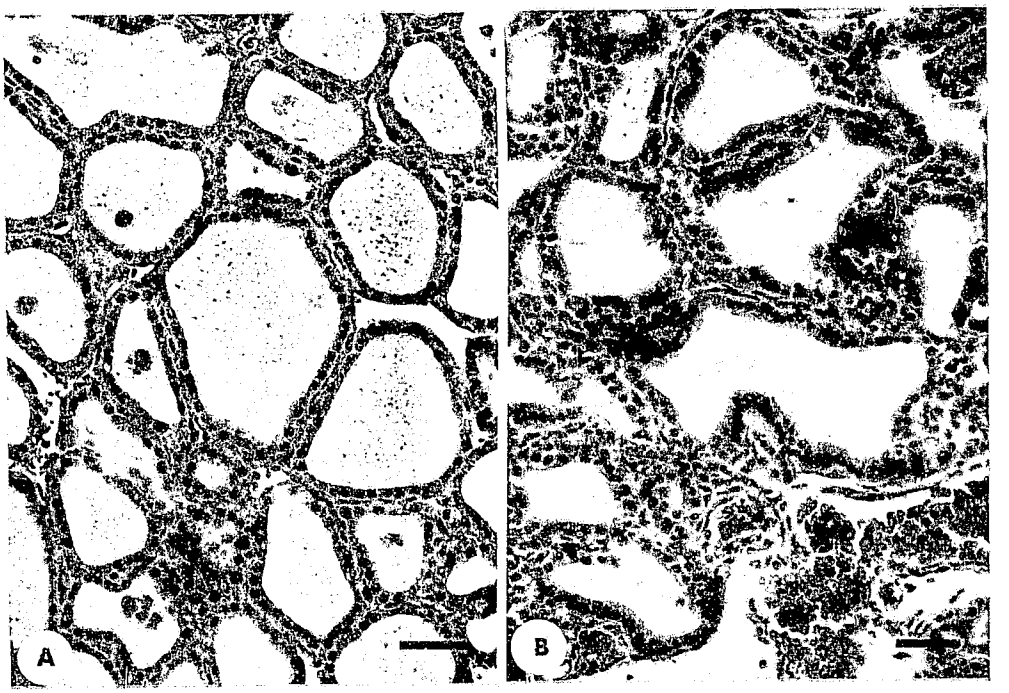
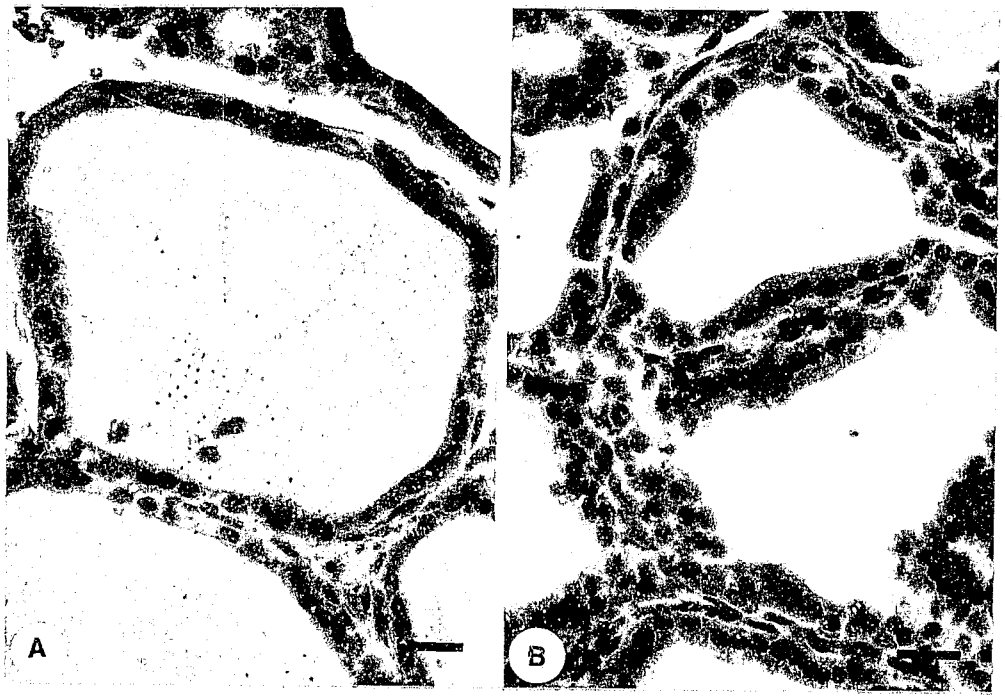

A
B

Plate 8.2. Thyroid gland histology of A) thermoneutral-acclimated (30°C) control naked mole-rats, and B) cold-acclimated (25°C) naked mole-rats showing increased follicular cell height and a relative abundance of binucleated follicular cells in the cold. Sections were stained with haematoxylin and eosin. 10mm scale bar is equivalent to 12μm.


A
B

8.4.4 Immunocytochemistry

Distribution of thyrotrophs within the adenohypophysis appeared similar in both groups, and appeared to be more concentrated in the middle region of the gland, with fewer cells in the more ventral and dorsal regions (Plate 8.3). The cells ranged from circular to spindle shaped, all with eccentrically placed nuclei. They were often found paired or in clusters closely associated with capillaries. Incubation with rabbit serum or buffer as replacement for rabbit anti-rat TSH showed no specific staining. The TSH antigen abolished staining at a concentration of 20μg/ml.

8.4.5 Electron Microscopy

Immuno-identified thyrotrophs in cold-acclimated animals had a large nucleus which was eccentrically situated (Plate 8.4). The membrane bound secretory granules were clustered mainly towards the periphery of the cell, close to the plasmalemma. There were numerous mitochondria and extensively dilated rough endoplasmic reticulum (RER) cisternae (Plate 8.4). Thyrotrophs in the control animals also had large eccentrically placed nuclei, but the secretory granules were scattered throughout the cytoplasm, closely associated with dilated RER cisternae. Numerous mitochondria were also present (Plate 8.4).

Plate 8.3. Immunocytochemical detection (streptavidin-biotin) of adenohypophyseal thyrotrophs, A) middle region of the adenohypophysis, and B) dorsal/ventral region of the adenohypophysis. 10mm scale bar is equivalent to 18μm.

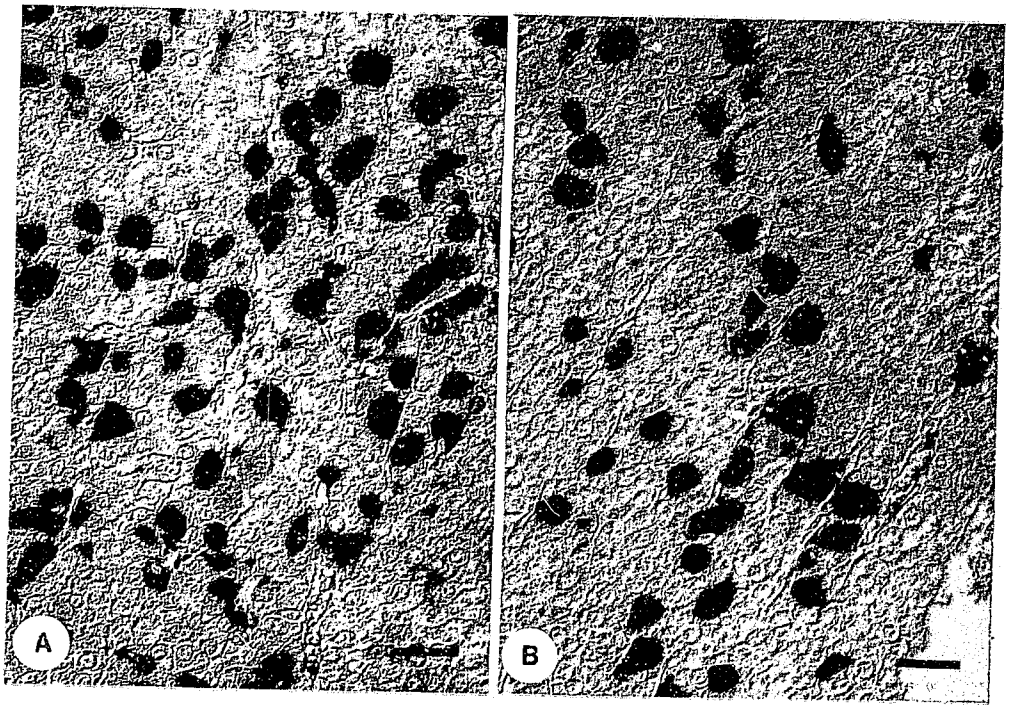
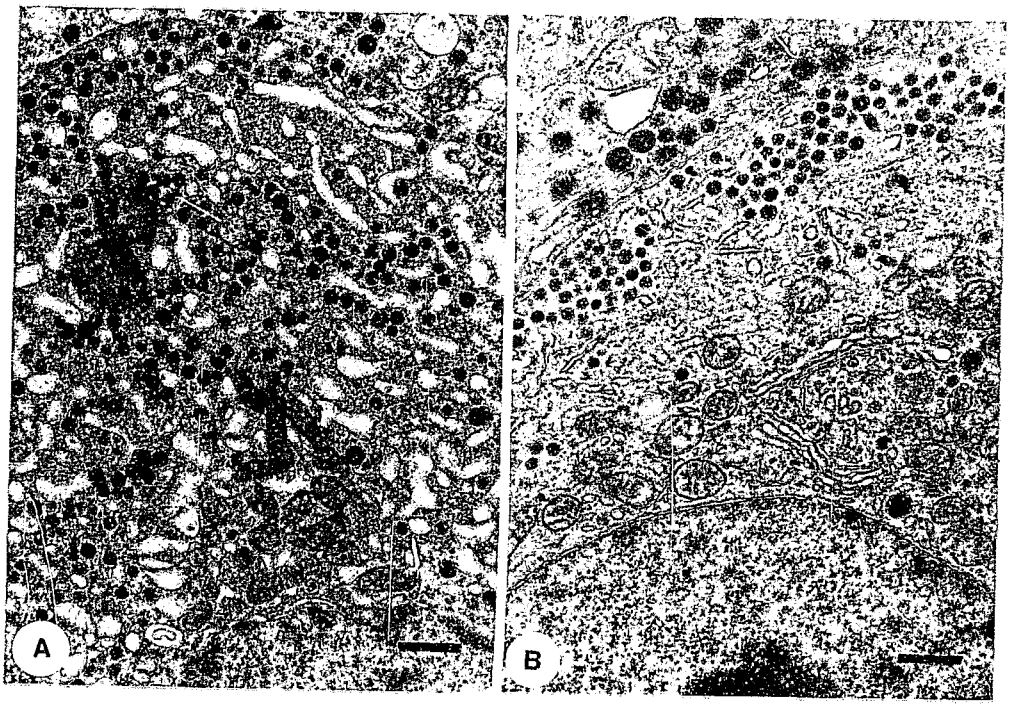

A
B

Plate 8.4. Ultrastructure of immuno-identified thyrotrophs in the adenohypophysis of A) thermoneutral-acclimated (30°C) control naked mole-rats with evenly dispersed secretory granules, and B) cold-acclimated (25°C) naked mole-rats showing peripherally located secretory granules closely associated with the plasmalemma. 10mm scale bar is equivalent to 1μm.


A
B

8.5 DISCUSSION

Thyroid hormones have a major influence on metabolism, lipogenesis and lipolysis (Hulbert, 1978; Bianco *et al.*, 1988; Oppenheimer *et al.*, 1991; Tomasi, 1991), facilitating the plasticity of phenotypic responses to changing ambient conditions (Hulbert, 1978; Wang, 1989). While the thyroid gland is structurally conserved in all vertebrates, responses of the thyroid gland and concomitant hormone concentrations are considerably different across the animal kingdom. Assuming that 0.05% of T_4 remains unbound (i.e. free T_4), the total T_4 concentrations for naked mole-rats would range between 7.8ng/ml (control) and 11.0ng/ml (cold-acclimated). Thyroid hormone concentrations of naked mole-rats (Table 8.1), in keeping with their poikilothermic mode of thermoregulation, are in a similar range to that reported for reptiles (Bona-Gallo *et al.*, 1980; Hulbert and Else, 1981). Indeed, both free and total thyroid hormone concentrations are an order of magnitude lower than that reported for small mammals (Hulbert and Else, 1981; John-Alder, 1983). Similarly, TSH levels are also an order of magnitude below those of other small mammals (see, Hefco *et al.*, 1975). These low concentrations of metabolic regulatory hormones correlate well with the very low basal metabolism of these mammals and no doubt contribute substantially to the markedly reduced cellular heat production and poikilothermic profile of T_b with changing

T_a.

Contrary to most poikilotherms which show decreased thyroid activity in the cold, naked mole-rats conform to general mammalian trends by increasing thyroid activity, with a concomitant elevation in free T_4 serum concentrations. The 40% increase in free T_4 levels is of similar magnitude to the observed changes in basal metabolism (38-50%; Chapters 6 and 9), suggesting that the increase in thyroid activity is intimately linked to the change in basal heat production and the concomitant increased temperature differential (T_b-T_a). Thyroid responses of other small mammals to prolonged cold exposure vary considerably with the extent of other physiological and morphological changes, and also with the intensity of cold exposure and rate of change in ambient conditions (Gordon, 1993). Whilst thyroid activity in mammals is inversely dependent upon T_a, in contrast, the activity of the reptilian thyroid gland is directly dependent on T_a (Firth and Turner, 1982). Exceptions to these generalizations have been noted (Gordon, 1993) and indeed some species of winter-active reptiles respond in a similar manner and magnitude to naked mole-rats and show greater thyroid activity in winter than in summer (Binyon and Twigg, 1965; Lynn, 1970; Mason, 1977).

Changes in thyroid gland activity are regulated by the hypothalamic-pituitary axis and one would expect a

concomitant change in TSH concentration and anterior pituitary activity, which was not immediately obvious for TSH concentrations were unchanged. The observed absence of a significant increase in TSH levels in the cold may be explained by its short half-life (50-80 min; Toft, 1991) and the pulsatile nature of its release (Brabant *et al.*, 1986; Greenspan *et al.*, 1986; Brabant *et al.*, 1990). These contributing factors resulted in a large variation in TSH levels and similar mean values. However, thyrotrophs of cold-acclimated animals had numerous secretory granules concentrated at the periphery of the cell, suggesting enhanced secretory capacity and/or frequency (Plate 8.4). Similar thyrotrophic morphology has been noted in hypothyroid mice where TSH concentrations are raised in response to the lack of inhibitory regulation via negative feedback mechanisms (Noguchi *et al.*, 1986; Noguchi *et al.*, 1995). Other evidence of increased TSH production is provided by the dilated RER cisternae and changes in TSH-mediated thyroid gland morphology. Follicular cell height, an indicator of thyroid activity (Bloom and Fawcett, 1975; Gershon and Nunez, 1988), was significantly greater in the cold-acclimated animals (Table 8.1, Plates 8.1 and 8.2). In addition, follicular cells of the cold-acclimated animals were often binucleated, and the colloid volume was markedly reduced (Plates 8.1 and 8.2).

Despite showing a typical mammalian response to cold exposure by augmenting thyroid hormone concentrations and

presumably thyroid mediated metabolic activities, there was no evidence for improved T_b regulation. This is attributed to the high thermal conductivity of the naked mole-rat integument which lacks a functional hypodermis (Buffenstein and Yahav, 1991a; Daly and Buffenstein, 1998). Nevertheless, the temperature differential (T_b - T_a) for the cold-acclimated animals (6.1°C) was twice that of the control group (3.1°C) due to increased thyroid-mediated thermogenesis. Maintenance of an elevated temperature differential may be important to this poikilothermic mammal, where physiological function (e.g. gut activity, pregnancy) and locomotion are temperature dependent. The 40% elevation in free T_4 levels may be intimately linked to the 38-50% increase in BMR and the lowering of the apparent "thermoneutral zone" (Chapters 6 and 9). As such minimal metabolism can be maintained at a lower range of T_a's, thus facilitating an enormous energy saving.

In conclusion, thyroid hormone concentrations are of a similar order of magnitude to that reported for other poikilotherms. Nevertheless, these rodents exhibit typical mammalian cold-acclimation responses with increased thyroid activity and basal metabolism under cooler conditions.

CHAPTER 9

COLD-ACCLIMATION IN A POIKILOTHERMIC MAMMAL, THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*); EFFECTS ON THERMOREGULATION AND ENERGY BALANCE

9.1 ABSTRACT

Naked mole-rats (*Heterocephalus glaber*) are unusual mammals, in that they are both endothermic and poikilothermic, and consequently show both mammalian and reptilian features of cold-acclimation. Thermoregulatory shifts were investigated by monitoring changes in oxygen consumption, body temperature (T_b), and minimal thermal conductance (C_m) following acute exposure (3hr) to a range of ambient temperatures (T_a) from 13-36°C, in long term cold-acclimated (25°C; >18months) and thermoneutral-acclimated (30°C) control animals. Differences in regional skin temperatures, rates of cooling and reheating, body composition, food consumption and digestibility were also investigated. Peak resting metabolic rate was not increased with cold-acclimation (2.65 ± 0.23mlO_2/g/h, control; 2.01 ± 0.39mlO_2/g/h, cold-acclimated; P>0.05). However, basal metabolism (BMR) was 1.5-fold greater in the cold (0.78 ± 0.29mlO_2/g/h) compared to that of control animals (0.52 ± 0.07mlO_2/g/h), and was matched by a similar percent increment (45%) in food consumption. The augmented BMR together with decreased (42%) C_m, facilitated a left-shift of the thermoneutral zone from 32-34°C to a lower T_a range of 27-34°C. There was no change in energy balance, with percent body fat unaltered in the cold. Body temperature tracked T_a for both groups of naked mole-rats, although cold-acclimated animals exhibited greater T_b-T_a differentials with skin temperatures significantly lower

for all anatomical sites measured. The shivering threshold of control animals (28.2 ± 2.7°C) was significantly reduced with cold-acclimation (21.6 ± 0.6°C). Upon reheating, shivering intensity and frequency were greater in the control group with T_b rising faster. Moreover, cold-acclimated naked mole-rats selected a lower preferred T_b at higher T_a's. The observed thermoregulatory shifts facilitate an enormous reduction in energy expenditure allowing for the prolonged survival of these poikilothermic, poorly insulated mammals at the lower T_a encountered.

9.2 INTRODUCTION

Regardless of whether an animal is a bradymetabolic ectotherm, or a tachymetabolic endotherm, chronic cold exposure induces a suite of physiological, cellular, and behavioural responses that enable the animal to adjust to lower temperatures (Hochachka and Somero, 1984; Cossins and Bowler, 1987; Huey, 1991; Leroi *et al.*, 1994; Kingsolver and Huey, 1998). This plasticity of response is generally considered to be highly adaptive (Mangum and Hochachka, 1998; Huey *et al.*, 1999).

While poikilotherms can endure fluctuating thermal environments, tolerant of the concomitant effects on body temperature (T_b) and physiological function, homeotherms maintain a relatively constant T_b, albeit at high energetic

cost. Cold-acclimation in mammals essentially involves shifting the thermoneutral zone (TNZ) to lower ambient temperatures (T_a) (Scholander *et al.*, 1958; Bligh, 1973; Li and Tokura, 1997). This is facilitated by increasing basal metabolic rate (BMR) and/or augmenting insulation. Partial or complete compensation in response to changing T_a's may occur in some ectotherms also, as indicated by left and upward shifts of their metabolic-temperature curves, displaced along the T_a axis (Rieck *et al.*, 1960; White and Lasiewski, 1971; Eckert and Randall, 1983; Cossins and Bowler, 1987; Kingsbury, 1994). Most vertebrate ectotherms however, employ behavioural thigmothermy or heliothermy to raise T_b at low T_a's (e.g. lizards, Carrascal *et al.*, 1992; Martin *et al.*, 1995). In addition, many ectotherms are tolerant of lower T_b's and remain active during cold exposure, although physiological function (e.g. locomotory and digestive capacity) may be impaired (Kingsbury, 1994).

Prolonged cold exposure may impact considerably upon gut function and energy balance (Weiner, 1992; Carey, 1993; Hammond *et al.*, 1994; McDevitt and Speakman, 1994). In endotherms, the increased energy requirements of cold exposure necessitate increased food intake, facilitated by gastrointestinal hypertrophy and enhanced nutrient uptake capacity (Karasov and Diamond, 1985; McDevitt and Speakman, 1994; Zhao *et al.*, 1995; Hammond *et al.*, 1996). Gut function may however be rate-limiting, with compromised transit time resulting in a decrease in

digestibility and nutrient uptake, which may constrain maximal metabolic capacity (Nagy, 1987; Hammond *et al.*, 1994; McDevitt and Speakman, 1994; Hammond and Diamond, 1997). Under these situations, if energy expenditure exceeds energy intake, body reserves must be mobilized (see Stott and Slee, 1985). Ectotherms generally show the opposite trend with a decline in food consumption accompanying cold-acclimation (Buffenstein and Louw, 1982; Cossins and Bowler, 1987; McKinon and Alexander, 1999). However, in some poikilotherms, intestinal volume, enzyme activity, and the rate of absorption may be augmented, even at lower T_b's, to maintain transport capacities and digestive efficiency (Smit, 1967; Karasov *et al.*, 1985; Houpe *et al.*, 1996; McKinon and Alexander, 1999).

Naked mole-rats (*Heterocephalus glaber*) are intriguing animals in which to examine the effects of cold-acclimation. These hairless rodents, under simulated field conditions, have extremely low metabolic rates, amongst the lowest recorded allometrically for mammals (McNab, 1979; Withers and Jarvis, 1980; Buffenstein and Yahav, 1991a), and are akin to that recorded for lizards of similar body mass (Goldman *et al.*, 1999). In addition, although capable of employing endothermy (Hislop and Buffenstein, 1994), these mammals lack fur and a functional hypodermis (Daly and Buffenstein, 1998), and are thus unable to effectively regulate T_b. As such, T_b tracks T_a over the entire range of temperatures measured

(12-37°C), being 1-2° C above ambient (Buffenstein and Yahav, 1991a). Thus these mammals are operatively poikilotherms (Buffenstein and Yahav, 1991a). Endothermic heat production is evident over a narrow T_a range of 28-31°C, such that peak metabolic rates are attained at T_a's only 2-3°C below the apparent TNZ (as T_b is at no stage regulated; Buffenstein and Yahav, 1991a). Similar incremental changes in metabolism, relative to BMR, are seen in other laboratory rodents exposed to extreme cold-stress (e.g. hamsters at 5°C, Dicker *et al.*, 1995). At T_a's below 28°C, naked mole-rats abandon endothermy and oxygen consumption varies in a typical poikilothermic manner, increasing exponentially with increasing T_a. These mammals show no signs of regulated hypothermia (torpor or hibernation), and are unable to substantially raise T_b by endogenous heat production.

With woefully inadequate skin insulatory properties, compensatory increases in heat production may be both energetically prohibitive and ineffective. The latter may also be impeded by constraints on gut capacity, given their already high fractional absorption efficiencies (Buffenstein and Yahav, 1991c). Given the anomalous and unique thermoregulatory mode of the naked mole-rat, I questioned how they would respond to prolonged cold exposure and whether cold-acclimation would follow typical mammalian or reptilian trends. This was assessed by monitoring 1) T_a-dependent thermoregulatory profiles, 2)

rates of heating and cooling, 3) body composition, and 4) daily energy intake and digestive efficiency, in animals housed for prolonged periods (>18 months) at 25°C (cold-acclimated) and comparing these data with those from naked mole-rats maintained at their preferred housing temperature (30°C; Jarvis, 1991b).

9.3 MATERIALS AND METHODS

9.3.1 Animal Care and Maintenance

Naked mole-rats used in this study were born in captivity from progenitors collected in northern Kenya during 1980. Fifteen adult (32 ± 4g) male naked mole-rats were housed at 25 ± 1°C (>18 months) together with their mates in standard laboratory rat cages in which lengths of perspex tubing were provided for burrowing. A control group of six adult (32 ± 5g) males were similarly maintained together with their mates at their preferred housing temperature which is equivalent to that experienced in their natural habitat (30°C; Jarvis, 1991b). Relative humidity was maintained above 60% for both groups. Sawdust was provided for nesting material and animals were fed an *ad lib.* diet of fresh fruit and vegetables as well as a high energy, protein rich cereal (Pronutro, Becketts, RSA).

9.3.2 Oxygen Consumption Measurements

Oxygen consumption was measured using an open circuit system (Depocas and Hart, 1957). Animals were placed on a wire stand inside an air-tight glass respiratory chamber which was immersed in a constant temperature waterbath. A positive flow pump (Ametek flow control R-1) maintained an air flow rate of 100ml/min through the respiratory chamber. Air was dried *en route* before passing through an oxygen analyser (Ametek S-3A/ii; Sensor model N-22, Applied Electrochemistry, Pittsburgh). Flow rate was measured by the rate of movement of a soap film through a calibrated bubble tube (internal diameter = 23mm; volume 200ml) situated after the metabolic chamber.

Animals were subjected to brief exposure (3 hours) to T_a's ranging between 13-36°C. Animals were allowed to acclimate for 2 hours, followed by an hour of measurement. At T_a's below 18°C and above 34°C, the acclimation period was reduced to 1 hour. At least 3 days lapsed between different T_a measurements for each animal. At each T_a, the 12 lowest consecutive readings measured over a 4 minute period were used to calculate resting metabolic rate (RMR). Oxygen consumption values were corrected to STP and expressed as $mlO_2/g/h$. Ambient temperature was monitored using a calibrated copper-constantan thermocouple.

9.3.3 Body Temperature Measurements

Body temperature was measured by inserting a copper-constantan thermocouple 2-3cm into the rectum. The thermocouple was connected to a calibrated digital display (Model-Bat-12, Physitemp, New York, USA), and the T_b recorded once the reading had stabilized. All measurements were taken immediately after monitoring oxygen consumption.

9.3.4 Minimal Thermal Conductance

Minimal thermal conductance, which includes evaporative water loss, was calculated at T_a's below thermoneutrality from individual measurements of metabolic rate and T_b by applying the formula:

$$C_m = M/(T_b-T_a) \qquad \text{(McNab, 1980)}$$

where C_m is the minimal thermal conductance ($ml O_2/g/°C/h$) and M is the RMR ($ml O_2/g/h$).

9.3.5 Skin Temperature Measurements

Skin temperature of naked mole-rats at the two respective housing temperatures was measured at four anatomical sites, viz. subscapular, abdominal, tail and hindfoot. A flattened copper disc was soldered to the tip of a copper-constantan thermocouple and the disc held against the skin surface until the temperature had stabilized and the temperature recorded to within 0.1°C via a calibrated

digital display (Model-Bat-12, Physitemp, New York, USA).

9.3.6 Rates of Heating and Cooling

Five male naked mole-rats from both temperature groups were moved to a cold room (10°C) and their T_b recorded at 10 minute intervals for 40 minutes. Animals were then moved to a hot room (31°C) and T_b monitored at similar intervals until it had stabilized. Time and T_b at onset and termination of shivering were noted.

9.3.7 Body Composition Analysis

Body composition was determined for both cold-acclimated and control animals using an EM-SCAN Model SA-2 Small Research Animal Body Composition Analyser (Buffenstein *et al.*, 1996). Six to ten measurements were made and the average percent body fat and lean mass (g) recorded.

9.3.8 Food Consumption and Faecal Output Measurements

Food consumption was monitored over a 24 hour period for 7 consecutive days for 6 non-breeding naked mole-rats from both groups. A pre-weighed *ad lib.* supply of apple was added to each cage and a similar quantity kept as a desiccation control at the same temperature. All food and faecal samples were removed after 24 hours and dried to constant mass. After correcting for water loss, food consumption was calculated and expressed as grams dry mass (DM)/40g body mass (BM)/day. Energy content of food and faeces was determined by bomb calorimetry (dds CP500

Calorimeter, Digital Data Systems) and digestible DM energy consumption calculated as kJ/40g BM/day.

The proportion of assimilated energy (i.e. energy content of food - energy content of faeces) apportioned to BMR was also calculated for each group.

9.3.9 Statistical Analysis

All data are expressed as means ± SEM. For the oxygen consumption and T_b data sets, the T_a range was divided into 1°C intervals, and data points within each interval were averaged. Best fit curves for the relationship of T_b against T_a were determined (Tablecurve, Sigma). Data were compared by paired- and unpaired T-tests, and linear regression analyses where appropriate. Data were considered significantly different at $P<0.05$.

9.4 RESULTS

9.4.1 Oxygen Consumption

Ambient temperature dependent metabolic profiles for both groups of naked mole-rats showed the typical endothermic, poikilothermic pattern. Control group data were similar to that previously reported, with an apparent TNZ between 32-34°C (Buffenstein and Yahav, 1991a; 31-34°C) and low BMR's (0.52 ± 0.02mlO_2/g/h). Basal metabolism was 1.5-times greater (P=0.0207) in the cold-acclimated animals (0.78 ± 0.07mlO_2/g/h; Fig. 9.1). This resulted in a substantial

extension and left-shift of the apparent TNZ to a lower range of T_a's (27-34°C; Fig. 9.1). A left-shift of the poikilothermic component of the metabolic profile was also evident, with metabolism greater in the cold-acclimated animals at each test temperature below the peak resting metabolic rate (PRMR). This PRMR was surprisingly not increased with cold-acclimation (2.01 ± 0.22mlO_2/g/h, cold-acclimated; 2.65 ± 0.17mlO_2/g/h, control; P>0.05), but occurred at a lower T_a (20°C) compared to that of the control animals (25°C; Fig. 9.1). Consequently, the percent increase in metabolism (above basal levels) per degree Celsius drop in T_a was greater for the control animals (± 60%) than for the cold-acclimated group (± 25%).

9.4.2 Body Temperature

Body temperature tracked T_a for both cold-acclimated and control animals (Fig. 9.1; Table 9.1). Control animals exhibited greater T_b's (± 1°C higher) than their cold-acclimated counterparts over the T_a range of 19-26°C (Fig. 9.1), whereas T_b of cold-acclimated naked mole-rats was greater at low T_a's, but especially within their TNZ (27-34°C; 1-2°C higher than that of control animals; Fig. 9.1).

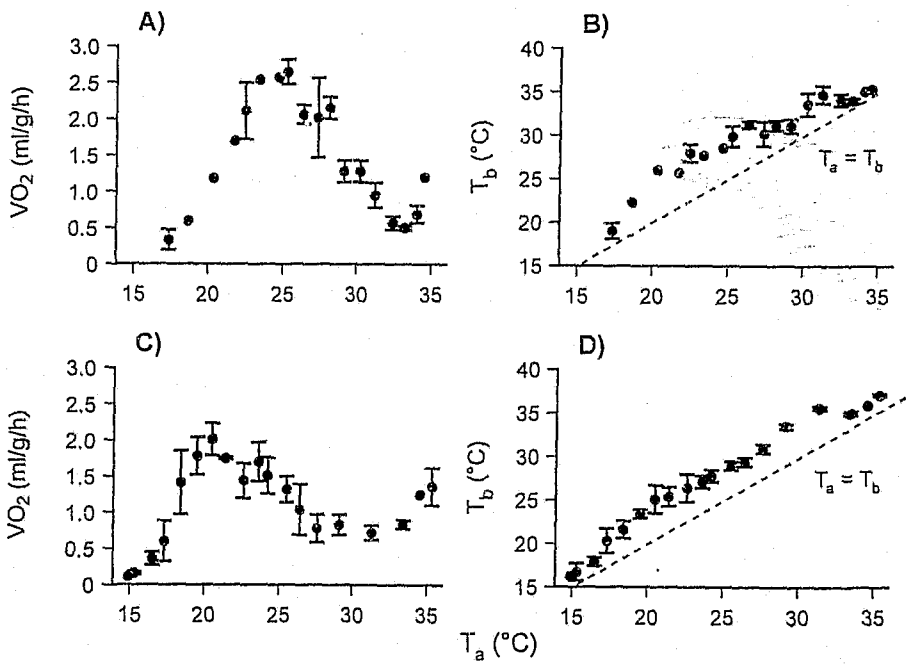


Figure 9.1 Ambient temperature (T_a) dependent A) oxygen consumption (VO_2) and B) rectal temperature (T_b)of thermoneutral acclimated (30°C; n=6) control naked mole-rats and the cold-induced thermoregulatory shifts in T_a -dependent C) VO_2 and D) rectal temperature of 15 cold-acclimated naked mole-rats.

Table 9.1. Body temperature (T_b) tracked ambient (T_a) for both the cold-acclimated naked mole-rats (25°C) and the thermoneutral-acclimated control animals (30°C). Both the best-fit curvilinear and linear functions are given for comparison.

	Curvilinear Function	Linear Function
Control	$T_b^2 = 4735 - 12445/\ln T_a$ ($r^2 = 0.97$)	$T_b = 0.82T_a + 8.0$ ($r^2 = 0.92$)
Cold-Acclimated	$\ln T_b = 4 - 21/T_a$ ($r^2 = 0.99$)	$T_b = 1.02T_a + 2.3$ ($r^2 = 0.97$)

9.4.3 Minimal Thermal Conductance

Mean C_m in the metabolic regulatory zone (T_a=20-27°C; Fig. 9.1) of cold-acclimated naked mole-rats (0.42 ± 0.04mlO_2/g/°C/h; n=29) was 42% lower (P=0.0236) than that of the control animals (0.72 ± 0.15mlO_2/g/°C/h; n=18) over their respective metabolic regulatory zone (T_a=25-32°C; Fig. 9.1).

9.4.4 Skin Temperature

Regional skin temperatures were significantly lower in the cold-acclimated naked mole-rats (Table 9.2). The greatest temperature differential between the two groups was noted for tail temperature (5.1°C), and the lowest for the subscapular temperature (1.6°C). In the cold-acclimated animals, both foot and tail skin temperatures were significantly lower (P<0.001) than both abdominal and subscapular skin temperatures, whereas in the control group, only foot skin temperature was significantly lower (P<0.01).

9.4.5 Rates of Heating and Cooling

Cooling rates of cold-acclimated naked mole-rats were similar (P>0.05) to that of the control animals. Control animals however, exhibited faster rates of heating (P=0.0169; Fig. 9.2). The shivering threshold was significantly reduced in the cold-acclimated animals (21.6 ± 0.3°C) compared to the control group (28.2 ± 1.2°C), with the T_b at which paralysis of the shivering centre occurred

also reduced (Table 9.3). One cold-acclimated animal did not initiate shivering and became hypothermic rapidly and was therefore excluded. Similarly, one control animal also did not employ shivering during the cooling phase, resulting in loss of consciousness and a loss of the righting reflex at T_b's between 16-18°C. In both groups ataxia was noted at low T_b's culminating in rigidity, but occurred at lower T_b's in the cold-acclimated animals. Shivering was initiated at similar T_b's during reheating (Table 9.3). However, the frequency and intensity of shivering was notably greater in the control animals and shivering was maintained over a wider range of T_b's, resulting in a significant elevation in T_b above that of the cold-acclimated group after 40 minutes of reheating. Locomotory activity was restored at a lower T_b (± 26°C) in the cold-acclimated animals compared to that of the control group (± 28°C). Body temperature of cold-acclimated naked mole-rats equilibrated slower and at a lower level (32.2 ± 0.1°C) than that of control animals (33.5 ± 0.1°C; $P<0.0001$).

9.4.6 Body Composition

Body composition of male naked mole-rats was unchanged with cold-acclimation (Table 9.4) with similar ($P>0.05$; paired T-test) percent body fat and lean mass in cold-acclimated and control animals.

Table 9.2. Regional skin temperatures of cold-acclimated (25°C) and thermoneutral-acclimated (30 °C) control naked mole-rats.

Regional Skin Temperature (°C)	Cold-Acclimated (25°C)	Control (30°C)	P Value
Subscapular	31.7 ± 1.4	33.3 ± 0.6	0.0029
Abdominal	31.2 ± 1.4	33.4 ± 0.5	0.0002
Hindfoot	28.7 ± 1.0	32.4 ± 0.7	<0.0001
Tail	27.9 ± 1.0	33.0 ± 0.5	<0.0001

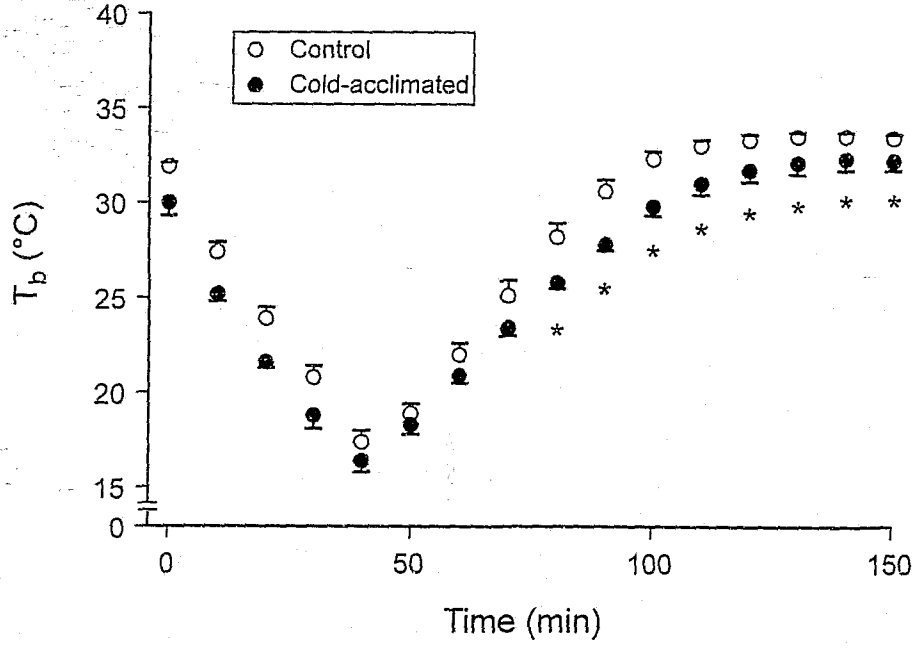


Figure 9.2 Rates of cooling (T_a = 10°C) and reheating (T_a = 31°C) of thermoneutral-acclimated (30°C) control naked mole-rats, and cold-acclimated (25°C) naked mole-rats.

* indicates significant differences in body temperature (T_b) between the two groups, $P < 0.05$.

Table 9.3. Comparison of the body temperature (T_b) of both onset and termination of shivering during both cooling (T_a=10°C) and rehcating (T_a=31°C) of cold-acclimated (25°C) and thermoneutral-acclimated (30°C) control naked mole-rats.

	Cold-Acclimated (25°C)	Control (30°C)	P Value
Cooling Phase: Shivering Onset	21.6 ± 0.6	28.2 ± 2.7	0.0021
Cooling Phase: Shivering End	16.4 ± 1.3	20.4 ± 0.9	0.0009
Heating Phase: Shivering Onset	22.5 ± 1.1	21.0 ± 1.2	0.0948
Heating Phase: Shivering End	25.8 ± 0.6	28.2 ± 1.6	0.0260

Table 9.4. Body composition of cold-acclimated (25°C) and thermoneutral-acclimated (30°C) control male naked mole-rats. No significant difference ($P>0.05$) was noted for all variables.

	Cold-Acclimated (25°C)	Control (30°C)
Body Mass (g)	45.1 ± 6.8	46.8 ± 9.2
Percent Body Fat	11.62 ± 9.50	12.96 ± 10.73
Lean Mass (g)	39.36 ± 0.87	39.78 ± 1.81

Table 9.5. Effect of cold-acclimation on body mass, average daily intake of digestible dry matter and energy in naked mole-rats (*Heterocephalus glaber*).

	Cold-Acclimated (25°C)	Control (30°C)	P Value
Body Mass (g)	39.4 ± 2.7	43.7 ± 1.2	
Digestible DM Intake (g DM/40g BM/day)	1.73 ± 0.12	1.19 ± 0.06	<0.0001
Digestible E Intake (kJ/40g BM/day)	28.58±1.99	19.62±0.91	<0.0001
Faecal Output (g/40g BM/day)	0.1194 ± 0.0079	0.0702 ± 0.0044	<0.0001
Faecal E Content (kJ/40g BM/day)	2.79 ± 0.21	1.63 ± 0.10	<0.0001
Digestibility of DM (%)	89.8 ± 0.9	91.4 ± 0.6	0.1430

Values are presented as means ± SEM's. DM = dry matter, BM = body mass, E = energy.

9.4.7 Food Consumption

Average food consumption over the 7 day period was 45% greater in the cold-acclimated animals ($P<0.0001$; Table 9.5). Energy content of faeces was not altered with cold-acclimation ($P>0.05$), but total faecal output was greater in the cold-acclimated animals ($P<0.0001$) with no resultant change in digestibility ($P=0.1430$; Table 9.5).

The proportion of total assimilated energy apportioned to basal metabolism was similar in both groups, ranging between 56-58%.

9.5 DISCUSSION

The naked mole-rat remains an anomaly of confounding contradictions, showing both mammalian and reptilian features of cold-acclimation. Typical mammalian responses included, 1) shifting the TNZ to a lower range of T_a's, this being facilitated by an augmented BMR and reduced thermal conductance, 2) a lowering of the shivering threshold, and 3) increased food consumption and energy assimilation. Reptilian features of cold-acclimation included, 1) a left-shift of their poikilothermic metabolic profile, 2) maintenance of higher T_b-T_a differentials, but selection of lower T_b's at high T_a's, and 3) greater cold tolerance of physiological and locomotory function. There was no indication of regulated hypothermia as T_b could not be raised independent of T_a,

and with acute cold exposure (10°C) T_b dropped rapidly, culminating in ataxia and the loss of the righting reflex within 30-40 minutes.

9.5.1 Metabolic Responses of Cold-Acclimation

Cold-acclimation in the naked mole-rat, like that of many mammalian species (e.g. Scholander *et al.*, 1958; Bligh, 1973; Li and Tokura, 1997), resulted in the apparent TNZ being extended and shifted to a lower T_a range of 27-34°C (Fig. 9.1), facilitating an enormous energy saving. For instance, at an T_a of 25°C, cold-acclimated naked mole-rats are operating just outside their TNZ, with metabolic rates just above 1.0mlO_2/g/h, whereas the control animals have reached PRMR's of just over 2.0mlO_2/g/h (Fig. 9.1). Moreover, the onset of heat production below apparent thermoneutrality occurred at a T_b of *ca.* 30°C, approximately 4°C lower than that of the control animals (*ca.* 34°C). Similarly, cold-acclimated golden hamsters and guinea pigs also exhibit a decreased threshold for metabolic heat production (Pohl, 1965; Bruck and Zeisberger, 1987). An energy saving is thus facilitated, for the cold-acclimated naked mole-rat allows some degree of "adaptive hypothermia" before endogenous heat production is stimulated.

The reduction of the lower critical temperature from 32 to 27°C can be attributed to a substantial increase (50%) in BMR and a decrease in conductance (Fig. 9.1). Thyroid hormone may be involved in these observed changes. Increased basal

metabolism following cold-acclimation has been reported for many small mammals (Webster, 1974; Wunder, 1979; Wiesinger *et al.*, 1990; Ellison *et al.*, 1992) but is costly to maintain as it represents obligatory energy expenditure, which cannot be reduced below a fundamental threshold. This increased BMR is regulated by changes in thyroid function (Chapter 8) with concomitant changes in cellular metabolism. In contrast to BMR, NST is considered an essential component of cold-acclimation in small mammals (Heldmaier *et al.*, 1982), and unlike basal metabolism, is only expressed when necessary to maintain homeothermy in the cold (Jansky, 1973). Thus improving NST capacity is more energetically expedient than increasing basal metabolism. Several studies have reported increases in PRMR of between 21-108% in cold-acclimated animals (see Heldmaier *et al.*, 1985; Marsh and Dawson, 1989a), indicative of increased thermogenic capacity and thermogenic endurance (Wickler, 1980; Marsh and Dawson 1989b). Thermogenic capacity was however not increased in cold-acclimated naked mole-rats, as PRMR in the cold (2.01 ± 0.39mlO_2/g/h) was no different from that of the thermoneutral-acclimated control animals (2.65 ± 0.23mlO_2/g/h; $P>0.05$; Fig. 9.1). These findings concur with previous observations of no increase in a) NST capacity following prolonged cold exposure (Chapter 6), nor b) BAT mitochondrial thermogenic capacity, although basal thermogenic activity was enhanced (Chapter 7). These findings suggest that BAT thermogenic capacity is maximal

even at the warm temperatures of thermoneutrality, and cannot be further enhanced in response to cold. Peak metabolic rates in this study are of similar magnitude to peak metabolism in pregnancy (Urison and Buffenstein, 1994 & 1995), and maximal metabolic rate in response to noradrenaline (Hislop and Buffenstein, 1994). Similar maximal increases in metabolism under a wide variety of physiological and/or experimental conditions, suggests that there is a common metabolic ceiling, possibly imposed by size constraints of energy supply organs (e.g. cardiovascular, gastrointestinal and pulmonary morphology), thereby limiting maximal transport rates and thus energy output.

Increased basal metabolism is usually sustained by increased energy intake facilitated by augmented gut capacity and/or function. This appears to be true of naked mole-rats, where the 50% increase in BMR following cold-acclimation was matched by a 45% increase in food intake (Table 9.5). Given their poikilothermic features, despite lower T_b's, digestibility was surprisingly not reduced in the cold. This may be attributed to improved caecal function, for this organ is normally maintained at a lower temperature than the abdominal surrounds, and functions optimally at a lower temperature than that of other hindgut fermenters (Yahav and Buffenstein, 1991a; Yahav and Buffenstein, 1992). Furthermore, caecum capacity reportedly is increased in cold-acclimated naked mole-rats

(Daly *et al.*, in prep). Below 25°C however, gut function is adversely affected as indicated by compromised survival when the temperature inadvertently dropped to 23°C. Postmortem data implicated caecal malfunction and concomitant rectal impaction and secondary infection as the cause of death (pers observ). Further evidence of impaired gut function was evident in one pair of cold-acclimated animals which were unable to maintain the normal T_b-T_a differential. These animals had to be excluded from the food consumption data set for they were unable to maintain energy balance and exhibited markedly reduced food consumption and a decline in digestibility as indicated by the presence of large undigested particles, and visibly larger and lighter coloured faecal pellets. Although the augmented gut capacity usually meets the additional basal metabolic needs, such that total energy balance is maintained, gut capacity may nevertheless still restrict their metabolic scope and peak metabolic rates. The proportion of the total energy budget that is apportioned to basal metabolism is disproportionately large (56-58%), and this would limit the energy available for other functions that normally contribute to their large metabolic scope (300-500%; Lovegrove, 1989; Chapter 6).

A left-shift of the poikilothermic metabolic profile was also evident for cold-acclimated naked mole-rats, such that metabolic rates were higher than those of the control

animals for any given experimental T_a below PRMR (Fig. 9.1). Similar responses have been noted in other cold-acclimated poikilotherms (see Cossins and Bowler, 1987). Furthermore, over a similar drop in T_a of approximately 7°C below PRMR, estimates of the Q_{10} for metabolic rate reduction range from 1.5 for the control group to 14 for the cold-acclimated animals. Greater Q_{10} effects (from the normal 2-3) may be facilitated by physiological inhibition (Geiser, 1988), which strongly suggests the presence of such mechanisms in the cold-acclimated naked mole-rat, such that the reduction in metabolic rate is not determined solely by T_b. The significance of such a strong inhibition of metabolism, independent of T_b, is enormous when one considers the concomitant energy savings, although the mechanisms involved are unknown.

9.5.2 Insulatory Responses of Cold-Acclimation

Minimal thermal conductance was reduced by 42% in the cold-acclimated naked mole-rats, contributing to their slower rates of heating. Since no increase in percent body fat was noted (Table 9.4), the enhanced insulation must be primarily attributed to peripheral vasoconstriction, and possible closure and decreased density of arteriovenous anastomoses, resulting in a marked decline in peripheral blood flow. Indeed, when the T_a inadvertently dropped to below 23°C, a few animals developed necrosis of the distal tail and distal aspects of the hindfoot, and were subsequently euthanased. Similarly, severe cold exposure

(5°C) has also been reported to lead to infarcted and necrotic tail tissue in 22 month old rats (Owen *et al.*, 1991). Cold-acclimated naked mole-rats also showed periodic reductions in T_b which may be ascribed to cold-induced vasodilations, thus preventing hypoxic damage to ischaemic tissue (Henshaw, 1986). For endotherms which regulate T_b poorly and for poikilotherms, the decrease in C_m with decreasing T_b may be due to a reduced heart rate (direct effects of cold on SA node) and concomitant reduction in skin blood flow, rather than as a result of an active reduction in conductance *per se*. This may be true of the naked mole-rat, where housing at a lower T_a results in a reduced T_b, which may effect heart rate and conductance in a similar manner.

Diverting blood away from the appendages and reducing skin blood flow, may significantly reduce heat loss. Indeed, skin temperatures of cold-acclimated naked mole-rats were significantly lower for all anatomical sites measured (Table 9.2). Notable vasoconstriction was only evident in the feet of the control animals, with foot skin temperature significantly lower than both abdominal and subscapular temperatures. The greater thermal stress endured by the cold-acclimated group resulted in both foot and tail skin temperatures being significantly lower than that of abdominal and subscapular temperatures. Blood flow to the tail was stemmed to the greatest extent, thus resulting in the largest temperature differential for this

anatomical site. The lowest temperature differential was noted for the subscapular area, indicative of increased BAT thermogenesis and increased blood flow to the region.

Surprisingly, fat deposition was not enhanced in the cold. Increased food intake compensated for the increased basal metabolic demands, with no evidence of any positive energy balance and additional fat deposition. Evolution may have selected against excessive fat deposition as this would impede the high thermal conductance necessary in their hot, humid burrow environment in reducing the risk of thermal death. Evolution of eusociality may also be adaptive in this regard as the foraging costs are shared amongst all individuals, thus obviating the need for energy storage in their harsh, subterranean environment in which individual energetic costs of foraging are high and food is sparsely distributed (Brett, 1991b).

The high conductance of their skin precludes the maintenance of T_b independent of ambient conditions. The observed reduction in the shivering threshold of cold-acclimated naked mole-rats is a typical mammalian response (e.g. golden hamster and guinea pig; Pohl, 1965; Bruck and Zeisberger, 1987). By shifting the shivering threshold to lower T_b's, the cold-acclimated naked mole-rat allows for the use of NST at a lower range of temperatures before the less economical shivering mechanism is evoked. Furthermore, there is normally no increase in the capacity

for shivering thermogenesis in cold-acclimated small mammals, with the shift in the shivering threshold usually following an increase in NST capacity (Heldmaier *et al.*, 1985). Shivering, therefore, is only utilized at low T_a's when the magnitude of the NST response is insufficient (Banet and Hensel, 1977; Bockler and Heldmaier, 1983; Heldmaier *et al.*, 1989). As such, physiological mechanisms exist to ensure that NST is used preferentially (Bruck, 1970). Indeed, control animals exhibited a greater shivering response with T_b's greater within the T_a range of 19-26°C. Body temperatures of the cold-acclimated animals were greater over the lowest T_a range due to their superior insulation, and also between 27-34°C (TNZ) due to the 50% increase in BMR. The elevated BMR allows for the maintenance of T_b's 2.8-4.7°C higher than T_a within their TNZ, thus facilitating more optimal physiological function. Upon reheating, shivering was terminated sooner in the cold-acclimated naked mole-rats, with the concomitant selection of a lower preferred T_b at higher T_a's. This suggests that their metabolic enzyme systems have adjusted to a new thermal optimum. Similarly, many cold-acclimated reptiles have been reported to select lower preferred T_b's with concomitant down-regulation of physiological systems (Huey, 1982; Geiser *et al.*, 1992; Rome *et al.*, 1992; Hulbert, 1993). At low T_a's all thermogenesis is aborted for both groups due to cold-induced paralysis of the regulatory centres, this occurring at lower T_b's in the cold-acclimated animals.

In conclusion, thermal acclimation to chronic cold exposure was evident with pronounced left-shifts in both the endothermic and ectothermic components of the thermoregulatory curves and lower rates of thermal conductance. Cumulatively, these cold-acclimation responses allow for more optimal functioning at lower T_b's and considerable energy savings at T_a's only slightly below their apparent TNZ. However, the increased basal metabolism results in more energy apportioned to basal activities and thermoregulation at the expense of other energetically costly processes. Indeed, even NST thermogenic capacity was unchanged (Chapters 6 and 7). In addition, both activity levels and reproduction were markedly reduced in response to this repartitioning of the energy budget with cold-acclimation (Chapter 10). Although naked mole-rats do show both typical and atypical mammalian and reptilian cold-acclimation responses, these features are comparatively inconsequential and would preclude their successful exploitation of colder habitats. The distribution of naked mole-rats to the thermally stable equatorial milieu of the north-east African underground is therefore not surprising.

CHAPTER 10

PROFOUND TEMPERATURE SENSITIVITY OF REPRODUCTIVE FUNCTION IN A POIKILOTHERMIC MAMMAL, THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*)

10.1 ABSTRACT

A longitudinal study was undertaken to evaluate the effects of chronic cold exposure (25°C) on body temperature (T_b), gestation, fecundity, and maternal body composition in a thermoconforming mammal, the naked mole-rat (*Heterocephalus glaber*). Pregnant females were also subjected to acute cold bouts (5 days; 25°C) during early (EP), mid (MP), or late pregnancy (LP), to determine at what stage of pregnancy these animals are most sensitive to cold. Predictably, T_b of cold-acclimated pregnant females was significantly lower (1-2°C) than that of control pregnant females housed under simulated burrow conditions (30°C), although the temperature differential (T_b-T_a) was greater (7.8°C, cold-acclimated; 4°C, control). Reproductive success was markedly reduced (4.5-fold; $P<0.0001$) by prolonged cold exposure. Furthermore, interbirth interval was significantly prolonged in the cold (152 ± 77 days, cold-acclimated; 77 ± 2 days, control), with evidence that the gestation period was also lengthened (76 ± 5 days) compared to the control females (68 ± 2 days; $P<0.0001$). Whilst no reduction in litter size was observed (8 ± 4, cold-acclimated; 9 ± 3, control), average pup mass in the cold (1.70 ± 0.29g) was 14% greater than that in the warm (1.49 ± 0.27g; $P=0.0227$). Maternal energy reserve depletion was greater in cold-acclimated lactating females. Pup growth rate post-weaning was 38% lower in the cold, but no difference

in pup mortality rate was noted. At all stages of pregnancy, females lost body mass during the acute cold bouts, the majority (94%) attributed to fat loss, with lean mass only being drawn upon during the EP cold bouts. Extension of the interbirth interval with acute cold exposure was very variable, particularly during EP and MP where the greatest effects were seen. Cold exposed naked mole-rats incur substantial thermoregulatory costs which limit energy allocation to reproduction, such that reproductive success is dramatically reduced. In successful pregnancies, prolonged cold exposure significantly lengthened the gestation period, culminating in the birth of larger pups that may be more adept at surviving the colder temperatures.

10.2 INTRODUCTION

Reproductive fecundity in small viviparous vertebrates is comparatively more sensitive to environmental factors than that of larger animals. In small mammals, this is attributed to higher rates of heat loss due to larger surface area to volume ratios, with concomitantly more of the total energy budget apportioned to thermoregulation. Not surprisingly, since small mammals have relatively little fat storage to counter acute energy-demanding emergencies during reproduction (Gyug and Millar, 1980; Merson and Kirkpatrick, 1981), reproductive success declines with severe cold-stress (Biggers *et al.*, 1958;

Perrigo and Bronson, 1985; Marstellar and Lynch, 1987; Schneider and Wade, 1991). In poikilotherms and torpid pregnant metatherians and eutherians, physiological functions, including foetal development rates and reproduction are directly influenced by body temperature (T_b) and this may impact considerably upon gestation length (Racey, 1973; Racey and Swift, 1981; Beuchat, 1988; Geiser and Masters, 1994).

Prolonged cold exposure, without a change in photoperiod in small homeotherms, reportedly results in a) delayed puberty, b) the termination of adult oestrus cycles, c) reduced ovulation rates and reproductive organ weights, d) decreased litter size at birth and weaning, and e) increased intervals between successive litters in mice (Biggers *et al.*, 1958; Barnett, 1965; Millar and Gyug, 1981; Marstellar and Lynch, 1983; Perrigo and Bronson, 1985; Marstellar and Lynch, 1987; Manning and Bronson, 1990), rats (Chang and Fernandez-Cano, 1959; Piacsek and Nazian, 1981), and hamsters (Reiter, 1968; Schneider and Wade, 1990 & 1991). Reproductive outcome in response to cold exposure is however species specific and dependent on the stage of pregnancy at which the cold-stress occurs. For instance, rats are more sensitive to cold during the latter half of their gestation cycle, with gestation being halted and uterine haemorrhage more common (Courrier and Marois, 1953), whereas mice exposed to cold during EP show delayed implantation and higher rates of

intrauterine mortality (Stankiewicz, 1974).

Reproductive outcome in naked mole-rats (*Heterocephalus glaber*) may be particularly sensitive to changes in ambient temperature (T_a), for these small mammals, lacking adequate insulation (Daly and Buffenstein, 1998), are effectively poikilotherms outside the narrow confines of their warm, equatorial and social burrow milieu (Buffenstein and Yahav, 1991a). Indeed, a drop in T_a of less than 5°C below apparent thermoneutrality represents a severe cold-stress to these naked rodents (Chapter 6).

When breeding under simulated burrow conditions, the single reproductive female within the eusocial colony produces litters of 1-27 pups (average 12 ± 6) throughout the year on a 70 day gestation cycle with an 8-11 day postpartum oestrus, such that the interbirth interval ranges from 77-84 days (Jarvis, 1991a). Body mass increases by approximately 30-50% during pregnancy (Jarvis, 1991a; Urison and Buffenstein, 1995; Woodley and Buffenstein, in prep), largely due to foetal fat deposition (Woodley and Buffenstein, in prep). Reproduction in the naked mole-rat is an energetically costly process, with metabolic rate substantially elevated (37%) from the second week of pregnancy and maximal during LP and lactation (Urison and Buffenstein, 1994 & 1995). Indeed the lactating female is compelled to draw on her

fat stores for fuel substrate, thus losing up to 16% of her body mass (Jarvis, 1991a). There is little maternal fat storage during pregnancy, most of which occurs during the early stages of pregnancy when metabolic demands are lowest (Woodley and Buffenstein, in prep).

Given the high energetic costs of pregnancy, minimal fat stores and ineffective insulation, I questioned whether breeding naked mole-rats would be able to sustain pregnancy when subjected to acute or chronic cold exposure and whether the reproductive responses to cold would follow reported poikilothermic patterns (Beuchat, 1988). This was assessed by monitoring changes in body composition after exposing pregnant females to an ambient temperature 5°C lower than their preferred housing temperature (30°C; Jarvis, 1991b) for 5 days at different stages of pregnancy. The effect of prolonged cold exposure on gestation length and reproductive outcome was also assessed, with the hypothesis being that lower T_c's during prolonged cold exposure would increase gestation length.

10.3 MATERIALS AND METHODS

10.3.1 Animal Care and Maintenance

Naked mole-rats used in this study were born in captivity from stock animals originally collected in Kenya in 1980. Animals were housed under simulated burrow conditions with no day-night cycle in climatically controlled rooms in

which relative humidity was maintained above 60% (Jarvis, 1991b). Sawdust and shredded paper were provided for bedding. Animals were fed an *ad lib.* diet of apple and sweet potato, as well as receiving a high energy, protein enriched commercial cereal (Pronutro, Becketts, RSA).

10.3.2 Experimental Protocol

Experiment 1 - Effects of acute cold exposure on maternal body composition and interbirth interval

Established reproductive pairs (n=33) that had exhibited regular breeding cycles for at least 3 consecutive litters were housed under simulated burrow conditions (30 ± 1°C; Jarvis, 1991b). Body mass and body composition of breeding females was monitored before and after a period of 5 days during either EP (approximately 15 days postpartum), MP (approximately 38 days postpartum) or LP (approximately 63 days postpartum). Reproductive pairs were then randomly moved to a colder T_a of 25 ± 1°C and the body mass and body composition of the pregnant female monitored over the same time intervals. No lactating females were used for the EP measurements. Body mass and body composition were measured as outlined below and the interbirth interval monitored for both groups.

Experiment 2 - Effects of chronic cold exposure on maternal body composition and reproductive outcome

Established reproductive pairs (n=29), that had displayed regular breeding cycles for at least 3 consecutive

litters, were moved to an T_a of 25 ± 1°C, three weeks into their gestation cycle. An equivalent control group (n=22) were maintained at their preferred housing temperature and that experienced in their natural habitat (30 ± 1°C; Jarvis, 1991b).

Body Mass and Body Temperature Measurements

To prevent undue stress to the pregnant female, body mass and T_b were recorded on a weekly basis only, at a similar time each day. Body mass was recorded to the nearest gram (Ohaus LS2000, Ohaus Scale Corporation, New York, USA). A copper-constantan thermocouple was inserted ±2cm into the rectum and the T_b recorded to the nearest 0.1°C via a calibrated digital display (Model-Bat-12, Physitemp, New York, USA).

Body Composition Analysis

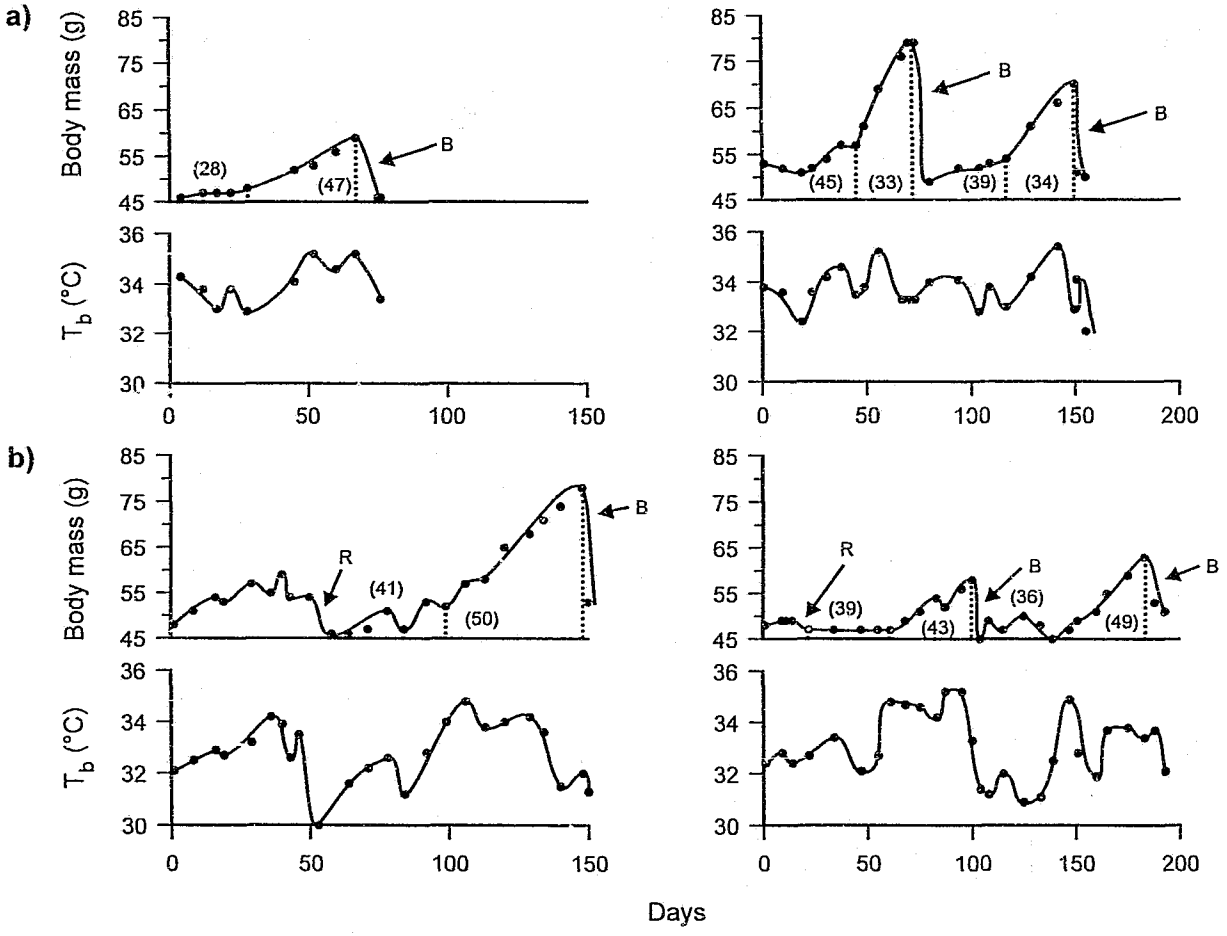
Body composition was measured using an EM-SCAN Model SA-2 Small Research Animal Body Composition Analyser (EM-SCAN Inc, Springfield, Illinois, USA), following the methods of Buffenstein *et al.*, (1996). Six to ten measurements were made and the average percent body fat and lean mass (grams) recorded.

Pregnancy Outcome

Pregnancy outcome was assessed by monitoring litter size, litter mass, average pup mass and pup mortality rate. The percent reproductive success was calculated as the actual

number of pregnancies over the number of potential pregnancies. Interbirth intervals were monitored and gestation length estimated assuming a postpartum oestrus of 9 days (Jarvis, 1991a). As many females in the cold stopped cycling for prolonged periods, and exhibited increased frequency of resorption, gestation length was extrapolated from an analysis of body mass and T_b. It was frequently noted that post-resorption bleeding was accompanied by parallel declines in both body mass and T_b (Fig. 10.1), and this was taken as the start of the next reproductive cycle. The difficulty in obtaining isolated urine samples from pregnant females, and the invasive and stressful nature of blood sampling, precluded the measurement of hormone levels as an indicator of the start of pregnancy. Time of mating could also not be used as an indicator of the start of pregnancy, as it was infrequently observed. Foetal growth rate was estimated from the rate of increase in body mass during the latter half of pregnancy, as the majority of foetal growth occurs during this period (Woodley and Buffenstein, in prep; Fig. 10.1).

Figure 10.1 Three representative gestation cycles are shown for both a) warm-acclimated, and b) cold-acclimated naked mole-rats. The warm-acclimated females cycled consistently every ± 76 days. Resorptions (R) were common in the cold-acclimated females and were noted by post-resorption bleeding and parallel declines in both body mass and body temperature (T_b). This was used as an indicator of the start of the next reproductive cycle. In so doing, an estimate of gestation length was made assuming a postpartum oestrus of 9 days (Jarvis, 1991a). Foetal growth rate was estimated as the rate of growth of the products of conception during the latter half of the gestation cycle, as foetal tissue accounts for the majority of the mass increment, with most of the total growth occurring during the latter half of pregnancy (Woodley and Buffenstein, in prep). Parturition is indicated by (B). Values in parenthesis represent the lengths of the two halves of the gestation period.


a)
b)
Body mass (g)
T_b (°C)
Days
B
R
(28)
(47)
(45)
(33)
(39)
(34)
(41)
(50)
(43)
(36)
(49)

10.3.3 Statistical Analysis

Data are presented as means and standard deviations, except where otherwise indicated. Data sets for the short-term cold bouts were compared with their respective control groups using unpaired T-tests. Data from the long-term cold exposure experiments were pooled into four groups: non-pregnant (NP) (1-7 days postpartum), EP (weeks 2-4), MP (weeks 5-7), and LP (weeks 8-10). Statistical analyses performed include repeated measures ANOVA and unpaired T-tests. Linear regression analyses were also performed for appropriate data sets. Results were considered significant at $P<0.05$.

10.4 RESULTS

10.4.1 Experiment 1 - Acute Cold Exposure

Body mass of pregnant females decreased significantly during the acute cold bouts and was most pronounced during the earlier stages of pregnancy (Fig. 10.2). Most of this mass loss (94%) can be attributed to a loss of body fat ($r=0.8865$, $P<0.0001$, $n=24$; Fig. 10.3). Lean mass was only drawn upon during the EP cold bouts (Fig. 10.2).

Whilst the control females cycled regularly with an interbirth interval of 77 ± 2 days, extension of the interbirth interval following acute cold exposure was very variable, especially during EP and MP (EP, 113 ± 47 days; MP, 125 ± 93 days). Late pregnant cold bouts did not

affect gestation in any way, with the interbirth interval (79 ± 3 days) similar to that of the control group (78 ± 3 days).

10.4.2 Experiment 2 - Chronic Cold Exposure

Body Mass

Changes in body mass with pregnancy were similar at both housing temperatures, with similar percent incremental changes and similar time-based responses evident ($P>0.05$; Table 10.1). Body mass increased approximately 30% during gestation, with the largest increment occurring during the latter half of pregnancy (Fig. 10.1).

Body Temperature

Body temperatures of cold-acclimated pregnant females were significantly lower than that of the control group for all stages of pregnancy (Fig. 10.4), but the temperature differential (T_b-T_a) was greater (6.9-8.7°C, cold-acclimated; 3.7-4.5°C, control). Body temperature changes during pregnancy were similar for both groups with MP and LP T_b's significantly greater than NP temperatures ($P<0.01$ and $P<0.05$, respectively; Fig. 10.4). Body temperature peaked at MP for both groups (33.7 ± 0.9°C, cold-acclimated; 34.5 ± 0.6°C, control), with the NP-MP increment substantially greater for the cold-acclimated females (1.8°C) compared to the control group (0.7°C).

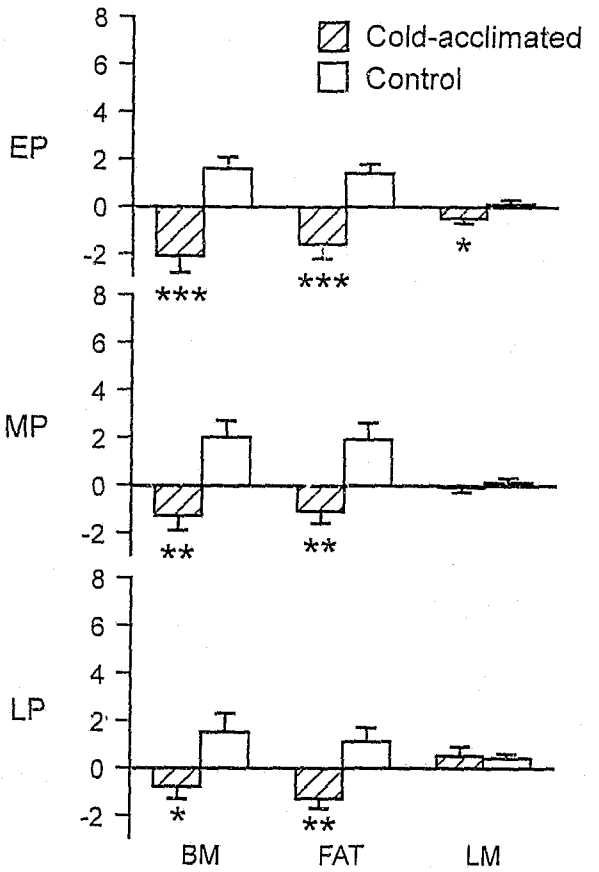


Figure 10.2 Changes in body mass (BM) and body composition with acute cold exposure in pregnant naked mole-rats. Data represent gram changes in body mass and its composition, when pregnant females at different stages of pregnancy (EP, early pregnancy; MP, mid-pregnancy; LP, late pregnancy) were moved to a room 5°C lower (25°C) than standard housing conditions (30°C) for 5 days. These data are compared with that of the warm-acclimated control females housed at 30°C. Significant differences between these two groups are noted by the following: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$. Data are presented as means ± SEM. LM = lean body mass.

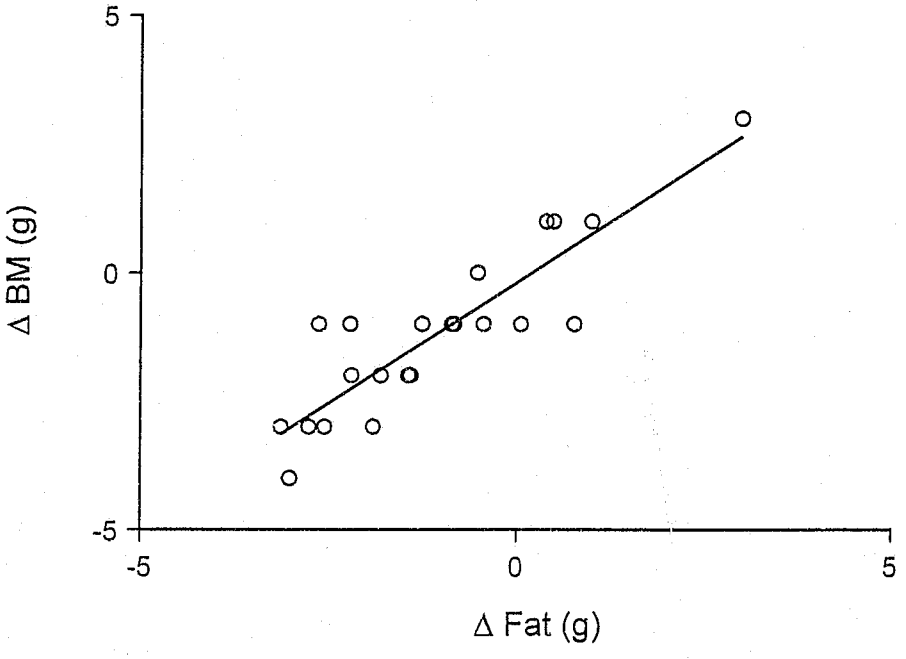


Figure 10.3 Body mass (BM) loss is directly correlated with fat loss in acutely cold exposed pregnant naked mole-rats. BM loss = 0.94 (fat loss) - 0.2.

Table 10.1. Body mass changes with pregnancy in cold-acclimated (25°C) and warm-acclimated (30°C) control naked mole-rats. Body mass at different stages of pregnancy was similar for both groups, with similar percent incremental changes ($P>0.05$).

Body Mass (grams)	NP	EP	MP	LP
Cold-Acclimated	42.6 ± 5.5	44.8 ± 5.7	47.9 ± 6.7	57.8 ± 9.2
Warm-Acclimated	50.5 ± 12.7	51.5 ± 12.7	55.3 ± 13.5	64.0 ±13.9

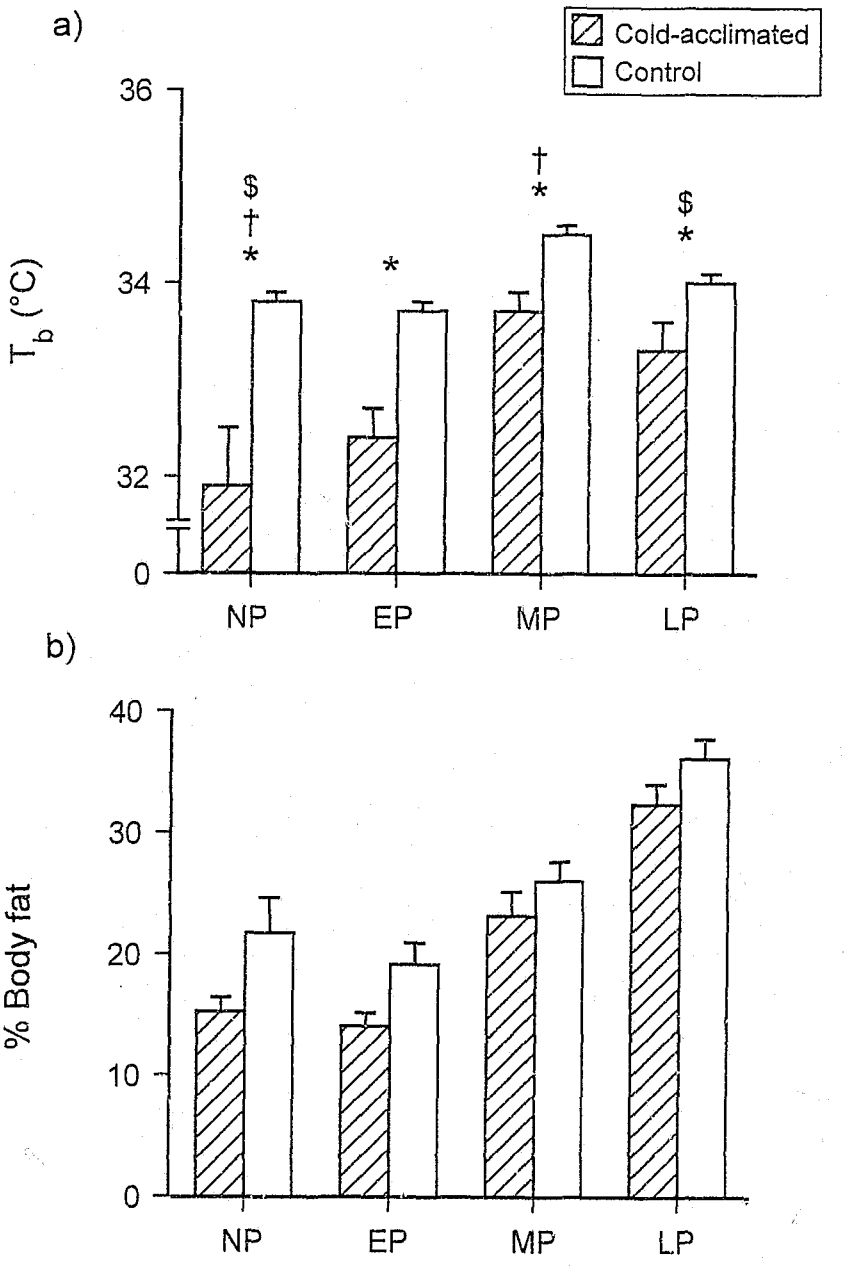


Figure 10.4 Changes in a) body temperature (T_b), and b) percent body fat with pregnancy in cold-acclimated (25°C) and warm-acclimated (30°C) control naked mole-rats. Data are presented as means ± SEM's.

Body Composition

No significant change in lean mass with pregnancy was observed for both groups, with similar average lean masses for both the cold-acclimated and the control females (41.8 ± 1.7g, cold-acclimated; 41.5 ± 2.2g, control; $P>0.05$). Whilst average percent body fat at different stages of pregnancy were similar between the two groups ($P>0.05$), the temporal pattern of fat deposition differed (Fig. 10.4). For cold-acclimated females, the greatest percent increment occurred between EP and MP (65%) and was twice that of the warm-acclimated group (36%). The percent increment from MP to LP was similar for both groups (39%).

Pregnancy Outcome

4 of the 29 breeding females (13.5%) successfully bred in the cold whereas 20 of the 33 (61%) breeding females successfully bred when housed at the warmer temperature ($P<0.0001$), with resorptions far more common in the cold (27 in cold, 3 in warm). In addition, one pregnant female in the cold aborted 7 partially developed foetuses.

The interval between successive litters in the cold (152 ± 77 days; n=14) was twice that in the warm (77 ± 2 days, n=22; Table 10.2). For the few females that successfully completed a pregnancy in the cold, the estimated gestation period (76 ± 5 days; range 67-84 days; n=14) was significantly longer than in the warm (68 ± 2 days; range 65-74 days; n=22; Table 10.2).

Weight gain occurred in two distinct phases during the gestation period for both groups (Fig. 10.1), conceptually dividing the gestation period into 2 periods. The duration of the first half of pregnancy was similar for both groups (32 ± 7 days, cold-acclimated; 32 ± 9 days, control). The second half of the gestation period was significantly longer in the cold (44 ± 5 days; P=0.0031) compared to that in the warm (36 ± 8 days). Foetal growth rate in the cold (0.43 ± 0.15g/day; n=10) was not significantly different from that in the warm (0.53 ± 0.26g/day; n=20; P>0.05). No reduction in litter size was evident in the cold (8 ± 4 pups) compared to that in the warm (9 ± 3 pups), but pups born in the cold were significantly larger (Table 10.2). An indication of the lean mass per pup was obtained from the difference in body composition measurements made immediately prior to and immediately after parturition. Lean mass of pups born in the cold was significantly greater than that in the warm (Table 10.2), whilst percent body fat was unchanged.

Body fat decreased during lactation by a similar magnitude for both groups (Fig. 10.4). Lactating females in the cold showed a slight decrease in lean mass per day (-0.2356g/day), whilst their warm-acclimated counterparts gained lean mass (+0.0228g/day; P=0.0021). Pup growth rate post-weaning was 38% lower in the cold (Table 10.2), but pup survival was not compromised.

Table 10.2. Interbirth interval, gestation length and litter demographics for cold-acclimated (25°C) and warm-acclimated (30°C) control naked mole-rats.

	Cold-Acclimated	Warm-Acclimated	P Value
Interbirth Interval (Days)	152 ± 77	77 ± 2	<0.0001
Gestation Length (Days)	76 ± 5	68 ± 2	<0.0001
Pup Mass (grams)	1.70 ± 0.29	1.49 ± 0.27	0.0227
Pup Lean Mass (grams)	0.46 ± 0.39	0.11 ± 0.06	0.0022
Pup Growth Rate (grams/day)	0.13 ± 0.04	0.18 ± 0.05	0.0437

10.5 DISCUSSION

Prolonged exposure to T_a's only 5°C lower than simulated burrow conditions, resulted in a 1-2°C drop in T_b. These comparatively small temperature changes severely compromised reproductive success with a 4.5-fold reduction in the number of successful pregnancies. Not only were fewer females able to sustain pregnancy in the cold, but those that did, exhibited a significant extension of the gestation period that culminated in the birth of larger pups. Naked mole-rats appeared to be more vulnerable to the adverse effects of cold exposure during the early stages of pregnancy, where depletion of fat and lean mass were most pronounced, resulting in the longest extension of the interbirth interval. These changes in reproductive fecundity with a 5°C drop in T_a, occurred even though all other housing and husbandry conditions were identical and food was supplied *ad libitum*. Naked mole-rats exhibit a marked temperature sensitivity of pregnancy, intermediate to that previously reported for typical poikilothermic ectotherms and heterothermic bats and marsupials (Racey and Swift, 1981; Beuchat, 1988; Geiser and Masters, 1994). Such a profound sensitivity to environmental fluctuations in T_a may explain their limited distribution to the thermally stable, subterranean milieu of the equatorial north-east African soils.

10.5.1 Chronic Cold Exposure

Although T_b of cold-acclimated females was only 1-2°C lower than the control group at each stage of pregnancy, pronounced adverse effects in reproductive outcome were evident. Even in those females that successfully maintained pregnancy, parturition was delayed and gestation extended.

The optimal temperature for foetal development in the naked mole-rat, reportedly, is higher than eccritic non-pregnant T_b (Buffenstein *et al.*, 1996). This higher T_b presumably facilitates or enhances the rate of maternal-foetal energy transfer, resulting in higher foetal growth and development rates. The T_b of the cold-acclimated females showed an earlier and more substantial rise than the control group. This coincided with the greatest increment in percent body fat (64%, EP-MP), suggesting that fat deposition during the early stages of pregnancy may have a thermoregulatory role, thus facilitating the maintenance of the greater T_b-T_a differential. An earlier and more substantial elevation of T_b by cold-acclimated females may facilitate the observed maintenance of optimal foetal growth rates.

Several females which had previously been cycling regularly, ceased to do so, resulting in a marked reduction of litters born in the cold with a substantial extension of the interbirth interval. Although only

inferred from the observations of post-resorption bleeding and concomitant declines in body mass and T_b, gestation also appeared to be significantly prolonged in the cold. The first half of pregnancy appeared to be unaltered, whereas the second half of the gestation period appeared to be profoundly affected by cold, increasing by more than 20% or approximately 8 days. Thus the lengthening of the gestation period cannot be attributed to delayed implantation as has been noted for other cold exposed rodents (Oswald and McClure, 1987). Nor can it be ascribed to a cold-induced lengthening of the oestrus cycle such as occurs in rats (Chang and Fernandez-Cano, 1959; Nazian and Piacsek, 1977), but rather, is due to a real lengthening of the gestation period.

For most small mammals, even severe cold-stress has little effect on gestation length (e.g. rats, mice, and hamsters; see Racey, 1981). Notable exceptions are only reported when the T_b of the pregnant female is considerably reduced. Courrier and Marois (1953) reported delayed implantation, retarded development and postponed parturition when they cooled rats to 16-20°C during the first 11 days of pregnancy. Similarly, foetal developmental rates of heterothermic bats and marsupials are retarded or even halted in response to a fall in T_b during torpor, resulting in a lengthening of the gestation period (Racey, 1973; Racey and Swift, 1981; Geiser and Masters, 1994). Gestation length of viviparous reptiles is

even more profoundly sensitive to temperature and can nearly double with a 2°C decline in T_b (Beuchat, 1988). A 2°C decline in the T_b of naked mole-rats resulted in a 12% increase in gestation length. Such a striking temperature dependence of pregnancy is normally characteristic of poikilothermic ectotherms, and although the response is less than that reported for lizards and snakes (Beuchat, 1988), the extended gestation length is nevertheless substantially greater than that of torpid mammals.

Despite the lower T_b's in the cold, foetal growth rate was surprisingly unchanged, with the longer (12%) gestation period culminating in the birth of larger (14%) pups with significantly greater lean mass which are perhaps more adept at surviving at the lower T_a. Indeed, no increase in pup mortality rate was noted in the cold. Similarly, ectotherms are larger in size if they develop at lower temperatures (Beuchat, 1988; Atkinson and Sibly, 1997), and large size often affords an ecological advantage (Barbault, 1988).

10.5.2 Acute Cold Exposure

Acute cold exposure (5 days; 25°C) had the greatest negative impact during EP and MP. Like other small mammals, naked mole-rats responded to short-term cold exposure by rapidly mobilizing fat stores. Fat loss was however more pronounced than that seen in other small mammals housed at considerably lower T_a's (e.g. hamsters

housed at 6°C; Kodama and Pace, 1964), and accounted for 94% of the mass loss (Figs. 10.2 & 10.3). Lean mass conservation may be particularly important to naked mole-rats for their natural diet is low in protein and essential amino acids, limiting protein synthesis and tissue restoration (Jarvis, 1985; Brett, 1991b). The only time a negative lean mass balance was observed was during the EP cold bouts (Fig.10.2), which were also associated with an increase in the interbirth interval.

Lean mass did not change significantly during pregnancy for both the cold-acclimated and the control groups. It appears that the protein needed for foetal accretion in naked mole-rats is stored in maternal tissues prior to pregnancy and is gradually transferred to foetal tissues as pregnancy progresses (Woodley and Buffenstein, in prep). The protein costs of pregnancy are therefore distributed over the entire gestation period, rather than accrued during the period of high demand only. Thus it appears that during pregnancy, every effort is made to conserve lean body mass at the expense of stored fat.

Breeding naked mole-rat females do not store large amounts of fat during pregnancy, as immediately postpartum, body mass returns to near pre-pregnant levels. As such, postpartum fat reserves are comparatively low. Increased thermoregulatory costs when housed in the cold during EP must therefore be met by an increase in food

consumption, necessitating an increase in gut capacity. Although gut capacity is comparatively plastic, substantial hypertrophy appears to take approximately 7-10 days to develop (Himms-Hagen, 1997). In the interim, the cold exposed EP naked mole-rat must draw upon stored energy reserves to meet her increased thermoregulatory requirements. Although fat stores are utilized preferentially, in EP these stores are inadequate and lean mass is catabolized to meet the energy requirements for the first few days (Fig. 10.2). Similar responses are shown by other small mammals, but at much more severe temperatures. For instance, mice when kept at -8°C start utilizing protein in addition to fat stores and at these temperatures ovulation is suppressed and maternal survival is compromised (Manning and Bronson, 1990).

Maternal fat stores are maximal by MP with concomitant enhanced insulation (Woodley and Buffenstein, in prep), and during LP, rates of heat loss decline in response to body shape changes and also higher rates of heat production as foetal lean tissue becomes metabolically active. These features of the latter stages of pregnancy, together with augmented gut capacity, negate the effects of cold bouts, such that protein reserves are spared.

10.5.3 Energy Balance during Reproduction in the Cold

Energy balance during pregnancy and lactation is compromised in the cold, with depletion of maternal fat and protein reserves, especially with successive reproductive efforts. The greater depletion of whole body lean mass immediately postpartum in the cold-acclimated females, suggests that their pups had significantly greater lean mass. Increased pup lean mass is both beneficial and detrimental in that it results in more mature larger pups, however with this increase in metabolically active tissue, the lactational demands upon the mother would be considerably greater.

The production of larger mature pups, may lead to the shortening of the proportion of the reproductive event spent lactating, thus facilitating an energy saving given the high energetic costs incurred during this reproductive effort (Urison and Buffenstein, 1995). The greater protein turnover rates are however energetically costly to maintain (Kelly and McBride, 1990), and the greater pup lean mass results in concomitantly higher lactational demands. The depletion of maternal lean mass reserves may also impact upon future reproductive success, as it has been suggested that protein reserves, rather than adiposity may influence reproductive function (Britt *et al.*, 1988). The substantial metabolic cost incurred by the breeder in the cold probably explains why few cold-exposed females produced more than one litter. One breeding female lost 34% of body mass over 7 days after

having given birth to her second litter in the cold and subsequently died.

Contrary to most small mammals where the production costs of gestation are far greater than the maintenance costs (e.g. rat; Spray, 1950), in the naked mole-rat, maintenance costs are 34% greater than production costs (Woodley and Buffenstein, in prep). Thus a 12% lengthening of gestation necessitates that the maintenance costs be carried for longer, at considerable cost to the pregnant female. Moreover, lengthening of gestation occurs during the latter stages, at which time sustained metabolic rates of the pregnant female are maximal (Urison and Buffenstein, 1994). The observed marked reduction in reproductive success is therefore not surprising.

Post-weaning pup growth rates were 38% lower in the cold. This may reflect the well documented temperature effect on growth and development in ectotherms (Cloudsley-Thompson, 1971). Indeed, the 38% decrease in pup growth rate is consistent with that which might be expected due to a purely Q_{10} effect. By increasing protein deposition *in utero*, early growth, rather than being retarded at low T_a's, occurs in the warmer, more favourable uterine environment and provides the pups with larger energy reserves to draw upon during the first few critical days of life.

In conclusion, it appears that cold exposure results in a depletion of energy apportioned to reproduction, thus impacting profoundly upon reproductive outcome in the naked mole-rat. The cessation of reproduction, rather than representing a physiological dysfunction at low T_b's, may serve as an adaptation to conserve maternal resources, especially lean body mass, to allow for a repartitioning of available energy in favour of physiological processes essential for maternal survival.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUSION

THERMOREGULATORY RESPONSES OF COLD-ACCLIMATION

Across the animal kingdom, morphological, physiological, and behavioural responses serve to offset the direct effects of prolonged cold exposure (Hochachka and Somero, 1984; Cossins and Bowler, 1987; Huey, 1991; Leroi *et al.*, 1994; Kingsolver and Huey, 1998). These responses differ considerably between poikilotherms and homeotherms, but are nevertheless generally considered highly adaptive (Mangum and Hochachka, 1998; Huey *et al.*, 1999), with the most pronounced changes occurring in animals encountering extreme temperature fluctuations within their natural environment (Aleksuik, 1971; Feder, 1982; Huey, 1982; Tsuji, 1988; Huey and Berrigan, 1996).

Naked mole-rats in the wild inhabit a thermally buffered environment, encountering only small daily and seasonal changes in ambient conditions. In captivity, these unusual mammals exhibit many anomalous features of mammalian thermoregulation, with pronounced thermolability and a typical poikilothermic T_b profile. Their unusual mode of thermoregulation led me to question whether chronic cold exposure would induce typical mammalian responses, or whether, because they are poikilotherms, cold-acclimation responses would conform rather to typical reptilian trends. This was investigated by monitoring changes in thermogenic capacity, thermoregulatory profiles, basal metabolism, and the hypothalamic-pituitary-thyroid axis.

In addition, the consequences of a prolonged reduction in T_b on energy balance and reproductive outcome was also addressed.

Cold-acclimation in most mammals, particularly those that do not employ winter dormancy, consists essentially of shifting the TNZ to a lower range of T_a's (Scholander *et al.*, 1958; Bligh, 1973; Li and Tokura, 1997). This is achieved by a variety of means, including 1) increased basal metabolism, 2) thicker and more extensive insulation, 3) a reduced sensitivity to low temperatures in extremities (e.g. ears and limbs), and 4) peripheral vascular changes to reduce heat loss. Alternatively, cold exposed mammals may increase heat production by increasing 1) BMR under the regulation of thyroid hormones, 2) muscle contraction (shivering or exercise), or 3) NST. These modes of increased heat production have been reported in many species of small mammals, but are metabolically expensive to maintain (Scholander *et al.*, 1950 & 1958; Bligh, 1973; Cossins and Bowler, 1987; Heldmaier *et al.*, 1990).

In contrast, most poikilotherms can endure colder T_a's with concomitant effects on T_b and physiological function. Some reptiles respond to prolonged cold exposure by partial or complete metabolic compensatory mechanisms involving left-shifts of their temperature-dependent metabolic curves (Rieck *et al.*, 1960; White and Lasiewski,

1971; Eckert and Randall, 1983; Cossins and Bowler, 1987; Kingsbury, 1994). Most reptiles are reliant on less costly behavioural responses to counter cold-stresses (Carrascal *et al.*, 1992; Kingsbury, 1994; Martin *et al.*, 1995). However, they have also been reported to select lower preferred T_b's and remain active, although locomotor and digestive capacity may be reduced (Huey, 1982; Geiser *et al.*, 1992; Rome *et al.*, 1992; Hulbert, 1993; Kingsbury, 1994).

The naked mole-rat remains an anomaly of confounding contradictions, and does not obey any one particular thermoregulatory dogma, but exhibits both typical and atypical mammalian and reptilian responses to prolonged cold exposure (Table 11.1 and 11.2).

Table 11.1. Typical and atypical mammalian responses of cold-acclimated (25°C) naked mole-rats (*Heterocephalus glaber*).

Typical Mammalian Responses	Atypical Mammalian Responses
• Left-shift of the TNZ to lower T_a's • Elevated BMR and BAT thermogenic activity • Increased activity of hypothalamic-pituitary-thyroid axis • Decreased thermal conductance • Decreased shivering threshold • Increased food consumption	• Unchanged NST capacity • Unchanged BAT thermogenic capacity (GDP binding) • Thyroid hormone levels within the reptilian range • No augmentation of insulatory fat deposition

Table 11.2. Typical and atypical reptilian responses of cold-acclimated (25°C) naked mole-rats (*Heterocephalus glaber*).

Typical Reptilian Responses	Atypical Reptilian Responses
• Left-shift of their poikilothermic-T_a profile • Increased T_b-T_a differentials • Selection of lower preferred T_b's • Greater cold tolerance of physiological and locomotor function • Decreased vascular-mediated thermal conductance	• Increased thyroid activity • Increased food consumption and energy assimilation

Basal metabolism of cold-acclimated naked mole-rats increased by 50%. This, together with a vascular-mediated reduction in conductance, rather than increased insulatory fat deposition, resulted in a left-shift of the endothermic-T_a curve (Fig. 9.1). Although T_b was at no point regulated, the apparent TNZ (32-34°C) was extended and shifted to a lower T_a range of 27-34°C (Fig. 9.1), with the onset of heat production occurring at a T_b *ca.* 4°C lower than that exhibited by animals housed under simulated burrow conditions (Chapter 9). A substantial energy saving is thus facilitated for minimal metabolic expenditure is possible at a lower T_a range.

The increased BMR exhibited by naked mole-rats housed in the cold follows similar responses to that of many other cold-acclimated small mammals (Webster, 1974; Wunder, 1979; Whitaker *et al.*, 1990; Wiesinger *et al.*, 1990; Ellison *et al.*, 1992; Wunder and Gettinger, 1996). Like these other small mammals (Whitaker *et al.*, 1990; Wunder and Gettinger, 1996), the increased BMR of the naked mole-rat appears to be mediated by thyroid hormone activity as indicated by the similar incremental changes in these two variables (Chapter 8). However, thyroid hormone levels, even with cold-acclimation, fall within the reptilian range, an order of magnitude below mammalian levels (Chapter 8) and these low levels of metabolic regulatory hormones may explain their very low basal metabolism in both control and cold conditions (Chapter

8). Despite the advantages of the left-shift in the TNZ, an elevated BMR is energetically costly to maintain as it represents obligatory heat production which cannot be reduced below a fundamental threshold even when there is no immediate heat drain on the animal. An elevated BMR is normally sustained by increased food intake, facilitated by augmented gut capacity and improved digestibility. Despite lower T_b's of cold-acclimated naked mole-rats, food consumption was increased (45%) and digestibility maintained, with the increased energy intake matching the augmented BMR (50%). Sustained digestibility at extremely efficient levels was unexpected and may be attributed to the contribution of microbial fermentation processes in the hindgut. Caecal temperature is normally maintained lower than core T_b, and functions optimally at lower temperatures than that of other hindgut fermenters (Yahav and Buffenstein, 1991a & 1992). The lower T_b may therefore not negatively impact upon fermentation processes and this together with increased caecal capacity may enable the sustained processing of the additional food intake.

In contrast to augmented basal metabolism, NST is considered an essential component of cold-acclimation in small mammals (Heldmaier *et al.*, 1982), and unlike BMR, NST is only expressed when there is an immediate cold stress challenging homeothermic maintenance (Jansky, 1973). Improving NST capacity in the cold is thus more energetically expedient than increasing basal metabolism.

In keeping with their unusual thermal biology, naked mole-rats exhibited atypical NST responses to that of most small mammals, by not increasing BAT mass nor NST capacity in the cold (Table 7.1). This was evident by:

1) no increase in noradrenaline-induced NST (Chapter 6)
2) unchanged BAT mitochondrial GDP binding (Chapter 7), a
3) no change in peak resting metabolic rate (Chapter 9).

As naked mole-rats have inhabited a thermally stable, subterranean milieu for millennia (Lavocat, 1978), from an evolutionary perspective, one might argue that they have lost the ability to exhibit long-term cold-acclimatory responses. Indeed, cold-acclimated naked mole-rats showed only typical acute responses (increased BAT thermogenic activity) to prolonged cold exposure, rather than the expected chronic response (increased BAT thermogenic capacity; Chapter 7). Furthermore, their social lifestyle under simulated burrow temperatures, facilitates homeothermy by behavioural means (Yahav and Buffenstein, 1991b). Indeed, huddling behaviour in small mammals has been shown to reduce the requirements for BAT thermogenesis and results in a suppression of thermogenic capacity (Himms-Hagen and Villemure, 1992), although BAT thermogenesis may nevertheless be an essential part of successful huddling during cold exposure (Sokoloff and Blumberg, 2000). In naked mole-rats however, BAT thermogenic capacity, rather than being suppressed,

appears to be maximal even at temperatures within the apparent TNZ and is constantly primed for thermogenesis. Regardless of thermal history, naked mole-rats have enormous BAT reserves, twice that of cold-acclimated (4°C) mice, with similar GDP binding properties (Chapter 7). Further indications of their extreme cold sensitivity and inherently high thermogenic capacity is illustrated by the pronounced rate of increase in metabolism at T_a's only a few degrees below thermoneutrality. This change in metabolism is of similar magnitude to that exhibited by other small mammals during severe cold stress (5°C; e.g. hamsters, Dicker *et al.*, 1995). Extreme cold sensitivity (as indicated by these markers of thermogenic activity and capacity), is attributed to the extremely high thermal conductance of their naked skin, resulting in rapid heat flux (Buffenstein and Yahav, 1991a; Chapter 9). It would be energetically wasteful and extremely costly for cold-acclimated naked mole-rats to increase BAT thermogenic capacity further, when the insulatory properties of their skin are such that heat cannot be retained. Indeed, the costs of endothermy become prohibitive at comparatively warm T_a's (20°C, cold-acclimated; 25° C, thermoneutral-acclimated), with the result that endothermy is abandoned at T_a's below these limits (Fig. 9.1).

The shivering threshold with prolonged cold exposure was shifted to lower T_b's (Table 9.1), thus allowing the cold-acclimated naked mole-rat to utilize NST at a lower

range of temperatures before the less economical shivering mechanism is evoked. This is typical of cold-acclimated mammals, although the shift in the shivering threshold usually coincides with an increase in NST capacity (Heldmaier *et al.*, 1985). Shivering would then only be utilized at low T_a's when the magnitude of the NST response is insufficient (Banet and Hensel, 1977; Bockler and Heldmaier, 1983; Heldmaier *et al.*, 1989). Indeed, both the intensity and frequency of shivering were greater in the thermoneutral-acclimated animals, such that they maintained higher T_b's than the cold-acclimated animals over the T_a range at which shivering was noted (Chapter 9).

Cold-acclimated naked mole-rats exhibited greater T_b's than the thermoneutral-acclimated control group at T_a's below 18°C and within their TNZ (27-34°C; Fig. 9.1). The temperature differential (T_b-T_a = 2.8-4.7°C) maintained by cold-acclimated naked mole-rats within their TNZ is 3 times greater than that of the control group over their respective TNZ (0.9-1.6°C). This is attributed to the combined effects of augmented basal heat production and pronounced changes in thermal conductance. Changes in thermal conductance are attributed to vascular mediated modifications rather than increased insulatory fat deposition. At high T_a's however, cold-acclimated naked mole-rats selected lower preferred T_b's, suggesting a change in the thermal optimum of their metabolic enzyme systems. This was also evident from their superior

physiological and locomotor function at lower T_b's (Chapter 9). Similarly, many cold-acclimated reptiles are reported to select lower preferred T_b's but with concomitant down-regulation of physiological systems (Huey, 1982; Geiser *et al.*, 1992; Rome *et al.*, 1992; Hulbert, 1993).

Although only total fat mass was measured and not regional locations of fat deposition, it is highly likely that insulatory fat depositions will be subcutaneous and that the regional distribution of body fat could be an important component of the measured changes in thermal conductance of the body. Surprisingly, insulatory fat deposition was also not augmented by prolonged cold exposure (Chapter 9; Table 9.3). This may be due to energetic constraints imposed by the increased thermoregulatory demands and gut capacity limitations. Evolution may however have selected against excess fat deposition as this would impede heat loss, thereby increasing the chances of overheating and thermal death in their hot, humid closed burrows which preclude heat loss by evaporative and convective means. Evolution of eusociality may also be adaptive in this regard, as the energetic demands of foraging are shared amongst many individuals in the colony, and in so doing, may preclude the need for energy storage.

Thermoregulatory and energetic responses of cold-acclimation in naked mole-rats, results in a greater

proportion of the available energy being apportioned to thermoregulation, and this impacts upon other components of the energy budget. For instance, activity levels of naked mole-rats in the cold appeared to be markedly reduced and they exhibited pronounced declines in reproductive success.

REPRODUCTIVE CONSEQUENCES OF COLD EXPOSURE

Reproduction in naked mole-rats is profoundly sensitive to changes in T_a and concomitant changes in T_b. Their endothermic, yet poikilothermic mode of thermoregulation coupled with their small size and limited energy reserves impact considerably upon reproductive function.

The response of naked mole-rats to short-term cold exposure is similar to that of most small mammals, with fat preferentially mobilized to fuel thermogenesis, accounting for 94% of the mass loss (Figs. 10.2 & 10.3). Thus during pregnancy every effort is made to conserve lean mass at the expense of stored fat, with protein reserves only being drawn upon during the early pregnancy cold bouts (Fig. 10.2), when fat reserves are low. Similar responses are shown by other small mammals, but at much more severe temperatures. For instance, mice when kept at -8°C, start utilizing protein in addition to fat stores and at these temperatures ovulation is suppressed and survival becomes compromised (Manning and Bronson, 1990).

Preferential use of fat reflects the importance of lean mass conservation, for their natural diet is low in protein (Jarvis, 1985; Brett, 1991b), and the costs of increased protein turnover are great (Kelly and McBride, 1990). Furthermore, protein reserves rather than adiposity are implicated to be of greater importance in reproductive function (Britt *et al.*, 1988). As such, maternal lean mass depletion may impact negatively on reproductive success. Indeed, naked mole-rats appear to be more vulnerable to the adverse effects of cold exposure during the early stages of pregnancy, when maternal fat and lean mass depletion were greatest (Chapter 10; Fig. 10.2). The repercussions of this were far reaching with a substantially longer extension of the interbirth interval (Chapter 10).

Despite the metabolic and behavioural responses of pregnant naked mole-rats to short-term cold bouts, pregnancy in these unusual mammals is profoundly sensitive to temperature variations. Such a striking temperature dependence of pregnancy is normally characteristic of poikilothermic ectotherms (e.g. reptiles; Beuchat, 1988).

The optimal temperature for foetal development in naked mole-rats appears to be higher than eccritic non-pregnant temperature (Buffenstein *et al.*, 1996), and presumably enhances the rate of maternal-foetal energy transfer resulting in higher foetal growth and development

rates. Although T_b of the cold-acclimated females was significantly lower at each stage of pregnancy, the temperature differential (T_b-T_a) was much greater (6.9-8.7°C, cold-acclimated; 3.7-4.5°C, control). Furthermore, cold-acclimated females showed an earlier and more substantial rise in T_b (Fig. 10.4), coinciding with the largest increment in percent body fat (64% increment from early- to mid-pregnancy), suggesting that fat deposition during the early stages of pregnancy may have a thermoregulatory role. The earlier elevation of T_b and the higher temperature differential maintained by the cold-acclimated females may facilitate optimal foetal growth rates despite the lower T_b's.

Chronic cold exposure in the naked mole-rat prolonged both the interbirth interval and gestation length, the latter due to a significant lengthening (20%) of the second half of the gestation period by approximately 8 days. For most mammals even severe cold has little effect on gestation length (e.g. rats, mice, and hamsters; see, Racey, 1981). Notable exceptions however are those pregnant animals with labile T_b's, with reports of extended gestation in torpid pregnant metatherians and eutherians (Racey, 1973; Racey and Swift, 1981; Geiser and Masters, 1994). Similarly, T_b influences gestation length of viviparous reptiles. These animals however, are profoundly sensitive to temperature and gestation length can vary nearly two-fold for a change in T_b of only 2°C (Beuchat,

1988). Changes in gestation length with prolonged cold exposure in the naked mole-rat is not as extreme as in viviparous reptiles, although these rodents are more sensitive to declines in T_b than other mammals. A drop in T_a of only 5°C resulted in T_b's approximately 2°C lower than normal and a 12% lengthening of the gestation period.

Despite lower T_b's in the cold, foetal growth rate was surprisingly unchanged, suggesting that foetal growth rates may be less sensitive to temperature effects. The longer gestation period therefore culminated in the birth of larger pups with significantly greater lean mass. Similar findings have been reported in viviparous lizards, such that those incubated at lower temperatures were significantly larger than those incubated at higher temperatures (Be chat, 1988). Barnett *et al.* (1975) found that house mice maintained at a colder temperature reared fewer young, but their litters were larger at birth and their young heavier. Large pup size may reduce the heat flux that a young pup may be subjected to in the cold, and contribute to their unchanged survival rates in the cold. In mice, the high energetic costs of sustaining thermoregulation and reproduction impact upon the proportion of energy available for foetal development and this may account for the smaller litter size. This was not apparent in the naked mole-rat, for litter size did not decline in the cold.

Post-weaning pup growth rates were markedly reduced (38%) in the cold. This was not unexpected and may reflect the well known temperature effect on growth and development in ectotherms (Cloudsley-Thompson, 1971). Indeed, the 38% reduction in pup growth rate is consistent with that which might be expected due to a purely Q_{10} effect.

A substantial metabolic cost is incurred by breeding females in the cold. This impacts considerably upon maternal body composition and in particular results in maternal lean mass depletion, and probably accounts for the fact that very few females gave birth to more than one litter in the cold. Indeed, one breeding female experienced a dramatic reduction in body mass (34% in 7 days) after having given birth to her second litter in the cold, was hypothermic and lethargic and subsequently died. Further reductions in both body fat and lean mass with lactation, may push protein reserves to critical limits, further suppressing reproductive effort. Increasing the duration of pregnancy and producing larger more mature pups, may enable the cold-acclimated female to shorten the proportion of the reproductive event spent lactating, thus facilitating an energy saving given the high energetic costs incurred during lactation (Urison and Buffenstein, 1995).

In conclusion, whilst a high thermal conductance and low BMR are advantageous in the hot, humid burrows, where they limit heat gain and the chances of thermal death, they markedly restrict the ability of the naked mole-rat to adapt to cold. Whilst capable of NST (Hislop and Buffenstein, 1994; Chapter 6), total NST capacity was not augmented by cold-acclimation. Indeed, no attempt is made to utilize this source of heat production even at moderately low T_a's (below 25°C for thermoneutral-acclimated animals and below 20°C for cold-acclimated animals; Fig. 9.1). Clearly, the rapid heat flux across the uninsulated, highly conductive skin of naked mole-rats makes it energetically untenable to sustain costly thermogenic activities. Rather, the cold-acclimated naked mole-rat increases basal metabolism by thyroid mediated processes. An elevated BMR together with increased insulation due to vascular responses rather than increased fat deposition, facilitate a lowering of the lower critical temperature, such that the TNZ is extended and shifted to a lower T_a range. Nevertheless, the increased BMR is energetically costly to maintain, necessitating a substantial increase in food consumption (45%), mediated by augmented caecal capacity. The large increase in food intake raises the question of whether caecal function is the limiting step inducing the metabolic ceiling evident under a wide range of energetically costly processes. Indeed, the additional thermoregulatory costs of cold exposure impacted negatively on reproductive success. The

cessation of reproduction, rather than representing a dysfunction, may serve as an adaptation to conserve maternal resources, allowing for the repartitioning of available energy in favour of physiological processes that are essential for the survival of the breeding female. For the few successful pregnancies in the cold (13.5% breeding success), the increased gestation length culminated in the birth of larger pups with significantly greater metabolically active lean mass which are more adept at surviving at the lower temperature. Naked mole-rats exhibit a marked temperature sensitivity of pregnancy, intermediate between that seen in typical poikilothermic ectotherms and that of heterothermic bats and marsupials. Such an extreme sensitivity to environmental fluctuations in T_a, both in reproductive and non-pregnant, non-lactating naked mole-rats may explain their limited distribution to the thermostable milieu of the north-east African soils.

REFERENCES

Abelenda M., and M.L. Puerta (1987). Inhibition of diet-induced thermogenesis during pregnancy in the rat. *Pflugers Archives* 409: 314-317.

Aleksuik M. (1971). Temperature-dependent shifts in metabolism in a cool temperate reptile *Thamnophis sirtalis parietalis*. *Comparative Biochemistry and Physiology* 39A: 495-503.

Alexander G., and D. Williams (1971). Heat stress and development of the conceptus in domestic sheep. *Journal of Agricultural Science* 76: 53-72.

Anon, IUPS Thermal Commission (1987). Glossary of terms of thermoregulatory physiology. *Pflugers Archives* 410: 567-587.

Ar A. (1986). Physiological adaptations to underground life in mammals. In: *Comparative Physiology of Environmental Adaptation*. Edited by P. Dejours. Basel, Karger. p. 208-211.

Ar A. (1987). Physiological adaptations to underground life in mammals. A case of mammalian neoteny? In: *Comparative Physiology of Environmental Adaptation*. Vol 2. Edited by P. Dejours. Basel, Kargel. p. 208-221.

Arieli R. (1977). The atmospheric environment of the fossorial mole-rat (*Spalax ehrenbergi*): effects of season, soil texture, rain, temperature and activity. *Comparative Biochemistry and Physiology* A63: 569-575.

Asano A., Nikami H., Morimatsu M., and M. Saito (1996). Vascular endothelial growth factor in brown adipose tissue: role in angiogenesis during tissue growth after cold exposure. In: *Adaptations to the Cold: Tenth International Hibernation Symposium*. Edited by F. Geiser, A.J. Hulbert, and S.C. Nicol. Armidale, University of New England Press. p. 299-303.

Ashwell M., Jennings S., Richard D., Stirling D.M., and P. Trayhurn (1983). Effects of acclimation temperature on the concentration of the "uncoupling" protein measured by radioimmuno-assay in mouse brown adipose tissue. *FEBS Letters* 161: 108-112.

Assali N.S., and B. Westin (1962). Effect of hypothermia on uterine circulation and on the fetus. *Proceedings of the Society of Experimental Biology and Medicine* 109: 485-488.

Atkinson D., and R.M. Sibly (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *TREE* 12: 235-239.

Banet M., and Hensel (1977). The control of shivering and nonshivering thermogenesis in the rat. *Journal of Physiology (London)* 269: 669-676.

Barbault R. (1988). Body size, ecological constraints, and the evolution of life-history strategies. In: *Evolutionary Biology*. Edited by M.K. Hecht, B. Wallace, and G.T. Prance. New York, Plenum Press. p. 261-286.

Barnett S.A. (1965). Adaptation of mice to cold. *Biological Reviews* 40: 5-51.

Barnett S.A. (1973). Maternal processes in the cold-adaptation of mice. *Biological Reviews* 48: 477-508.

Barnett S.A., Munro K.M.H., Smart J.L., and R.C. Stoddart (1975). House mice bred for many generations in two environments. *Journal of Zoology (London)* 177: 153-169.

Bartholomew G.A., and V.A. Tucker (1964). Size and body temperature: thermal conductance, oxygen consumption and heart rate in Australian varanid lizards. *Physiological Zoology* 37: 341-354.

Bartholomew G.A. (1977). Energy metabolism. In: *Animal Physiology: Principles and Adaptations*. Edited by M.S. Gordon, G.A. Bartholomew, A.D. Ginnel, C.B. Jorgensen, and F.N. White. New York, MacMillan. p. 57-110.

Bartness T.J., and G.N. Wade (1984). Photoperiodic control of body weight and energy metabolism in Syrian hamsters (*Mesocricetus auratus*): role of the pineal gland, melatonin, gonads and diet. *Endocrinology* **114**: 492-498.

Bennett A.F., and J.A. Ruben (1979). Endothermy and activity in vertebrates. *Science* **206**: 649-654.

Bennett N.C., Jarvis J.U.M., and K.C. Davis (1988). Daily and seasonal temperatures in the burrows of african rodent moles. *South African Journal of Zoology* **23**: 189-195.

Bennett N.C., and J.U.M. Jarvis (1993). Poikilothermic traits and thermoregulation in the Afrotropical social subterranean Mashona mole-rat (*Cryptomys hottentotus darlingi*) (Rodentia: Bathyergidae). *Journal of Zoology (London)* **231**: 179-186.

Bennett N.C., and J.U.M. Jarvis (1995). Coefficients of digestibility and nutritional value of geophytes and tubers eaten by southern African mole-rats (Rodentia: Bathyergidae). *Journal of Zoology (London)* **236**: 189-198.

Beuchat C.A. (1986). Reproductive influences on the thermoregulatory behaviour of a live-bearing lizard. *Copeia* **4**: 971-979.

Beuchat C.A., and S. Ellner (1987). A quantitative test of life history theory: thermoregulation by a viviparous lizard. *Ecological Monographs* 57: 45-60.

Beuchat C.A. (1988). Temperature effects during gestation in a viviparous lizard. *Journal of Thermal Biology* 13: 135-142.

Bianco A.C., and J.E. Silva (1987a). Intracellular conversion of thyroxine to triiodothyronine is required for optimal thermogenic function of brown adipose tissue. *Journal of Clinical Investigations* 79: 295-300.

Bianco A.C., and J.E. Silva (1987b). Optimal response of key enzymes and uncoupling protein to cold in BAT depends on local T_3 generation. *American Journal of Physiology* 253: E255-E263.

Bianco A.C., and J.E. Silva (1988). Cold exposure rapidly induces virtual saturation of brown adipose tissue nuclear T_3 receptors. *American Journal of Physiology* 255: E496-E503.

Bianco A.C., Sheng X., and J.E. Silva (1988). Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *Journal of Biological Chemistry* 263: 18168-18175.

Biggers J.D., Ashroub M.R., McLaren A., and D. Michie (1958). The growth and development of mice in three climatic environments. *Journal of Experimental Biology* 35: 144-155.

Binyon E.J., and G.I. Twigg (1965). Seasonal changes in the blood and thyroid of the grass snake, *Natrix natrix*. *Nature* 207: 779-780.

Bligh J. (1973). *Temperature Regulation in Mammals and other Vertebrates*. New York, North-Holland. p. 276-278.

Bloom W., and D.W. Fawcett (1975). *Textbook of Histology*. 10th Ed. W.B. East Sussex, Saunders Company.

Bockler H., and G. Heldmaier (1983). Interaction of shivering and nonshivering thermogenesis during cold exposure in seasonally-acclimated Djungarian hamsters (*Phodopus sungorus*). *Journal of Thermal Biology* 8: 97-98.

Bona-Gallo A., Licht P., Mackenzie D.S., and B. Lofts (1980). Annual cycles in levels of pituitary and plasma gonadotropin, gonadal steroids, and thyroid activity in the Chinese cobra (*Naja naja*). *General and Comparative Endocrinology* 42: 477-493.

Boulouard R. (1963). Effects of cold and starvation on adrenocortical activity in rats. *Federation Proceedings*

22: 750-753.

Boulouard R. (1966). Adrenocortical activity during adaptation to cold in the rat: role of Porter-Silber chromogens. *Federation Proceedings* 25: 1195-1199.

Brabant G., Ranft U., Ocran K., Hesch R.D., and A. von zur Muhlen (1986). Thyrotropin - an episodically secreted hormone. *Acta Endocrinologica.* 112: 315-322.

Brabant G., Prank K., Ranft U., Schuermeyer T., Wagner T.O., Hauser H., Kummer B., Feistner H., Hesch R.D., and A. von zur Muhlen (1990). Physiological regulation of circadian and pulsatile thyrotropin secretion in normal men and woman. *Journal of Clinical Endocrinology and Metabolism* 70: 403-409.

Brett R.A. (1991a). The population structure of naked mole-rat colonies. In: *The Biology of the Naked Mole-Rat.* Edited by P.W. Sherman, J.U.M. Jarvis, and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 97-136.

Brett R.A. (1991b). The ecology of the naked mole-rat: burrowing, food and limiting factors. In: *The Biology of the Naked Mole-Rat.* Edited by P.W. Sherman, J.U.M. Jarvis, and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 137-184.

Britt J.H., Armstrong J.D., and N.M. Cox (1988). Metabolic interfaces between nutrition and reproduction in pigs. *11th International Congress of Animal Reproduction and Artificial Insemination*. Dublin, Ireland. Vol. 5. p. 117-125.

Brody S. (1945). *Bioenergetics and growth, with special reference to the efficiency complex in domestic animals*. New York, Reinhold.

Bronson F.H., and S. Pryor (1983). Ambient temperature and reproductive success in rodents living at different latitudes. *Biology of Reproduction* 29: 72-80.

Bronson F.H. (1985). Mammalian reproduction: an ecological perspective. *Biology of Reproduction* 32: 1-26.

Bronson F.H. (1988). Effect of food manipulation on the gonadotropin releasing hormone-luteinizing hormone-oestradiol axis of the young female rat. *American Journal of Physiology* 254: R616-R621.

Bruck K. (1970). Nonshivering thermogenesis and brown adipose tissue in relation to age, and their integration in the thermoregulatory system. In: *Brown Adipose Tissue*. Edited by O. Lindberg. Amsterdam, Elsevier. p. 117-154.

Bruck K. (1986). Basic mechanisms in thermal long-term and short-term adaptation. *Journal of Thermal Biology* 11: 73-77.

Bruck K., and E. Zeisberger (1987). Adaptive changes in thermoregulation and their neurophysiological basis. *Pharmacology and Therapeutics* 35: 163-215.

Buffenstein R., and G. Louw (1982). Temperature effects on bioenergetics of growth, assimilation efficiency and thyroid activity in juvenile varanid lizards. *Journal of Thermal Biology* 7: 197-200.

Buffenstein R. (1984). Energy and water balance during torpor and hydropenia in the pigmy gerbil, *Gerbillus pusillus*. *Journal of Comparative Physiology* B154: 535-544.

Buffenstein R. (1985). The effect of starvation, food restriction, and water deprivation on thermoregulation and average daily metabolic rates in *Gerbillus pusillus*. *Physiological Zoology* 58: 320-328.

Buffenstein R., and S. Yahav (1991a). Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *Journal of Thermal Biology* 16: 227-232.

Buffenstein R., and S. Yahav (1991b). Cholecalciferol has no effect on calcium and inorganic phosphorus balance in a naturally cholecalciferol-deplete subterranean mammal, the naked mole-rat (*Heterocephalus glaber*). *Journal of Endocrinology* 129: 21-26.

Buffenstein R., and S. Yahav (1991c). The effect of diet on microfaunal population and function in the caecum of a subterranean mole-rat, *Heterocephalus glaber*. *British Journal of Nutrition* 65: 249-258.

Buffenstein R., Jarvis J.U.M., Opperman L.A., Cavaleros M., Ross F.P., and J.M. Pettifor (1994). Subterranean mole-rats naturally have an impoverished calciol status, yet synthesize calciol metabolites and calbindins. *European Journal of Endocrinology* 130: 402-409.

Buffenstein R. (1996). Ecophysiological responses to a subterranean habitat; a Bathyergid perspective. *Mammalia* 60: 591-605.

Buffenstein R., Urison N.T., Woodley R., van der Westhuizen L.A., and J.U.M. Jarvis (1996). Temperature changes during pregnancy in the subterranean naked mole-rat (*Heterocephalus glaber*); the role of altered body composition and basking behaviour. *Mammalia* 60: 619-628.

Bukowiecki L., Collet A.J., Follea N., Guay G., and L. Jahjah (1982). Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia. *American Journal of Physiology* 242: E353-E359.

Burger J., Zappalorti R.T., and M. Gochfeld (1987). Developmental effects of incubation temperature on hatchling pine snakes *Pituophis melanoleucus*. *Comparative Biochemistry and Physiology* 87A: 727-732.

Cagampang F.R.A., Maeda K.I., Yokoyama A., and K. Ota (1990). Effect of food deprivation on the pulsatile LH release in the cycling and ovariectomized female rat. *Hormone and Metabolic Research* 22: 269-272.

Cagampang F.R.A., Maeda K.I., Tsukamura H., Ohkura S., and K. Ota (1991). Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats. *Journal of Endocrinology* 129: 321-328.

Cameron I.L., and R.E. Smith (1964). Cytological responses of brown fat tissue in cold exposed rats. *Journal of Cell Biology* 23: 89-100.

Cannon B., and O. Lindberg (1979). Mitochondria from brown adipose tissue: Isolation and properties. *Methods in*

Enzymology 55: 65-78.

Cannon B., and J. Nedergaard (1983). Biochemical aspects of acclimation to cold. *Journal of Thermal Biology* 8: 85-90.

Cannon B., and J. Nedergaard (1985). The biochemistry of an inefficient tissue: brown adipose tissue. *Essays in Biochemistry* 20: 11063-11082.

Cannon B., and J. Nedergaard (1989). Regulation of the amount and activity of the uncoupling protein thermogenin in brown adipose tissue. In: *Anion Carriers of Mitochondrial Membranes*. Edited by A. Azzi, K.A. Naecz, M.J. Naecz and L. Wojtozak. Berlin, Heidelberg: Springer-Verlag. p. 269-281.

Cannon B., and J. Nedergaard (1998). Nonshivering thermogenesis and brown adipose tissue. In: *Physiology and Pathophysiology of Temperature Regulation*. Edited by C.M. Blatteis. Singapore, World Scientific Publishing.

Carey C. (1978). Factors affecting body temperature in toads. *Oecologia* 35: 179-219.

Carey H.V. (1993). Regulation of gut structure and function in hibernators. In: *Life in the Cold*. Edited by C.Carey, G.L. Florant, B.A. Wunder and B. Horwitz. Oxford,

Westview Press. p. 155-166.

Carneheim C., Nedergaard J., and B. Cannon (1984). ß-Adrenergic stimulation of lipoprotein lipase in rat brown adipose tissue during acclimation to cold. *American Journal of Physiology* 246: E327-E333.

Carrascal L.M., Lopez P., Martin J., and A. Salvador (1992). Basking and antipredator behaviour in a high altitude lizard: implications of heat exchange rate. *Ethology* 92: 143-154.

Case T.J. (1978). On the evolution and adaptive significance of post-natal growth rates in the terrestrial vertebrates. *Quarterly Review of Biology* 53: 243-282.

Chaffee R.R.J., and J.C. Roberts (1971). Temperature acclimation in birds and mammals. *Annual Review in Physiology* 33: 155-202.

Chang M.C., and L. Fernandez-Cano (1959). Effect of short changes of environmental temperature and low atmospheric pressure on the ovulation of rats. *American Journal of Physiology* 196: 653-655.

Charland M.B., and P.T. Gregory (1990). The influence of female reproductive status on thermoregulation in a viviparous snake, *Crotalus viridis*. *Copeia*: 1089-1098.

Cloudsley-Thompson J.L. (1971). *The Temperature and Water Relations of Reptiles*. Merrow. Watford.

Colquhoun E.Q., and M.G. Clark (1991). Open question: has thermogenesis in muscle been overlooked and misinterpreted. *News in Physiological Sciences* 6: 256-259.

Contreras L.C. (1984). Bioenergetics of huddling: test of a psycho-physiological hypothesis. *Journal of Mammalogy* 65: 256-262.

Cossins A.R., and K. Bowler (1987). *Temperature Biology of Animals*. London, Chapman and Hall.

Cottle W.H. (1960). Role of thyroid secretion in cold acclimation. *Federation Proceedings* 19: 59-63.

Courrier R., and M. Marois (1953). Action de I'hypothermie experimentale sur la gestation chez le rat. *C.r. Seanc. Soc. Biol.* 147: 1922-1924.

Czajkowski W. (1958). The effect of hypothermia on the duration of pregnancy in the Golden hamster. *Folia biol. Krakow* 6: 195-202.

Daly T.J.M., Williams L.A., and R. Buffenstein (1997). Catecholaminergic innervation of interscapular brown adipose tissue in the naked mole-rat (*Heterocephalus*

glaber). *Journal of Anatomy* 190: 321-326.

Daly T.J.M., and R. Buffenstein (1998). Skin morphology and its role in thermoregulation in mole-rats, *Heterocephalus glaber* and *Cryptomys hottentotus*. *Journal of Anatomy* 193: 495-502.

Daly T.J.M., Alison B., Thomadakis C., and R. Woodley. Cold-induced changes in gut structure and function in a poikilothermic mammal, the naked mole-rat (*Heterocephalus glaber*). in prep.

Dawson T.J., and J.M. Olson (1988). Thermogenic capabilities of the opossum *Monodelphis domestica* when warm- and cold-acclimated: Similarities between American and Australian marsupials. *Comparative Biochemistry and Physiology* A89: 85-91.

Dawson W.R., and R.L. Marsh (1989). Metabolic acclimatization to cold and season in birds. In: *Physiology of Cold Adaptation in Birds*. Edited by C. Bech and R.E. Reinertsen. New York, Plenum. p. 83-94.

De Graaf G. (1981). *The Rodents of Southern Africa.* Johannesburg, RSA, Butterworths.

Denckla W.D., and E. Marcum (1973). Minimal O_2 consumption as an index of thyroid status: standardization of method.

Endocrinology 93: 61-73.

Depocas F., and J.S. Hart (1957). Use of the Pauling oxygen analyser for measurement of oxygen consumption of animals in open circuit systems and in a short-lag closed-circuit apparatus. *Journal of Applied Physiology* 10: 388-392.

Depocas F. (1960). The calorigenic response of cold-acclimated white rats to infused noradrenaline. *Canadian Journal of Biochemistry and Physiology* 38: 107-114.

Dicker A., Cannon B., and J. Nedergaard (1995). Cold-acclimation-recruited nonshivering thermogenesis: the Syrian hamster is not an exception. *American Journal of Physiology* 269: R767-R774.

Dreisig H. (1984). Control of body temperature in shuttling ectotherms. *Journal of Thermal Biology* 9: 229-233.

Eckert R., and D. Randall (1983). *Animal Physiology - Mechanisms and Adaptations*. San Franscisco, W.H. Freeman and Co.

Eisentraut M. (1937). Die Wirkung niedriger Temperaturen auf die Embryonentwicklung bei Fledermausen. *Biol. Zbl.* 57: 59-74.

Ellison G.T.H., and J.D. Skinner (1990). Noradrenaline thermogenesis in conscious and anaesthetized pouch mice (*Saccostomus campestris*). *Comparative Biochemistry and Physiology* 97A: 23-26.

Ellison G.T.H., Skinner J.D., and A. Haim (1992). The relative importance of photoperiod and temperature as cues for seasonal acclimation of thermoregulation in pouched mice (*Saccostomus campestris*: Cricetidae) from southern Africa. *Journal of Comparative Physiology* B162: 740-746.

Ellner S.P., and C.A. Beuchat (1984). A model of optimal thermoregulation during gestation by *Sceloporus jarrovi*, a live-bearing lizard. In: *Mathematical Ecology*. Edited by S.A. Levin and T.G. Hallam. Proceedings, Trieste 1982, *Lecture Notes in Biomathematics*, 54. Springer, New York. p. 15-28.

Else P.L., and A.J. Hulbert (1981). Comparison of the "mammal machine" and the "reptile machine": energy production. *American Journal of Physiology* 240: R3-R9.

Else P.L., and A.J. Hulbert (1987). Evolution of mammalian endothermic metabolism: "leaky" membranes as a source of heat. *American Journal of Physiology* 253: R1-R7.

Evans L.T., and E. Hegre (1938). The effects of ovarian hormones and seasons on *Anolis carolinensis*. I. The

thyroid. *Anatomical Records* 72: 1-9.

Faulkes C.G., Abbott D.H., and J.U.M. Jarvis (1989). Reproductive suppression in female naked mole-rats, *Heterocephalus glaber*. In: *Comparative Reproduction in Mammals and Man. Proceedings of the N.C.R. Conference Nairobi*, November 1987. Edited by R.M. Eley. Nairobi, Institute for Primate Research/National Museums of Kenya. p. 155-161.

Faulkes C.G., Abbott D.H., and J.U.M. Jarvis (1990). Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats *Heterocephalus glaber*. *Journal of Reproduction and Fertility* 88: 559-568.

Feder M.E. (1982). Environmental variability and thermal acclimation of metabolism in tropical anurans. *Journal of Thermal Biology* 7: 23-28.

Feil S., and J. Rafael (1994). Effect of acclimation temperature on the concentration of uncoupling protein and GDP binding in rat brown fat mitochondria. *European Journal of Biochemisty* 219: 681-690.

Feist D.D., and C.F. Feist (1986). Effect of cold, short day and melatonin on thermogenesis, body weight and reproductive organs in Alaskan red-backed voles, *Clethrionomys rutilus dawsonii*. *Journal of Comparative*

Physiology B156: 741-746.

Firth B.T., and J.S. Turner (1982). Sensory, neural and hormonal aspects of thermoregulation. In: *Biology of the Reptilia*. Edited by C. Gans. London, Academic Press. p. 213-274.

Fishman J., Boyar R.M., and L. Hellman (1975). Influence of body weight on estradiol metabolism in young women. *Journal of Clinical Endocrinology and Metabolism* 41: 989-991.

Folk G.E. (1974). *Textbook of Environmental Physiology (2nd Ed)*. Philadelphia, Lea and Febiger. p. 9-15.

Foster D.O., and M.L. Frydman (1978). Non-shivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of calorigenesis induced by noradrenaline. *Canadian Journal of Physiology and Pharmacology* 56: 110-122.

Foster D.O., and M.L. Frydman (1979). Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Canadian Journal of Physiology and*

Pharmacology 57: 257-270.

Foster D.O. (1984). Quantitative contribution of brown adipose tissue thermogenesis to overall metabolism. *Canadian Journal of Biochemistry and Cell Biology* 62: 618-622.

Foster D.O. (1986). Quantitative role of brown adipose tissue in thermogenesis. In: *Brown Adipose Tissue*. Edited by P. Trayhurn and D.G. Nicholls. London, Edward Arnold. p. 31-51.

Fox W., Gordon C., and M.H. Fox (1961). Morphological effects of low temperature during the embryonic development of the garter snake *Thamnophis elegans*. *Zoologica* 46: 57-71.

Fraser F.C., and J. Skelton (1978). Possible teratogenicity of maternal fever. *Lancet* 2: 634.

Fregly M.J. (1989). Activity of the hypothalamic-pituitary-thyroid axis during exposure to cold. *Pharmacology and Therapeutics* 41: 85-142.

Fregly M.J. (1990). Activity of the hypothalamic-pituitary-thyroid axis during exposure to cold. In: *Thermoregulation: Physiology and Biochemistry*. Edited by E. Schonbaum and P. Lomax. New York, Pergamon. p. 437-494.

French A.R. (1993). Hibernation in birds: comparisons with mammals. In: *Life in the Cold: Ecological, Physiological and Molecular Mechanisms*. Edited by C. Carey, G.L. Florant, B.A. Wunder, and B. Horwitz. Oxford, Westview Press. p. 43-54.

Geiser F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *Journal of Comparative Physiology* **B158**: 25-37.

Geiser F., Firth B.T., and R.S. Seymour (1992). Polyunsaturated dietary lipids lower the selected body temperature of a lizard. *Journal of Comparative Physiology* **B162**: 1-4.

Geiser F. (1993). Dietary lipids and thermal physiology. In: *Life in the Cold: Ecological, Physiological and Molecular Mechanisms*. Edited by C. Carey, G.L. Florant, B.A. Wunder, and B. Horwitz. Oxford, Westview Press. p. 141-153.

Geiser F. (1994). Hibernation and daily torpor in marsupials: a review. *Australian Journal of Zoology* 42: 1-16.

Geiser F., and P. Masters (1994). Torpor in relation to reproduction in the mulgara, *Dasycercus cristicauda*

(Dasyuridae: Marsupialia). *Journal of Thermal Biology* 19: 433-442.

Genoud M., and P. Vogel (1990). Energy requirements during reproduction and reproductive effort in shrews (Sorcidae). *Journal of Zoology (London)* 220: 41-60.

Gershon M.D., and E.A. Nunez (1988). The thyroid gland. In: *Cell and Tissue Biology. A Textbook of Histology.* Edited by L. Weiss. Baltimore, Urban and Schwarzenberg, Inc. p. 1009-1020.

Gier P.J., Wallace R.L., and R.L. Ingerman (1989). Influence of pregnancy on behavioral thermoregulation in the Northern Pacific rattlesnake *Crotalus viridis oreganus*. *Journal of Experimental Biology* 145: 465-469.

Goldman B.D., Goldman S.L., Lanz T., Magaurin A., and A. Maurice (1999). Factors influencing metabolic rate in naked mole-rats (*Heterocephalus glaber*). *Physiology and Behaviour* 66: 447-459.

Goode J., and J. Russel (1968). Incubation of eggs of three species of chelid tortoises, and notes on their embryological development. *Australian Journal of Zoology* 16: 749-761.

Gordon C.J. (1993). *Temperature Regulation in Laboratory Rodents*. Cambridge, Cambridge University Press.

Gourou P. (1970). *L'Afrique*. Paris. Hachette.

Greenspan S.L., Klibanski A., Schoenfeld D., and E.C. Ridgeway (1986). Pulsatile secretion of thyrotropin in man. *Journal of Clinical Endocrinology and Metabolism* 63: 661-669.

Gregory P.T. (1982). Reptilian hibernation. In: *Biology of the Reptilia, Volume 13*. Edited by C. Gans and F.H. Pough. London, Academic Press. p. 53-154.

Gyug L.W., and J.S. Millar (1980). Fat levels in a subartic population of *Peromyscus maniculatus*. *Canadian Journal of Zoology* 58: 1341-1346.

Haim A., van Arde R.J., and J.D. Skinner (1990). Metabolic rates, food consumption and thermoregulation in seasonal acclimatization of the Cape porcupine *Hystrix africaeaustralis*. *Oecologia* 83: 197-200.

Hales J.R.S., Fawcett A.A., Bennett J.W., and A.D. Needham (1978). Thermal control of blood flow through capillaries and arteriovenous anastomoses in skin of sheep. *Pflugers Archives*. 378: 55-63.

Hammond K.A., Konarzewski M., Torres R.M., and J. Diamond (1994). Metabolic ceilings under a combination of peak energy demands. *Physiological Zoology* 67: 1479-1507.

Hammond K.A., Lam M., Lloyd K.C.K., and J. Diamond (1996). Simultaneous manipulation of intestinal capacities and nutrient loads in mice. *American Journal of Physiology* 271: G969-G979.

Hammond K.A., and J.M. Diamond (1997). Maximal sustained energy budgets in humans and animals. *Nature* 386: 457-462.

Heaton G.M., Wagenvoord R.J., Kemp A., and D.G. Nicholls (1978). Brown adipose tissue mitochondria: photoaffinity labelling of the regulatory site for energy dissipation. *European Journal of Biochemistry* 82: 515-521.

Hefco E., Krulich L., Illner P., and P.R. Larsen (1975). Effect of acute exposure to cold on the activity of the hypothalamic-pituitary-thyroid system. *Endocrinology* 97: 1185-1195.

Heldmaier G. (1971). Zitterfreie warmebildung und korpergrosse bei saugetieren. *Z. Vergl. Physiol.* 73: 222-248.

Heldmaier G., and S. Steinlechner (1981a). Seasonal pattern and the energetics of short daily torpor in the

Djungarian hamster, *Phodopus sangorus*. *Oecologia* 48: 265-270.

Heldmaier G., and S. Steinlechner (1981b). Seasonal control of energy requirements for thermoregulation in the Djungarian hamster, *Phodopus sungorus*, living under natural photoperiod. *Journal of Comparative Physiology* 142: 429-437.

Heldmaier G., Steinlechner S., and J. Raphael (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimation in the Djungarian hamster. *Journal of Comparative Physiology* 149: 1-9.

Heldmaier G., and A. Buchberger (1985). Sources of heat during non-shivering thermogenesis in Djungarian hamsters: a dominant role of brown adipose tissue during cold adaptation. *Journal of Comparative Physiology* B156: 237-245.

Heldmaier G., Bockler H., Buchberger A., Lynch G.R., Puchalski W., Steinlechner S., and H. Wiesinger (1985). Seasonal acclimation and thermogenesis. In: *Circulation, Respiration, and Metabolism*. Edited by R. Gilles. Berlin, Heidelberg. Springer-Verlag. p490-501.

Heldmaier G., Klaus S., Wiesinger H., Friedrichs U., and M. Wenzel (1989). Cold acclimation and thermogenesis. In:

Living in the Cold, 2nd International Symposium. Edited by A. Malan and B. Canguilhem. London. INSERM/John Libbey Eurotext Ltd. p 347-358.

Heldmaier G., Klaus S., and H. Wiesinger (1990). Seasonal adaptation of thermoregulatory heat production in small mammals. In: *Thermoreception and Temperature Regulation*. Edited by J. Bligh, and K. Voigt. Berlin, Springer Verlag. p. 235-243.

Henshaw R.E. (1986). Regulation of heat circulation to the feet of mammals in chronic cold. In: *Living in the Cold: Physiological and Biochemical Adaptations*. Edited by H.C. Heller, X.J. Musacchia, and L.C.H. Wang. Amsterdam, Elsevier. p. 167-176.

Heroux O. (1959). Comparison between seasonal and thermal acclimation in white rats. II. Surface temperature, vascularization and *in vitro* respiration of the skin. *Canadian Journal of Biochemistry and Physiology* 37: 1247-1253.

Herron D., Rehnmark S., Nechad M., Loncar D., Cannon B., and J. Nedergaard (1990). Norepinephrine-induced synthesis of the uncoupling protein thermogenin (UCP) and its mitochondrial targeting in brown adipocytes differentiated in culture. *FEBS Letters* 268: 296-300.

Hill W.C.O., Porter A., Bloom J., Seago J., and M.D. Southwick (1957). Field and laboratory studies on the naked mole-rat (*Heterocephalus glaber*). *Proceedings of the Zoological Society (London)* 128: 455-513.

Hillyard F.W., and F. Elsaesser (1983). Concentration of progesterone in the backfat of pigs during the oestrous cycle and over ovariectomy. *Journal of Reproduction and Fertility* 69: 73-80.

Himms-Hagen J. (1986a). Brown adipose tissue and cold acclimation. In: *Brown Adipose Tissue*. Edited by P. Trayhurn and D.G. Nicholls. London, Edward Arnold. P. 214-268.

Himms-Hagen J. (1986b). Cold- versus diet-induced thermogenesis in brown adipose tissue: different strategies in different species. In: *Living in the Cold.* Edited by H.C. Heller, X.J. Musacchia, and L.C.H. Wang. New York, Elsevier. p. 93-100.

Himms-Hagen J. (1990). Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB Journal* 4: 2890-2898.

Himms-Hagen J., and C. Villemure (1992). Number of mice per cage influences uncoupling protein content of brown adipose tissue. *Society for Experimental Biology and Medicine* 200: 502-506.

Himms-Hagen J. (1997). Neural and hormonal responses to prolonged cold exposure. In: *Handbook of Physiology. Section 4: Environmental Physiology Vol. 1.* Edited by M.J. Fregly and C.M. Blatteis. Oxford, Oxford University Press.

Hislop M.S., and R. Buffenstein (1994). Noradrenaline induces non-shivering thermogenesis in both the naked mole-rat (*Heterocephalus glaber*) and the damara mole-rat (*Cryptomus damarensis*) despite very different modes of thermoregulation. *Journal of Thermal Biology* 19: 25-32.

Hissa R., and P. Hirsimaki (1971). Calorigenic effects of noradrenaline and Inderal in the temperature-acclimated golden hamster. *Comparative and General Pharmacology* 2: 217-224.

Hissa R. (1988). Controlling mechanisms in avian temperature regulation: a review. *Acta Physiologica Scandinavica Suppl.* 567: 1-148.

Hochachka P.W., and G.N. Somero (1984). *Biochemical Adaptation.* Princeton, N.J., Princeton University Press.

Honeycutt R.L., Allard M.W., Edwards S.V., and D.A. Schlitter (1991). Systematics and Evolution of the family Bathyergidae. In: *The Biology of the Naked Mole-Rat.* Edited by P.W. Sherman, J.U.M. Jarvis, and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 45-65.

Horwitz P.A. (1989). Biochemical mechanisms and control of cold-induced cellular thermogenesis in placental mammals. In: *Advances in Comparative and Environmental Physiology*, Vol.4. Edited by L.C.H. Wang. Berlin, Heidelberg. Springer-Verlag. p. 83-116.

Houpe K.L., Malo C., Oldham P.B., and R.K. Buddington (1996). Thermal modulation of channel catfish intestinal dimensions, BBM fluidity, and glucose transport. *American Journal of Physiology* 270: R1037-R1043.

Howland B.E., and E.A. Ebrahim (1973). Increased LH-suppressing effect of oestrogen in ovariectomized rats as a result of underfeeding. *Journal of Reproduction and Fertility* 35: 545-548.

Hudson H.W. (1978). Shallow daily torpor: a thermoregulatory adaptation. In: *Strategies in the Cold: Natural Torpidity and Thermogenesis*. Edited by L.C.H. Wang and J.W. Hudson. New York, Academic Press. p. 67-108.

Huey R.B. (1982). Temperature, physiology and the ecology of reptiles. In: *Biology of the Reptilia*, Volume 12. Edited by C. Gans and F.H. Pough. London, Academic Press. p. 25-91.

Huey R.B. (1991). Physiological consequences of habitat selection. *American Naturalist* 137: S91-S115.

Huey R.B., and D. Berrigan (1996). Testing evolutionary hypotheses of acclimation. In: *Phenotypic and Evolutionary Adaptation to Temperature*. Edited by I.A. Johnston and A.F. Bennett. Cambridge, U.K., Cambridge University Press. p. 205-237.

Huey R.B., Berrigan D., Golchrist G.W., and J.C. Herron (1999). Testing the adaptive significance of acclimation: a strong inference approach. *American Zoologist* 39: 323-336.

Hulbert A.J. (1978). The thyroid hormones: a thesis concerning their action. *Journal of Theoretical Biology* 73: 81-100.

Hulbert A.J. (1980). On the evolution of energy metabolism in mammals. In: *Comparative Physiology: Primitive Mammals*. Edited by K. Schmidt-Nielsen. Cambridge, Cambridge University Press. p. 129-139.

Hulbert A.J., and P.L. Else (1981). Comparison of the "mammal machine" and "reptile machine": energy use. *American Journal of Physiology* 241: R350-R356.

Hulbert A.J. (1988). Metabolism and the development of endothermy. In: *The Developing Marsupial*. Edited by C.H. Tyndale-Biscoe and P.A. Janssens. Berlin, Heidelberg Springer Verlag. p. 148-161.

Hulbert A.J. (1993). Membrane adaptations in ectotherms and endotherms. In: *Life in the Cold - Ecological, Physiological, and Molecular Mechanisms.* Edited by C. Carey, G.L. Florant, B A. Wunder, and B. Horwitz. Colorado, USA, Westview Press.

Hutchison V.H., Dowling H.G., and A. Vinegar (1966). Thermoregulation in a brooding female Indian python *Molorus bivittatus*. *Science* 151: 694-696.

Ingram D.L., and K.F. Legge (1971). The influence of deep body temperature and skin temperature on peripheral blood flow in the pig. *Journal of Physiology (London)* 215: 693-707.

Ismail-Beigi F., and I.S. Edelman (1970). Mechanism of thyroid calorigenesis: role of active sodium transport. *Proceedings of the National Academy of Sciences U.S.A.* 67: 1071-1078.

Iverson S.L., and B.N. Turner (1974). Winter weight dynamics in *Microtus pennsylvanicus*. *Ecology* 55: 1030-1041.

Jacobson A., Nedergaard J., and B. Cannon (1986). Alpha and beta adrenergic control of thermogenin mRNA expression in brown adipose tissue. *Bioscience Reports* 6: 621-631.

Jansky L. (1973). Non-shivering thermogenesis and its thermoregulatory significance. *Biological Reviews* 48: 85-132.

Jarvis J.U.M. (1969). The breeding season and litter size of African mole-rats. *Journal of Reproduction and Fertility (suppl)* 6: 237-248.

Jarvis J.U.M. (1978). Energetics of survival in *Heterocephalus glaber* (Ruppell), the naked mole-rat (Rodentia: Bathyergidae). *Bulletin of the Carnegie Museum of Natural History* 6: 81-87.

Jarvis J.U.M. (1981). Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. *Science* 212: 571-573.

Jarvis J.U.M. (1985). Ecological studies on *Heterocephalus glaber*, the naked mole-rat, in Kenya. *National Geographic Society Research Report* 20: 429-437.

Jarvis J.U.M. (1991a). Reproduction of naked mole-rats. In: *The Biology of the Naked Mole-Rat*. Edited by P.W. Sherman, J.U.M. Jarvis, and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 384-425.

Jarvis J.U.M. (1991b). Methods for capturing, transporting, and maintaining naked mole-rats in captivity. In: *The Biology of the Naked Mole-Rat*. Edited

by P.W. Sherman, J.U.M. Jarvis, and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 467-481.

Jarvis J.U.M., and N.C. Bennett (1991). Ecology and behaviour of the Family Bathyergidae. In: *The Biology of the Naked Mole-Rat*. Edited by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 66-97.

Jarvis J.U.M., and N.C. Bennett (1993). Eusociality has evolved independently in two genera of Bathyergid mole-rats - but occurs in no other subterranean mammals. *Behavioral Ecology and Sociobiology* 33: 253-260.

Jarvis J.U.M., Bennett N.C., and A.C. Spinks (1998). Food availability and foraging by wild colonies of Damaraland mole-rats (*Cryptomys damarensis*): implications for sociality. *Oecologia* 113: 290-298.

Johanssen K., Lykkeboe G., Weber R.E., and G.M. Maloiy (1976). Blood respiratory properties in the naked mole-rat, *Heterocephalus glaber*, a mammal of low body temperature. *Respiratory Physiology* 28: 303-314.

John-Alder H.B. (1983). Effects of thyroxine supplementation on metabolic rate and aerobic capacity in a lizard. *American Journal of Physiology* 244: R659-R666.

Karasov W.H., and J.M. Diamond (1985). Digestive adaptations for fueling the cost of endothermy. *Science* 228: 202-204.

Karasov W.H., Solberg D.H., and J.M. Diamond (1985). What transport adaptations enable mammals to absorb sugars and amino acids faster than reptiles. *American Journal of Physiology* 249: G271-G283.

Kelly J.M., and B.W. McBride (1990). Symposium on 'Thermogenesis: mechanisms in large animals' The sodium pump and other mechanisms of thermogenesis in selected tissues. *Proceedings of the Nutrition Society* 49: 185-202.

Kennedy P.M., Christopherson R.J., and L.P. Milligan (1986). Digestive responses to cold. In: *Control of Digestion and Metabolism in Ruminants*. Edited by L.P. Milligan, W.L. Grovum and A. Dobson. Englewood Cliffs, New Jersey, Prentice-Hall. p. 285-306.

Kingdom J. (1974). *East African Mammals. Vol 2b. (Hares and Rodents)*. London, Academic Press. p. 506-517.

Kingsbury B.A. (1994). Thermal constraints and eurythermy in the lizard *Elgaria multicarinata*. *Herpetologica* 50: 266-273.

Kingsolver J.G., and R.B. Huey (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* 38: 545-560.

Klaus S., Heldmaier G., and D. Ricquier (1988). Seasonal acclimation of bank voles and wood mice: non-shivering thermogenesis and thermogenic properties of brown adipose tissue mitochondria. *Journal of Comparative Physiology* B158: 157-164.

Klaus S., Casteilla, Bouillaud F., and D. Ricquier (1991). The uncoupling protein UCP: A membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. *International Journal of Biochemistry* 23: 791-801.

Klingenberg M. (1990). Mechanism and evolution of the uncoupling protein of brown adipose tissue. *TIBS* 15: 100-112.

Kodama A.M., and N. Pace (1964). Effect of environmental temperature on hamster body fat composition. *Journal of Applied Physiology* 19: 863-867.

Kolb A. (1950). Beitrage zur Biologie einhemischer Fledermause. *Zool. Jb. Syst.* 78: 547-572.

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

Korn H. (1989). The annual cycle in body weight of small mammals from the Transvaal, South Africa, as an adaptation to a subtropical seasonal environment. *Journal of Zoology (London)* 218: 223-231.

Laburn H.P., Mitchell D., and K. Goelst (1992). Fetal and maternal body temperature measured by means of radiotelemetry in near term sheep during thermal stress. *Journal of Applied Physiology* 72: 894-900.

Lacey E.A., and P.W. Sherman (1991). Social organisation of naked mole-rat colonies: evidence for divisions of labour. In: *The Biology of the Naked Mole-Rat*. Edited by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander. Princeton, N.J., Princeton University Press. p. 275-336.

La Rochelle F.T, and M.E. Freeman (1974). Superimposition of thyroid hormone regulation on gonadotropin secretion. *Endocrinology* 95: 379-387.

Lavocat R. (1978). Rodentia and Lagomorpha. In: *Evolution of African Mammals*. Edited by V.H. Magio and H.B.S. Cooke. Cambridge, Harvard University Press. p. 68-89.

Leroi A.M., Lenski R.E., and A.F. Bennett (1994). Evolutionary adaptation to temperature. III. Adaptation of *Escheria coli* to a temporally varying environment. *Evolution* 48: 1222-1229.

Li X., and H. Tokura (1997). Lower critical temperature under the influence of seasonal cold acclimatization due to two types of clothing. *Journal of Thermal Biology* 22: 357-363.

Licht P., and W.R. Moberley (1965). Thermal requirements for embryonic development in the tropical lizard *Iguana iguana*. *Copeia* 1965: 515-517.

Lindquist J.M., and S. Rehnmark (1998). Ambient temperature regulation of apoptosis in brown adipose tissue. *The Journal of Biological Chemistry* 273: 30147-30156.

Louw G.N., Young B.A., and J. Bligh (1976). Effect of thyroxine and noradrenaline on thermoregulation, cardiac rate and oxygen consumption in the monitor lizard, *Varanus albigularis*. *Journal of Thermal Biology* 1: 189-193.

Louw G.N. (1993). *Physiological Animal Ecology*. UK. Longman Scientific and Technical. p. 1-74.

Lovegrove B.G., and C. Wissel (1988). Sociality in mole-rats. Metabolic scaling and the role of risk sensitivity. *Oecologia* 74: 600-606.

Lovegrove B.G. (1989). The cost of burrowing by the social mole-rat (Bathyergidae) *Cryptomus damarensis* and *Heterocephalus glaber*: the role of soil moisture. *Physiological Zoology* 62: 449-469.

Lyman C.P., Willis J.S., Malan A., and L.C.H. Wang (1982). *Hibernation and Torpor in Mammals and Birds.* New York, Academic Press.

Lynn W.G. (1970). The thyroid. In: *Biology of the Reptilia, Volume 3.* Edited by C. Gans and F.H. Pough. London, Academic Press. p. 216.

Malan A. (1996). The origins of hibernation: a reappraisal. In: *Adaptations to Cold.* Edited by F. Geiser, A.J. Hulbert, and S.C. Nichol. Armidale, University of New England Press. p. 1-6.

Mangum C.P., and P.W. Hochachka (1998). New directions in comparative physiology and biochemistry: mechanisms, adaptations, and evolution. *Physiological Zoology* 71: 478-484.

Manning J.M., and F.H. Bronson (1990). The effects of low temperature and food intake on ovulation in domestic mice. *Physiological Zoology* 63: 938-948.

Marsh R.L., and W.R. Dawson (1989a). Avian adjustments to cold. In: *Advances in Comparative and Environmental Physiology*, Vol. 4. Edited by L.C.H. Wang. Berlin, Heidelberg. Springer-Verlag. p. 205-253.

Marsh R.L., and W.R. Dawson (1989b). Energy substrates and metabolic acclimatization in small birds. In: *Physiology of Cold Adaptation in Birds*. Edited by C. Bech and R.E. Reinertsen. New York, Plenum. p. 105-114.

Marsteller F.A., and C.B. Lynch (1983). Reproductive consequences of food restriction at low temperatures in lines of mice divergently selected for thermoregulatory nesting. *Behavioral Genetics*. 13: 397-410.

Marsteller F.A., and C.B. Lynch (1987). Reproductive responses to variation in temperature and food supply by house mice: I. Mating and pregnancy. *Biology of Reproduction* 37: 838-843.

Martin J., Lopez P., Carrascal L.M., and A. Salvador (1995). Adjustment of basking postures in the high altitude Iberian rock lizard (*Lacerta monticola*). *Canadian Journal of Zoology* 73: 1065-1068.

Mason E.B. (1977). Serum thyroxine levels in *Chrysemys picta marginata* (Reptilia, Testudines, Testudinidae) exposed to different thermal environments. *Journal of Herpetology* 11: 232-234.

McDevitt R.M., and J.R. Speakman (1994). Central limits to sustainable metabolic rate have no role in cold acclimation of the short-tailed field vole (*Microstus agrestis*). *Physiological Zoology* 67: 1117-1139.

McKinnon W., and G.J. Alexander (1999). Is temperature independence of digestive efficiency an experimental artifact in lizards? A test using the common flat lizard (*Platysaurus intermedius*). *Copeia* 1999: 299-303.

McNab B.K. (1979). The influence of body size on the energetics and distribution of fossorial and burrowing mammals. *Ecology* 60: 1010-1021.

McNab B.K. (1980). On estimating thermal conductance in endotherms. *Physiological Zoology* 53: 145-156.

Merson M.H., and R.L. Kirkpatrick (1981). Relative sensitivity of reproductive activity and body-fat level to food restriction in white-footed mice. *American Midland Naturalist* 106: 305-312.

Merson M.H., and R.L. Kirkpatrick (1983). Role of energy intake in the maintenance of reproduction in female white-footed mice, *Peromyscus leucopus*. *American Midland Naturalist* 109: 206-208.

Millar M.R. (1955). Cyclic changes in the thyroid and interrenal glands of the viviparous lizard, *Xantusia vigilis*. *Anatomical Records* 123: 19-31.

Millar J.S., and L.W. Gyug (1981). Initiation of breeding by northern *Peromyscus* in relation to temperature. *Canadian Journal of Zoology* 59: 1094-1098.

Milner R.E., Wang L.C.H., and P. Trayhurn (1989). Brown fat thermogenesis during hibernation and arousal in Richardson's ground squirrel. *American Journal of Physiology* 256: R42-R48.

Mount L.E. (1979). *Adaptation to Thermal Environment*. London, Edward Arnold. p. 145-159.

Muth A. (1980). Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* 61: 1335-1343.

Nagy K.A. (1987). Field metabolic rate and food requirement scaling in mammals and birds. *Ecological Monographs* 57: 111-128.

Nazian S.J., and B.E. Piacsek (1977). Vaginal opening and early oestrous cycles in rats raised at a low ambient temperature. *Journal of Experimental Zoology* 198: 13-16.

Nedergaard J., Becker W., and B. Cannon (1983). Effects of dietary essential fatty acids on active thermogenin content in rat brown adipose tissue. *Journal of Nutrition* 113: 1717-1724.

Nevo E. (1979). Adaptive convergence and divergence of subterranean mammals. *Annual Review of Ecology and Systematics* 10: 269-308.

Nevo E. (1999). *Mosaic Evolution of Subterranean Mammals. Regression, progression and global convergence*. Oxford, Oxford University Press.

Nicholls D.G., and O. Lindberg (1973). Brown-adipose-tissue mitochondria. The influence of albumin and nucleotides on passive ion permeabilities. *European Journal of Biochemistry* 37: 523-530

Nicholls D.G. (1976). Hamster brown-adipose-tissue mitochondria. Purine nucleotide control of the ion conductance of the inner membrane, the nature of the nucleotide binding site. *European Journal of Biochemistry* 62: 223-228.

Nicholls D.G., and R.M. Locke (1984). Thermogenic mechanisms in brown fat. *Physiological Reviews* 64: 1-69.

Nicholls D.G., Cunningham S.A., and E. Rial (1986). The bioenergetic mechanisms of brown adipose tissue thermogenesis. In: *Brown Adipose Tissue*. Edited by P. Trayhurn and D.G. Nicholls. London, Edward Arnold. p. 52-85.

Nimrod A., and K.J. Ryan (1975). Aromatization of androgens by human abdominal and breast fat tissue. *Journal of Clinical Endocrinology and Metabolism* 40: 367-372.

Noguchi T., Kudo M., Sugisaki T., and I. Satoh (1986). An immunocytochemical and electron microscopic study of the *hyt* mouse anterior pituitary gland. *Journal of Endocrinology* 109: 163-168.

Noguchi T., Kuroki T., Yamada T., Sugisaki T., Kubota C., and K. Takamatsu (1995). Immunocytochemical study of the hypothyroid mouse thyrotroph. *Hormone and Metabolic Research* 27: 201-203.

Norris D.O. (1985). *Vertebrate Endocrinology*. Philadelphia, Lea and Febiger.

Olivecrona T., Bengtsson G., Marklund S.E., and M. Hook (1977). Heparin-lipoprotein lipase interactions. *Federation Proceedings* 36: 60-65.

Oppenheimer J.H., Schwartz H.L., Lane J.T., and M.P. Thompson (1991). Functional relationship of thyroid hormone-induced lipogenesis, lipolysis and thermogenesis in the rat. *Journal of Clinical Investigations* 87: 125-132.

O'Riain M.J., Jarvis J.U.M., and C.G. Faulkes (1996). A dispersive morph in the naked mole-rat. *Nature* 380: 619-621.

O'Riordan J.L.H., Malan P.G., and R.P. Gould (1988). *Essentials of Endocrinology*. London, Blackwell Scientific Publications. p. 619.

Oswald C., and P.A. McClure (1987). Energy allocation during concurrent pregnancy and lactation in Norway rats with delayed and undelayed implantation. *Journal of Experimental Zoology* 241: 343-357.

Owen T.L., Spencer R.L., and S.P. Duckles (1991). Effect of age on cold acclimation in rats: metabolic and behavioral responses. *American Journal of Physiology* 260: R284-R289.

Pandey B.N., and J.S. Munshi (1976). Role of the thyroid gland in regulation of metabolic rate in an air-breathing siluroid fish, *Heteropneustes fossilis*. *Journal of Endocrinology* 69: 421-425.

Patterson J.W. (1991). Emergence, basking behaviour, mean selected temperature and critical thermal minimum in high and low altitude subspecies of the tropical lizard *Mabuya striata*. *African Journal of Zoology* 29: 330-339.

Perkins M.N., Rothwell N.J., Stock M.J., and T.W. Stone (1981). Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* 289: 401-402.

Perrigo G., and F.H. Bronson (1985). Behavioral and physiological responses of female house mice to foraging variation. *Physiology and Behavior* 34: 437-440.

Piacsek B.E., and S.J. Nazian (1981). Thermal influences on sexual maturation in the rat. In: *Environmental Factors in Mammalian Reproduction*. Edited by D. Gilmore and B. Cook. London, MacMillan. p. 215-231.

Piacsek B.E. (1985). Altered negative feedback response due to ovariectomy and estrogen in prepubertal restricted-diet rats. *Biology of Reproduction* 32: 1062-1068.

Pitcher T., and R. Buffenstein (1994). Passive uptake in the small intestine and active uptake in the hindgut contribute to the highly efficient mineral metabolism of the common mole-rat, *Cryptomys hottentotus*. *British Journal of Nutrition* 71: 573-582.

Pohl H. (1965). Temperature regulation and cold acclimation in the golden hamster. *Journal of Applied Physiology* 20: 405-410.

Precht H., Christophersen J., Hensel H., and W. Larcher (1973). *Temperature and Life*.

Puerta M.L., and M. Abelenda (1987). Cold acclimation in food-restricted rats. *Comparative Biochemistry and Physiology* A87: 31-33.

Racey P.A. (1973). Environmental factors affecting the length of gestation in heterothermic bats. *Journal of Reproduction and Fertility (Suppl)* 19: 175-189.

Racey P.A. (1979). The prolonged storage and survival of spermatozoa in Chiroptera. *Journal of Reproduction and Fertility* 56: 391-402.

Racey P.A. (1981). Environmental factors effecting gestation length in mammals. In: *Environmental Factors in Mammalian Reproduction*. Edited by D. Gilmore and B. Cook.

Macmillan, London. p. 199-213.

Racey P.A., and S.M. Swift (1981). Variation in gestation lengths in a colony of pipistrelle bats (*Pipistrellus pipistrellus*) from year to year. *Journal of Reproduction and Fertility* 61: 123-129.

Rafael J., Vsiansky P., and G. Heldmaier (1985). Seasonal adaptation of brown adipose tissue in the Djungarian hamster. *Journal of Comparative Physiology* B155: 521-528

Rafael J., Fesser W., and D.G. Nicholls (1986). Cold adaptation in the guinea pig at the level of the isolated brown adipocyte. *American Journal of Physiology* 250: C228-C235.

Reiter R.J. (1968). Changes in the reproductive organs of cold-exposed and light-deprived female hamsters (*Mesocricetus auratus*). *Journal of Reproduction and Fertility* 16: 217-222.

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

Rieck A.F., Belli J.A., and M.E. Blaskovics (1960). Oxygen consumption of whole animals and tissues in temperature

acclimated amphibians. *Proceedings of the Society of Experimental Biology and Medicine* 103: 436-439.

Roberts J.C., and R.E. Smith (1967). Time-dependent responses of brown fat in cold-exposed rats. *American Journal of Physiology* 212: 519.

Rome L.C., Stevens E.D., and H.B. John-Alder (1992). The influence of temperature and thermal acclimation on physiological function. In: *Environmental Physiology of the Amphibians*. Edited by M.E. Feder and W.W. Buggren. Chicago, University of Chicago Press. p. 183-205.

Rosenmann M., Morrison P., and D. Feist (1975). Seasonal changes in the metabolic capacity of red-backed voles. *Physiological Zoology* 48: 303-310.

Roth J., Merker G., Nurnberger F., Pauly B., and E. Zeisberger (1990). Changes in physiological and neuroendocrine properties during thermal adaptation of golden hamsters (*Mesocricetus auratus*). *Journal of Comparative Physiology* B160: 153-159.

Schmid J. (1996). Oxygen consumption and torpor in mouse lemurs (*Microcebus murinus* and M. *myoxinus*): Preliminary results in a study in western Madagascar. In: *Adaptations to Cold*. Edited by F. Geiser, A.J. Hulbert, and S.C. Nicol. Armidale, University of New England Press. p. 47-

54.

Schmidt-Nielsen K. (1990). *Animal Physiology: Adaptation and Environment*. Cambridge, UK. Cambridge University Press.

Schneider J.E., and G.N. Wade (1990). Effects of diet and body fat content on cold-induced anestrus in Syrian hamsters. *American Journal of Physiology* 259: R1198-R1204.

Schneider J.E., and G.N. Wade (1991). Effects of ambient temperature and body fat content on maternal litter reduction in Syrian hamsters. *Physiology and Behavior* 49: 135-139.

Scholander P.F., Hock R., Walters V., Johnson F., and L. Irving (1950). Heat regulation in some arctic and tropical mammals and birds. *Biological Bulletin* 99: 237-258.

Scholander P.F., Hammel H.T., Hart J.S., LeMessurier D.H., and J. Steen (1958). Metabolic acclimation to cold in man. *Journal of Applied Physiology* 12: 1-8.

Seitz H.J., Muller M.J., and S. Soboll (1985). Rapid thyroid-hormone effect on mitochondrial and cytosolic ATP/ADP ratios in the intact liver cell. *Biochemistry Journal* 227: 149-153.

Sergeev I.N., Buffenstein R., and J.M. Pettifor (1993). Vitamin D receptors in a naturally vitamin D deficient subterranean mammal, the naked mole-rat (*Heterocephalus glaber*): biochemical characterization. *General and Comparative Endocrinology* 90: 338-345.

Sexton O.J., and K.R. Marion (1974). Duration of incubation of *Sceloporus undulatus* eggs at constant temperature. *Physiological Zoology* 47: 91-98.

Sherman P.W., Jarvis J.U.M., and S.H. Braude (1992). Naked mole-rats. *Scientific American* 267: 72-78.

Silva J.E., and P.R. Larsen (1986). Regulation of thyroid hormone expression at the prereceptor and receptor levels. In: *Thyroid Hormone Metabolism*. Edited by G. Hennemann. New York, Dekker. p. 441-500.

Sinsch U. (1989). Behavioral thermoregulation of the Andean toad (*Bufo spinulosus*) at high altitude. *Oecologia* 80: 32-38.

Skinner D.C., Moodley G.P., and R. Buffenstein (1991). Is vitamin D_3 essential for mineral metabolism in the damara mole rat (*Cryptomys damarensis*)? *General and Comparative Endocrinology* 81: 500-505.

Smit H. (1967). Influence of temperature on the rate of gastric juice secretion by the brown bullhead, *Ictalurus nebulosus*. *Comparative Biochemistry and Physiology* 21: 125-132.

Smith R.E., and D.J. Hoijer (1962). Metabolism and cellular function in cold acclimation. *Physiological Reviews* 42: 60-142.

Smith E.N. (1976). Cutaneous heat flow during heating and cooling in *Alligator mississipiensis*. *American Journal of Physiology* 230: 1205-1210.

Smith B.K., and T.J. Dawson (1985). Use of helium-oxygen to examine the effect of cold acclimation on the summit metabolism of a marsupial, *Dasyuroides byrnei*. *Comparative Biochemistry and Physiology* A81: 445-449.

Smithers R.H.N. (1983). *The Mammals of Southern African Subregion*. Pretoria, University of Pretoria.

Sokoloff G., and M.S. Blumberg (2000). An infrared thermographic study of the thermal advantage gained by huddling in infant rats, contributions of endothermy and behavior. *FASEB* 14: A594.

Speakman J.R., and P.A. Racey (1987). The energetics of pregnancy and lactation in the brown long-eared bat,

Plecotus auritus. In: *Recent Advances in the Study of Bats*. Edited by M.B. Fenton, P.A. Racey and J.M.V. Rayner. Cambridge, UK, Cambridge University Press. p. 367-393.

Speakman J.R., and J. McQueenie (1996). Limits to sustained metabolic rate: The link between food intake, basal metabolic rate and morphology in reproducing mice, *Mus musculus*. *Physiological Zoology* 69: 746-769.

Spray C.M. (1950). A study of some aspects of reproduction by chemical analysis. *British Journal of Nutrition* 4: 354-360.

Stankiewiez D. (1974). The course of pregnancy and the development of mice under conditions of hypothermy and hibernation with hypothermy. *Folia biol. Krakow* 22: 91-102.

Stott A.W., and J. Slee (1985). The effect of environmental temperature during pregnancy on thermoregulation in the newborn lamb. *Animal Production* 41: 341-347.

Sundin U., and B. Cannon (1980). GDP-binding to brown fat mitochondria of developing and cold-adapted rats. *Comparative Biochemistry and Physiology* B65: 463-471.

Sundin U., Moore G., Nedergaard J., and B. Cannon (1987). Thermogenin amount and activity in hamster brown fat mitochondria: effect of cold acclimation. *American Journal of Physiology* **252**: R822-R832.

Thomson J.F., Habeck D.A., Nance S.L., and K.L. Beetham (1969). Ultrastructural and biochemical changes in brown fat in cold-exposed rats. *Journal of Cellular Biology* **41**: 312-334.

Toft A.D. (1991). Thyrotropin: assay, secretory physiology, and testing of regulation. In: *Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text.* 6th Ed. Edited by L.E. Braverman and R.D. Utiger. p. 287.

Tomasi T.E. (1991). Utilization rates of thyroid hormones in mammals. *Comparative Biochemistry and Physiology* **100A**: 503-516.

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

Trayhurn P., and R.E. Milner (1989). A commentary on the interpretation of *in vitro* biochemical measures of brown adipose tissue thermogenesis. *Canadian Journal of Physiology and Pharmacology* **67**: 811-819.

Trayhurn P., and R.E. Milner (1989). Adaptive changes in uncoupling protein in brown adipose tissue during cold acclimation and hibernation. In: *Living in the Cold II.* Edited by A. Malan and B. Canguilhem. London, Libbey. p. 399-406.

Tsuji J.S. (1988). Thermal acclimation of metabolism in *Sceloporus* lizards from different latitudes. *Physiological Zoology* 61: 241-253.

Urison N.T., Goelst K., and R. Buffenstein (1993). A positive fever response by a poikilothermic mammal, the naked mole-rat (*Heterocephalus glaber*). *Journal of Thermal Biology* 18: 245-249.

Urison N.T., and R. Buffenstein (1994). Shifts in thermoregulatory patterns with pregnancy in the poikilothermic mammal - the naked mole-rat (*Heterocephalus glaber*). *Journal of Thermal Biology* 19: 365-371.

Urison N.T., and R. Buffenstein (1995). Metabolic and body temperature changes during pregnancy and lactation in the naked mole-rat (*Heterocephalus glaber*). *Physiological Zoology* 68: 402-420.

Van Soest P.J. (1982). Gastrointestinal fermentation. In: *Nutritional Ecology of the Ruminant.* Edited by P.J. van Soest. Corvallis Oregon, O&B Books. p. 152-229.

Vidovic V. (1952). Kalte- und Hypoxietoleranz von Ratten Embryonen. *Experientia* 8: 304-306.

Vinegar A. (1974). Evolutionary implications of temperature induced anomalies of development in snake embryos. *Herpetologica* 30: 72-74.

Vogt F.D., and G.R. Lynch (1982). Influence of ambient temperature, nest availability, huddling, and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus*. *Physiological Zoology* 55: 56-63.

Vybiral S., and L. Jansky (1974). Non-shivering thermogenesis in the golden hamster. *Physiol. Bohemoslovaca* 23: 235-243.

Vybiral S., Andrews J.F., Bostik J., Langer P., and L. Jansky (1985). Thyroid hormones in rats during long-term cold exposure and hypometabolic effect of reverse triiodothyronine on adrenaline induced thermogenesis. *Endocrinologia Experimentalis* 19: 179-185.

Wade G.N., and J.E. Schneider (1992). Metabolic fuels and reproduction in female mammals. *Neuroscience and Biobehavioral Reviews* 16: 235-272.

Wang L.C.H. (1989). *Animal Adaptation to Cold. Volume 4.* Berlin, Springer.

Webster A.J. (1974). Adaptation of cold. In: *Environmental Physiology*. Edited by D. Robertshaw. Baltimore, University Park Press. p. 71-106.

Weiner J. (1992). Physiological limits to sustainable energy budgets in birds and mammals; ecological implications. *TREE* 7: 384-388.

Whitaker E.M., Hussain S.H., Hervey G.R., Tobin G., and K.M. Rayfield (1990). Is increased metabolism in rats in the cold mediated by the thyroid? *Journal of Physiology* 431: 543-556.

White F.N., and R.C. Lasiewski (1971). Rattlesnake denning: Theoretical considerations on winter temperatures. *Journal of Theoretical Biology* 30: 553-557.

Wickler S.J. (1980). Maximal thermogenic capacity and body temperatures of white-footed mice (*Peromyscus*) in summer and winter. *Physiological Zoology* 53: 383-346.

Wiesinger H., Klaus S., Heldmaier G., Champigny O., and D. Ricquier (1990). Increased nonshivering thermogenesis, brown fat cytochrome-c oxidase activity, GDP binding, and uncoupling protein mRNA levels after short daily cold exposure of *Phodopus sungorus*. *Canadian Journal of Physiology and Pharmacology* 68: 195-200.

Williams D.D. (1968). The effects of cold exposure and guanethidine on norepinephrine thermogenesis in the golden hamster. *Comparative Biochemistry and Physiology* 27: 557-573.

Withers P.C., and J.U.M. Jarvis (1980). The effect of huddling on thermoregulation and oxygen consumption for the naked mole-rat. *Comparative Biochemistry and Physiology* A66: 215-219.

Wolfenson D. (1983). Blood flow through arteriovenous anastomoses and its thermal function in the laying hen. *Journal of Physiology (London)* 334: 395-407.

Woodley R., and R. Buffenstein. Body composition changes, and the effects thereof, during pregnancy and lactation in established reproductive naked mole-rats (*Heterocephalus glaber*). in prep.

Wunder B.A. (1979). Hormonal Mechanisms. In: *Comparative Mechanisms of Cold Adaptation*. Edited by L.S. Underwood, L.L. Tieszen, A.B. Callahan, and G.E. Folk. London, Academic Press. p. 143-158.

Wunder B.A. (1984). Strategies for, and environmental cueing mechanisms of, seasonal changes in thermoregulatory parameters of small mammals. In: *Winter Ecology of Small Mammals*. Edited by J.F. Merritt. *Carnegie Museum of*

Natural History Special Publication 10: 165-172.

Wunder B.A., and R.D. Gettinger (1996). Effects of body mass and temperature acclimation on the nonshivering thermogenic response of small mammals. In: *Adaptations to Cold. Tenth International Hibernation Symposium.* Edited by F. Geiser, A.J. Hulbert, and S.C. Nicol. Armidale, University of New England Press. p. 131-139.

Yacoe M.E. (1981). Changes in mitochondrial components of hamster brown adipose tissue in response to cold acclimation. *Biochemistry Journal* 194: 653-665.

Yahav S., and I. Choshniak (1990). Response of the digestive tract to low quality dry food in the fat jird (*Meriones crassus*) and in the levant vole (*Microtus guentheri*). *Journal of Arid Environments* 19: 209-215.

Yahav S., and R. Buffenstein (1991a). The effect of temperature on caecal fermentation processes in a poikilothermic mammal, *Heterocephalus glaber*. *Journal of Thermal Biology* 16: 345-349.

Yahav S., and R. Buffenstein (1991b). Huddling facilitates homeothermy in the naked mole-rat *Heterocephalus glaber*. *Physiological Zoology* 64: 871-884.

Yahav S., and R. Buffenstein (1992). Caecal function provides energy without liberating heat in the poikilothermic mammal, *Heterocephalus glaber*. *Journal of Comparative Physiology* **B162**: 216-218.

Zeisberger E., Roth J., and E. Simon (1988). Changes in water balance and in release of arginine vasopressin during thermal adaptation in guinea pigs. *Pflugers Archives* **412**: 285-291.

Zhao X., Jorgensen H., and B.O. Eggum (1995). The influence of dietary fibre on body composition, visceral organ weight, digestibility and energy balance in rats housed in different thermal environments. *British Journal of Nutrition* **73**: 687-699.