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The Physiological Ecology of Mammals in Extreme Environments

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ABSTRACT OF THE DISSERTATION

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One of the most significant evolutionary advancements in vertebrates is the evolution of homeothermic endothermy. The ability to maintain a high and constant body temperature has freed endotherms from many of the metabolic effects of fluctuating temperature and allowed endotherms to inhabit a wide range of habitats. However, endothermy is very energetically costly and not an ideal solution for many vertebrates. Despite the drawbacks, endotherms have evolved many physiological adaptations to overcome the challenges posed by challenging environmental conditions. In this dissertation I investigate several physiological adaptations endotherms have evolved to deal with the challenges imposed by these environments. Mammals living in deserts must prevent hyperthermia while maintaining proper water balance. This is problematic because the only mechanism for cooling is to evaporate water, the scarcity of which defines desert habitats. I

used a phylogenetic comparative analysis to show that mammals living in arid regions have evolved mechanisms to reduce rates of total evaporative water loss. In this analysis I used many continuous variables to describe habitat in addition to classifying habitats as arid or mesic. Mammals living in cold habitats must prevent hypothermia by increasing internal heat production, which requires increased energy intake, a problem confounded by the lack of primary productivity in cold habitats. Mammals can increase heat production either by increasing their capacity for shivering or nonshivering thermogenesis. I acclimated deer mice to cold ambient temperature to show that they can increase metabolic heat production by increasing their capacity for nonshivering thermogenesis. Endotherms living at high altitudes face a two-fold problem, cold temperatures combined with limited oxygen availability. The ability to maintain body temperature and aerobic activity is dependent on oxygen. The ability to carry excess oxygen in the blood would likely be beneficial to animals living at high altitude. I tested whether or not deer mice originally from high altitude have an excess ability to carry oxygen in their blood and found that the circulatory distribution of oxygen likely sets an upper limit for aerobic performance in these animals.

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Introduction

Physiological Adaptations that Enable Endotherms to Survive in Environmentally Challenging Habitats

Abstract

Temperature affects animals at all levels of biological organization. Effects at the biochemical level can have a tremendous impact on the physiological processes of the whole animal. The evolution of endothermy has somewhat freed animals from the physiological effects of temperature changes by allowing for constantly high body temperatures. However, endothermy is incredibly energetically expensive and the success of endotherms is largely dependent on proper thermoregulation and the availability of large amounts of oxygen. The success of endotherms can be challenged by physiological stressful environmental conditions that exist in various habitats. Endotherms living in deserts risk hyperthermy and must rely on evaporation of water within a desiccating environment in order to thermoregulate. Endotherms living in extremely cold environments must use considerable energy to stay warm while food resources may be limited. Endotherms at high altitude are challenged by cold temperatures and decreased levels of oxygen, making thermoregulation and aerobic activity increasingly difficult. Despite these challenges, endotherms successfully occupy all of these habitats and have been assisted by several physiological adaptations. In this review, I discuss many of the adaptations that endotherms have evolved to deal the specific challenges posed by living in hot, cold and oxygen limiting environments.

Introduction

Despite the large range of environmental temperatures that exist on Earth, active animal life is often restricted to a relatively narrow range. Temperature change poses a significant challenge to animals and affects all levels of biological organization (McNab 2002). The molecular structure of proteins is affected by temperature, as is enzyme activity. Increasing temperatures cause enzyme activity to increase up to a point, with each enzyme having an optimal range of temperatures over which it functions best. The fluidity of cell membranes is also dependent on temperature and a certain degree of fluidity to function properly and this fluidity is dependent on temperature. Neural activity is largely affected by temperature and its effects on both protein and lipid components of the membrane (Horchachka and Somero 1984). The effects of temperature at the biochemical level can have pronounced effects on the physiological processes that occur level of the whole organism. Consequently, animals have evolved many mechanisms to compensate for changes in temperature in order to maintain physiological processes. In biochemical reactions, enzyme or substrate concentration can be altered to account for changes in enzyme activity. Variation in the lipid concentration of cell membranes can alter the fluidity of the membrane at given temperatures. In addition to biochemical adaptations, animals have evolved different methods to regulate body temperature.

Heterothermic organisms allow their internal body temperature to fluctuate while homeothermiic organisms maintain a rather constant body temperature.

Many heterotherms obtain their body heat from the environment and their body temperature often varies with environmental temperature (referred to as ectotherms from here on). The reactions that occur within ectotherms still have optimum temperatures and these animals must use behavioral thermoregulation to obtain appropriate body temperatures. In contrast, many homeotherms obtain their body heat from internal metabolic reactions and maintain a fairly constant internal body temperature (referred to as endotherms from her on). Several benefits have been attributed to endothermy and are primarily centered on the idea that endothermy produces high body temperatures, constant body temperatures and high aerobic activity levels (McNab 2002). It has been suggested that high body temperatures can maximize rate of metabolism and growth along with increasing reproduction rate (Hamilton 1973). High body temperatures may have also allowed for enzymes to specialize for high activity rates (Heinrich 1977) with constant body temperature allowing for enzymes to specialize on a narrow range of temperatures (Hammel 1976). Constant body temperature also allows animals to function over a wide range of environmental temperatures (Hammel 1976). High rates of aerobic activity accompany endothermy with maximal metabolic rates being between 5 and 10 times resting rates of ectotherms (Bennett and Ruben 1979; Taigen 1983; Bennett 1991; Ruben 1995) and endotherms (McNab 2002). Thus, endothermy can confer many benefits, but maintaining a high, constant body temperature can be metabolically expensive. Endotherms occupy all of earth's available habitats and have evolved mechanisms to deal with the challenges each habitat presents. When

living in areas in which one or more environmental variables are less than optimal endotherms have three options: avoidance, tolerance, or acclimatization. These are not mutually exclusive and often occur together. I will discuss several mechanisms terrestrial endotherms (with an emphasis on mammals) have evolved to deal with physiologically challenging environments

Life in the desert

Deserts are the ultimate terrestrial environments and much research has focused on the physiological and behavioral adaptations of animals that inhabit these regions. Deserts can be defined as environments in which precipitation is so low, infrequent and variable that it has a major impact on biological productivity (Noy-Mier 1973). In many deserts the scarcity of water is combined with high air temperatures and intense solar radiation along with high surface temperatures, producing a challenging environment for endothermic animals. A crucial problem for endotherms living in the desert is dealing with the heat; animals must not become hyperthermic. Secondly, animals must maintain proper water balance while living in desiccating conditions. The environmental temperature of deserts often exceeds the body temperature of most endotherms living there. Therefore, these animals are faced with the challenge of moving heat out of the body against a temperature gradient and the only available mechanism available is evaporative cooling. So in order for endotherms to thermoregulate in deserts they must lose the one element whose scarcity defines the habitat (Walsberg 2000). Despite these challenges, many species of endotherms inhabit and thrive in deserts. For example,

the Sonoran Desert is home to more than 61 species of mammals (Hall 1981; Walsberg 2000). The ability of animals to thrive in deserts has fascinated biologists for many years and research on desert animals by scientists such as Bartholomew, Cowles, Dawson, and the Schmidt-Nielsens was largely responsible for the advancement of the field of physiological ecology (Walsberg 2000).

Avoidance is the easiest way for animals to deal with the environmental extremes that exist in the desert. Many desert animals avoid activity during the hottest parts of the day by remaining in underground burrows and becoming active only at night. In fact, 93% of mammals inhabiting the Sonoran Desert use burrows during the day (Walsberg 2000). The most widely distributed kangaroo rat in the Southwestern United States, *Dipodomys merriami* is a nocturnal, burrow-dwelling rodent that usually lacks access to free standing water (Kenagy and Bartholomew 1985). The burrows of *D. merriami* were traditionally thought to provide a cool, humid refuge during the hottest part of the day (Schmidt-Nielsen 1964; Louw and Seely 1982) by providing a moist microclimate that reduced water loss. However, the early work performed by Knut Schmidt-Nielsen on this species was conducted outside of the most arid part of this species range. This work also coincided with an atypical rainfall. It has recently been shown that these generalizations include some important misconceptions (Tracy and Walsberg 2002) and *D. merriami* typically experiences conditions less favorable for water conservation than originally thought. Burrow temperatures experienced by *D. merriami* in the Sonoran Desert average 35.3ºC with a relative humidity around 22% rather than cooler

temperatures with humidity near saturation as proposed by Schmidt-Nielsen and Schmidt-Nielsen (1950b, 1951, 1952). These kangaroo rats are active just after sundown while temperatures are quite high instead of during the cooler nighttime temperatures (Tracy and Walsberg 2002). Avoidance of environmental extremes by seeking refuge in a burrow is not wholly sufficient for the survival of desert rodents. Estivation is another avoidance strategy employed by desert-dwelling endotherms during periods of drought. Estivating animals exhibit behavioral lethargy and save considerably on both food and water by reducing metabolic rates and regulating body temperature at a low level (McNab 2002).

While some animals avoid extreme temperatures, others are active for prolonged periods during the hottest times of the day. I cannot think of a more iconic desert-adapted animal than the camel (*Camelus dromedarius*). Many stories exist about the camel's amazing abilities and many of these stories are just that. The most classic misconceptions regard the camel's hump. Despite what small children may say, the camel's hump consists of adipose tissue. It has often been said that this fat store can be used for energy when food is scarce and also used to produce metabolic water through lipid oxidation. The truth is that as metabolic pathways require oxygen, which results in evaporation from the lungs and the amount of water lost through pulmonary evaporation is greater than the potential water formed from fat metabolism (Schmidt-Nielsen 1964). One trait that helps camels endure hot, arid conditions is their fluctuating body temperature. A camel's body temperature can fluctuate about 6°C in a day reaching well over 40°C (Schmidt-

Nielsen 1964; Schmidt-Nielsen et al. 1957). Allowing absorbed heat to raise the body temperature reduces the amount of water that must be evaporated to reduce body temperature. Furthermore, heat transfer is driven by the difference between environmental temperature and body temperature; increasing body temperature decreases the difference between the camel and the air, reducing heat exchange. In deserts the temperature drops quickly as the sun goes down and the camel can then dump this stored heat back into the environment without evaporating any water. Some desert-dwelling gazelles also exhibit a similar pattern of temporal hyperthermy (Grant 1970). Smaller mammals, such as the antelope squirrels (*Ammospermophilus harrisi and Ammospermophilus leucurus*) and the round-tailed ground squirrel (*Spermophilus tereticaudus*) are also typically active above ground for prolonged periods during hot summer days (Vorhies 1945; Dengler 1967; Chappell and Bartholomew 1981). *A. leucurus* has been known to exhibit variations in body temperature of 4-5°C over the course of an hour or less (Chahppell and Bartholomew 1981). *S. tereticaudus* is active at air temperatures from 10-45°C and allows its body temperature to fluctuate between 30-42°C (Walsberg 2000). The hair of desert animals also assists in tolerating high temperatures by acting as an insulator, thereby reducing the temperature of the skin (Schmidt-Nielsen 1964).

Camels also have a great tolerance for dehydration. On one occasion a camel went without water for 17 days having lost 36.5 kg (16% of total mass). The camel was given water and drank 40 liters in 10 minutes, restoring the camel to its initial mass (Schmidt-Nielsen 1964)! Camels can tolerate at least a 25% reduction in body

weight due to dehydration and unlike humans their plasma volume is hardly affected (Schmidt-Nielsen 1964). While camels can tolerate substantial dehydration, not all mammals are capable of this. Many mammals rely on preventing water loss to the environment by reducing water in the urine and feces, and reducing evaporative water loss from the skin and respiratory tract. It has been hypothesized that natural selection should provide mammals from desert regions with mechanisms to reduce rates of water loss.

The kangaroo rat, *Dipodomys merriami*, is well known for its exceptional ability to produce concentrated urine and dry feces (Schmidt-Nielsen et al. 1948, Schmidt-Nielsen and Schmidt-Nielsen 1951, Kenagy 1973) and It was originally thought that *D. merriami* could survive on a diet of dry seeds alone without drinking water (Schmidt-Nielsen and Schmidt-Nielsen 1950a,1951, 1952; Schmidt-Nielsen 1964; MacMillen 1964; 1972; MacMillen and Hinds 1983; Hulbert and MacMillen 1988) Sperber (1944) showed that the kidneys of mammals from arid habitats had exceptionally long loops of Henle compared with more mesic species. Sperber (1944) proposed that the relative medullary thickness could be used as an index to identify the longest loops of Henle. Al-khatani et al. (2004) performed a phylogenetic analysis on 141 species of rodents and found that mammals with the largest values of residual medullary thickness were from arid habitats. So, it seems that there is much selection pressure for mammals living in deserts to evolve an enhanced urine concentrating ability.

Researchers have also proposed the hypothesis that natural selection has likely provided desert-dwelling mammals with reduced rates of total evaporative water loss (respiratory evaporative water loss (REWL) + cutaneous evaporative water loss (CEWL) when compared with species living in more mesic habitats. Several studies have investigated if species of arid mammals have reduced rates of TEWL compared with mesic species (Schmidt-Nielsen and Schmidt-Nielsen 1950a; Chew 1965; Hinds and MacMillen 1985; 1986; Withers et al. 2006). The most thorough study of this nature was performed by Chew (1965) and has been the benchmark for almost 50 years. Many studies of this type have been extremely limited in the taxa studied and did not take evolutionary relatedness into account (with the exception of Withers et al. (2006)), but these studies provide overall support for the hypothesis that mammals in arid habitats have lower rates of TEWL than species inhabiting more mesic habitats. A thorough analysis of this nature including all available data while using current statistical methods has been needed for a long time. The exact mechanisms that are responsible for reduced rates of TEWL are not known and this should be an area of future research.

It was long thought that cutaneous water loss was an insignificant contributor to TEWL. Tracy and Walsberg (2000) measured both CEWL and REWL in individuals of *D. merriami* from xeric and mesic habitats. Mesic-site animals had 70% greater TEWL and CEWL than xeric-site animals. REWL did not differ between populations and accounted for only 30-40% TEWL, while previously it was thought that REWL accounted for 70-84% of TEWL in kangaroo rats (Chew and Damman

1961; Schmidt-Nielsen 1964). Xeric-site animals increased CEWL after being acclimated to relaxed hydric conditions (Tracy and Walsberg 2000). These results indicate that CEWL is plastic and may be more important for regulating water loss than originally thought, but this has not yet been rigorously examined. Current research with birds suggests that cutaneous water loss can be altered by adjusting proportions of lipids within the stratum corneum to alter rates of CEWL in some species (Muñoz-Garcia and Williams 2005).

Another strategy for desert-dwelling mammals that has been hypothesized to reduce heat produced and thereby the need to actively dissipate heat to the environment is the reduction of basal metabolic rate (BMR). Studies of birds have shown that desert-dwelling species have BMR that is 17 - 25% lower than species from mesic habitats (Tieleman and Williams 2000). This pattern has also been found in mammals, but has been confounded by the impact of diet on BMR (McNab 2002). Many desert-dwelling rodents eat seeds, which also accompanies reduced BMR. So, it is not clear if BMR is reduced to accommodate the desert environment or as a side effect of the diet composition of many desert-dwelling mammals.

Life in the cold

Terrestrial environments that could be considered extremely cold are primarily encountered at higher latitudes, but much of the earth can be considered cold at least during certain times of the year. Small endotherms typically have a thermoneutral zone with a lower critical temperature of about 25-30°C. Animals living in areas where the temperature drops below this level for sustained periods

of time are essentially cold-stressed and are likely to make adjustments in behavior and physiology. Very cold environments, such as the polar zones, exhibit similar problems for endotherms as do the deserts. At very low temperatures there is little precipitation (including snowfall), the air is very dry, and food availability is severely limited. Unlike in deserts, animals living in the cold are faced with avoiding hypothermia rather than hyperthermia. The desiccating environment in the cold is not as problematic as in deserts because fortunately animals can generate heat without evaporating water from their bodies. Like desert-dwelling animals, animals inhabiting cold environments employ various strategies to survive the stresses of the environment.

One of the most obvious solutions for dealing with periods of seasonally cold temperatures is for animals to migrate to warmer areas. The diet of some animals has become so specialized that they have no choice to migrate. Herons cannot fish in ice-covered ponds and flycatchers cannot catch insects that are no longer present (Marchand 1996). Migration is very energetically expensive and is not a good choice for some animals simply due to the cost of transport involved. It has been estimated that a mammal would spend ten times more energy running a given distance than would a bird of equal mass flying the same distance (Marchand 1996). This may explain why many mammal species don't migrate with the notable exception of caribou, which can travel several hundred kilometers between their summer breeding grounds and over-wintering grounds. Hibernation is another avoidance strategy used by small mammals in which body temperature is allowed to

fall near ambient temperature. Hibernation is restricted to small mammals; the alpine marmot (*Marmota marmota*) is the largest mammal that hibernates and weighs 4 to 5 kg (Hill et al. 2008). Arctic ground squirrels (*Spermophilus parryii*) are amazing in their ability to effectively hibernate during the winter. The ground squirrels' hibernacula become so cold that sometimes there body temperature drops as low as -2.9°C, but they can avoid freezing by supercooling similar to some ectotherms (Barnes 1989). Arctic ground squirrels also thermoregulate maintaining a set point around -2° to -3°C by the use of brown adipose tissue and non-shivering thermogenesis.

Animals that endure the winter must make adjustments; altering the pelage is common response to winter. Some arctic species change their coat color so that they are less conspicuous; the arctic fox, arctic hare and ermine all shed their brownish summer coats and don pure white coats as winter approaches. In addition to changing color, changes in the insulatory properties of the pelage can be quite beneficial. The arctic fox has been observed to increase its fur thickness by 200% in certain regions of its body (Underwood 1971; Underwood and Reynolds 1980). There is a correlation between fur thickness and insulation, but animals the size of a fox and bigger seem to have reached a shared maximum insulation as there is no correlation with size and fur thickness or insulation for animals of increasing size (Scholander et al. 1950). Animals smaller than a fox must have shorter and lighter hair so that they can move, which compromises their insulation. However, smaller animals such as the shrews, weasels and lemmings all live underground in

burrows that are lined with leaves, grass caribou hair etc (Scholander et al. 1950). The burrows are warm enough and insulated enough to make up for the lack of insulation in the animal's natural coat.

In addition to increased insulation, some animals use regional heterothermy to shunt blood away from extremities to reduce heat loss from these regions. For example, the arctic fox uses a countercurrent heat exchanger in its legs to reduce the amount of heat lost through the feet, which are in direct contact with the snow (Prestrud 1991). Similarly, caribou have a counter current exchanger in their nasal cavity that prevents respiratory heat loss from exhaled air (Hammel et al. 1962). Preventing heat loss is important, but so is heat production and animals can make physiological changes to increase thermogenic capacity so that more heat can be produced. Thermogenic capacity can be defined as the total amount of heat an animal can produce and is the sum of the heat produced from shivering thermogenesis (ST), nonshivering thermogenesis (NST), and basal metabolic rate (BMR; Wunder and Gettinger 1996). Thermogenic capacity can be approximated by the maximal metabolic rate due to acute cold exposure (summit metabolism [$\dot{V}O_2$sum]; Chappell and Hammond 2004). There are many examples of small mammals responding to cold acclimation or acclimatization by increasing $\dot{V}O_2$sum (Heimer and Morrison 1978; Hayes and Chappell 1986; Nespolo et al. 1999, 2000; Chappell and Hammond 2004; Rezende et al. 2004). Several species of mammals show increased basal metabolic rate during winter (Lynch 1973; Rosenmann et al. 1975; Wunder et al. 1977; Heldmaier and Steinlechner 1981). NST has received a

lot of attention and is important for the survival of small mammals during the winter (Wunder 1985). Many mammals do not use ST unless the capacity of NST is insufficient to maintain body temperature (Brück 1970; Heldmaier 1972; Lilly and Wunder 1979). NST occurs in brown adipose tissue through the function of an uncoupling protein, UCP1. Proton fluxes across the mitochondria inner membrane are uncoupled from ATP production so that the net result is heat production (Cannon and Nedergaard 2003). Several studies involving multiple species have shown that NST increases after small mammals are exposed to prolonged cold exposure or decreased photoperiod (Lilly and Wunder 1979; Zegers and Merritt 1988; Wiesinger et al. 1989; Nespolo et al. 1999, 2000; Wang et al. 1999). With the exception of Nespolo et al. (1999, 2000), these studies only looked at changes in NST, but did not consider the effect this had on $\dot{V}O_2$sum.

Heat production by NST is regarded as more efficient than that by ST because both heat and muscle movement result from ST (Nespolo et al. 1999). Therefore, it is commonly assumed that NST is the most likely component of $\dot{V}O_2$sum to be changed in response to cold exposure, and it is often assumed that changes in $\dot{V}O_2$sum are due to changes in NST. However, Nespolo et al. (1999) demonstrated that this is not necessarily a safe assumption. *Phyllotis xanthopygus* acclimated to 15°C for one month showed a 94% higher $\dot{V}O_2$sum than animals acclimated to 30°C. This increase in $\dot{V}O_2$sum was due to a 76% increase in NST and a 200% increase in ST, suggesting that both NST and ST are important for maintaining body temperature. This relationship probably exists in other species, but may have been

missed as researchers often only look at one component of $\dot{V}O_2$sum and ignore the other components.

Life at high altitude

As mentioned before, endothermy requires high metabolic rates to maintain a constant, high body temperature. This is especially the case for small endotherms as heat flux is largely influenced by surface to volume rations and so very small organisms must maintain enormous mass-specific metabolic rates in order to regulate their body temperature (Horchachka and Somero 1984). In fact, some of the highest mass-specific metabolic rates known occur in the smallest endotherms such as shrews and hummingbirds (Horchachka and Somero 1984; Suarez 1998). Large amounts of oxygen are required for thermoregulation, a need that is complicated at high altitude. Endotherms living in high altitude environments are confronted with the combined challenges of low ambient temperature, decreased primary productivity and decreased partial pressure of oxygen (PO_2). Hypoxic conditions make it exceedingly difficult for highly aerobic endotherms to thermoregulate and maintain high levels of activity. Yet, research on animals living at high altitudes has shown that endotherms can be very successful at high altitudes and have evolved various physiological adaptations that enable them to do so. The physiological effects of altitude have been studied extensively in the deer mouse (*Peromyscus maniculatus sonoriensis*). The deer mouse is the most widely distributed mammal in North America and occupies a very large altitudinal range

from below sea level in Death Valley to 4300 m in the Sierra Nevada and White Mountains of California (Dunmire 1960).

The importance for increased maximal oxygen consumption ($\dot{V}O_2max$) in cold environments is likely enhanced at high altitudes. Animals at high altitude need a high $\dot{V}O_2max$ to meet increased thermogenic demands (Conley and Porter 1986). The lower partial pressure of oxygen at high altitudes reduces pulmonary oxygen loading and as a result many mammals suffer decreased $\dot{V}O_2max$ when PO_2 is reduced (Rosenmann and Morrison 1979; Ward et al. 1995) which in turn reduces thermogenic activity and ability for sustained activity (Hayes and O'Connor 1999). So, it is not surprising that Hayes and O'Connor (1999) found evidence for directional selection for increased $\dot{V}O_2max$ in wild populations of high altitude deer mice. Given that oxygen is reduced at high altitudes and having high $\dot{V}O_2max$ is important for survival there must be physiological mechanisms that allow for increasing $\dot{V}O_2max$ at altitude.

Physiologically, the body's first defense against hypoxia primarily involves improving oxygen delivery to account for reduced supply of atmospheric oxygen (Horchachka and Somero 1984). Wild deer mice from high altitudes have been found to have larger lungs and hearts than mice from nearby lower altitudes (Hock 1961; Hock 1964). Wyckoff and Frase (1990) found that deer mice from high altitudes had higher hematocrit, hemoglobin content, and mean red cell volume than white-footed mice (*Peromyscus leucopus*) from low altitudes. All of these traits would likely increase oxygen delivery, but these results are not sufficient to indicate

the differences are solely due to differences in oxygen availability. These traits likely increase thermogenic capacity and may have been a result of colder temperatures at high altitude, not hypoxic conditions. Hammond et al. (2001) investigated organ mass plasticity of deer mice across an altitudinal gradient while differentiating between the effects due to ambient temperature and PO_2. Ambient temperature was found to be the main determining factor for observed increases in heart mass while PO_2 was largely responsible for increased lung mass and increased hematocrit (Hammond et al. 2001).

In addition to changes in organ size, it is likely that species native to high altitudes have biochemical adaptations that alter the oxygen affinity of their respiratory pigments (Horchachka and Somero 1984). Classic studies on the genetics and physiology of hemoglobin have revealed that deer mice possess specialized adaptations potentially allowing for a higher oxygen binding affinity to accommodate lower oxygen availability at high altitudes (Snyder et al., 1988; Storz et al. 2007). Modifications that contribute to a higher binding affinity have occurred in both the α- and β- subunits of hemoglobin molecules (Storz et al 2009). Specific hemoglobin haplotypes have been shown to be advantageous at a particular altitude. Mice with the "high altitude haplotype" have increased oxygen binding affinity and higher VO_2max than mice with the "low altitude haplotype" when measured at high altitude, while mice with the "low altitude haplotype" perform best at low altitudes (Chappell and Snyder 1984). It is now widely accepted that high altitude animals typically have unusually high oxygen binding affinities

(Horchachka and Somero 1984; Powell 2003). A classic example can be seen in the camelid family. This family contains six species, four llamas living at high altitudes in the Andes Mountains (*Lama glama, L. pacos, L. guanacoe* and *L. vicugna*) and two camels living at low altitudes in Asia and Africa (*Camelus bactriamus* and *Camelus dromedarius*). The llamas all have higher oxygen binding affinities than the either of the two camels, but the llama that is active at the highest altitudes (*L. vicugna*) has the greatest oxygen binding affinity of all members of the family (Poyart et al. 1992).

Closing statements

The evolution of endothermy provided animals with numerous benefits. Endotherms are affected less by ambient temperature so that rapid changes in temperature will not immediately lead to rapid metabolic changes. This allows for flexibility in activity times so that endotherms can choose to be active when certain ectothermic predators are not. The ability to sustain high rates of metabolic activity may increase locomotory performance thereby increasing the ability to hunt prey and escape from predators or increase the time available to look for mates. High body temperatures allow for high rates of enzyme activity. Constant body temperature allows for neuromuscular coordination, sensory perception, and processing of neural inputs to occur at stable rates independent of changes in environmental temperature (Horchachka and Somero 1984). Despite all of the benefits endothermy confers, the reality is that endothermy is very energetically expensive. Many of the benefits of endothermy require maintaining a stable body temperature that is well above ambient temperature in most environments, which

places a large selection pressure on thermoregulatory ability. Proper thermoregulation, whether keeping cool or staying warm, requires high levels of oxygen. The need for proper thermoregulation and the dependence on oxygen make the presence of endotherms unlikely in extreme terrestrial environments such as the tops of mountains, the arctic poles and the earth's hottest deserts. However, as I have shown in the previous sections, endotherms occupy all of these habitats with great success by having evolved adaptations in their behavior and physiology.

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Chapter 1

A Phylogenetic Approach to Total Evaporative Water Loss in Mammals

Abstract

Maintaining appropriate water balance is a constant challenge for terrestrial mammals and this problem can be exacerbated in desiccating environments; therefore it has been proposed that natural selection has provided desert-dwelling mammals physiological mechanisms to reduce rates of total evaporative water loss. In this study we provide an updated allometric relationship for total evaporative water loss in mammals using a recent phylogenetic hypothesis. In addition we compared total evaporative water loss from 80 arid species to 56 mesic species ranging in size from 4 g to 3,500 Kg to test if mammals from arid environments have lower rates of total evaporative water loss compared with mammals from mesic environments. We found that arid species had lower rates of total evaporative water loss than mesic species when using a dichotomous variable to describe habitat (arid or mesic). We also found that total evaporative water loss was negatively correlated with the average maximum and minimum environmental temperature as well as the maximum vapor pressure deficit of the environment. Annual precipitation and the variable Q (a measure of habitat aridity) were positively correlated with total evaporative water loss. These results support the hypothesis that desert-dwelling mammals have lower rates of total evaporative water loss than mesic species after controlling for body mass and evolutionary relatedness regardless of whether categorical or continuous variables are used to describe habitat.

Introduction

Since the Devonian, when aquatic vertebrates first invaded land, terrestrial organisms have faced the challenge of reducing rates of water loss to their environment by minimizing evaporation from their respiratory passages (respiratory water loss, RWL) and/or across their skin (cutaneous water loss, CWL). Total evaporative water loss (TEWL), the sum of RWL and CWL, exceeds water loss in feces or urine by as much as 5 times, especially in small species (Chew 1951; MacMillen 1983; Lillywhite 2004). Endotherms have high mass-specific metabolic rates and, as a consequence, have high rates of respiration. As mammals exhale air, they lose considerable quantities of water from the saturated air in the lungs and respiratory passages. Mammals also lose water through their skin with rates of CWL being driven by the water vapor gradient between the animal's skin and its environment (Stoutjesdijk and Barkman 1992; Walsberg 2000).

Animals living in deserts face especially desiccating conditions due to high ambient temperatures, low ambient humidity and scarcity of drinking water (Schmidt-Nielsen 1964). In these regions, some animals rely on preformed water in their food and on metabolic water production to meet their water needs (Schmidt-Nielsen and Schmidt-Nielsen 1951). Most mammals typically maintain core body temperatures (T_b) of 37-38ºC, but this is particularly difficult in deserts where conditions usually produce environmental temperatures higher than T_b. When environmental temperatures exceed T_b, mammals must either seek more favorable

microclimates, or evaporate water from their skin or respiratory passages to thermoregulate (Schmidt-Nielsen and Schmidt-Nielsen 1950a; Chew 1951).

Several studies have investigated if species of arid mammals have reduced rates of TEWL compared with mesic species (Schmidt-Nielsen and Schmidt-Nielsen 1950b; Chew 1965; Hinds and MacMillen 1985; 1986; Williams et al. 2004; Withers et al. 2006). These studies provide evidence that mammals in arid habitats have lower rates of TEWL than species in mesic habitats. More than 40 years ago, Chew (1965) published an allometric equation for TEWL for 49 species of mammals that has been used to predict evaporative water losses in mammals for decades (Glenn 1970; Studier 1970; Hinds and Macmillen 1985; 1986; Anderson et al. 1997; Williams et al. 2001; 2004). Chew also compared rates of water loss for 7 mammals that live in arid environments with 42 mesic species and found that arid species had significantly lower rates of TEWL. Chew's (1965) allometric equation has been widely cited in comparative studies (Glenn 1970; Hinds and MacMillen 1985; 1986; Williams et al. 2002; 2004). However, there are aspects of Chew's study that potentially could lead to errors in interpretation. Sample size of arid species was small. Many more studies have been published since Chew's paper and addition of new data, particularly from arid species, could potentially alter his findings. Defining habitat as a categorical variable assumes that all habitats are identical within a given classification. However, within "arid" habitats there are numerous gradations of aridity with the least arid approaching the least mesic of the "mesic" habitats. For this reason the use of a continuous variable to characterize the

environmental conditions will reduce ambiguity over placing species into habitat categories (Muñoz-Garcia and Williams 2005). Chew's data set was confounded because it includes measurements of animals collected over a wide range of ambient temperatures (T_a) and included some species that were lactating; both of these factors can influence TEWL. Furthermore, methods to account for phylogenetic relatedness of species (Felsenstein 1985) were not available at the time of Chew's study (1965). Incorporation of more recent phylogenetic statistical models may improve our understanding of TEWL relative to body size. For these reasons we have revisited the relationship between TEWL and body mass in mammals.

In this study we present an allometric relationship for TEWL in mammals based on data from 136 species of mammals ranging in body size from 4 g to 3,500 Kg. We also examine the relationship between TEWL and habitat to determine if species from more arid environments have reduced rates of TEWL as has been demonstrated in other taxa (Schmidt-Nielsen and Schmidt-Nielsen 1950b; Chew 1965; Hinds and MacMillen 1985; 1986; Williams et al. 2004; Withers et al. 2006). Using phylogenetic comparative analyses our results suggest a different allometric relationship for TEWL than that of Chew; and that species from more arid environments have reduced rates of TEWL, when body size and phylogenetic relationships were taken into account.

Materials and Methods

Data collection

We obtained values for TEWL (g H_2O evaporated/d) measured directly (either gravimetrically or hygrometrically) and mass (g) for 136 species of adult mammals (Appendix 1). We used values of TEWL deemed to be standard rates of TEWL by the authors when stated; alternatively we used values of TEWL collected at temperatures closest to the lower critical temperature. Mass was not reported for one species, *Myotis lucifugus*, we obtained the data for mass from Whitaker (2000). We included data for 10 species that were in laboratory colonies, but excluded domesticated species. Digestive state of animals was often not mentioned in papers so we classified animals as post absorptive or not with those not specifically stated in the latter group. Although most mammals are nocturnal, we found data for 18 diurnal species. We excluded data for pregnant animals or for animals that were water deprived. When we found multiple sources of acceptable data for a species we average the data.

We used two different approaches to test if TEWL of mammals was related to habitat aridity. On one hand, we classified species of mammals in our data set into two discrete categories, arid or mesic species. On the other hand, we used continuous environmental variables to define climate and used these variables as predictors of TEWL. Classifying if a species is an arid or mesic species can be problematic because environmental aridity is based on a combination of factors including temperature, wind and rainfall (Noy-Meir 1973; Williams 1996). We

adopted the assignments of authors for a species' habitat type, and verified these assignments by overlaying range maps with arid areas of the world using McGinnies et al. (1968). Based on these assignments, we had 80 species from arid environments and 56 species from mesic habitats. Data on climate was collected from the International Water Management Institute's water and climate atlas (2012), Weather Reports (2012) and World Climate (2012). We acquired climate data for a location that was as close as possible to the reported collection site of animals in the original water loss studies. Collection sites were not reported for 14 species, so we used data on climate from the geographical midpoints of their range (see appendix 1). In the case of laboratory-reared and captive animals, we used climate data from the area where the stock population was collected. We used several independent variables to characterize habitat including annual precipitation (P mm), mean maximum temperature of the hottest month of the year (T_{max} °C), mean minimum temperature of the coldest month of the year (T_{min}, °C), mean yearly temperature (T_{mean}°C), precipitation variability (RV; coefficient of variation of annual rainfall calculated as SD/mean of the monthly average precipitation), mean saturation vapor pressure deficit of the driest month of the year (VPD_{max} mbar), mean saturation vapor pressure deficit of the wettest month of the year (VPD_{min} mbar), yearly average vapor pressure deficit (VPD_{mean} mbar), and the variable Q. Developed by Emberger (1955), Q measures primary productivity in arid and semi-arid habitats. Primary productivity and aridity are directly related, so Q has been used as a proxy for aridity (Tieleman et al. 2003). Q is calculated by the equation

$$Q = P/((Tmax + Tmin)(Tmax - Tmin)) * 1000 \quad (1)$$

where P (mm), Tmax and Tmin (°C) are described above. The index Q decreases with increasing aridity.

Construction of phylogenetic tree

We used the phylogenetic super tree published by Bininda-Emonds et al. (2007) to describe phylogenetic relationships and divergence times of the species of mammals in our dataset. We pruned the tree to remove all the taxa for which we had no data. Five taxa in our dataset were not represented in Bininda-Emonds et al.'s tree, two distinct populations of *Vulpes vulpes* (one montane and one desert-dwelling), *Molossus coibensis, Eothenomys miletus* and *Micoureus paraguayanus*. We treated the two populations of *Vulpes vulpes* as separate species, assuming they diverged similarly; we used the program Mesquite (Maddison and Maddison 2006) to split the branch of *Vulpes vulpes* in Bininda-Emonds et al. (2007) generating two branches of equal length (Oufiero et al. 2011). Although *Molossus coibensis, Eothenomys miletus* and *Micoureus paraguayanus* were not included in the supertree (Bininda-Emonds et al. 2007), they considered other species in these genera. Therefore, we merged the genus into one species and used that branch length for our species. We also used the taxonomic names used by Bininda-Emonds et al. (2007) and so some of the species names we use differ from those used in the original publications from which the data were collected.

Some species were included as polytomies since we had incomplete phylogenetic information. All of the polytomies in our tree were considered "soft"

and so degrees of freedom for hypothesis testing was determined by the more conservative (df = # nodes -1) method proposed by Purvis and Garland (1993).

Statistical analysis

When analyzing data from interspecific comparative studies, it can be important to incorporate information about the evolutionary relationships of the species (Garland et al. 1992 2005). Early on in the discussion about the importance of correcting for phylogeny, some authors warned about complete reliance on statistical methods, such as phylogenetic generalized least squares (PGLS), purported to control for phylogenetic relationships, and advocated for using both phylogenetically informed models and non-phylogenetically informed models, such as ordinary least squares models (OLS) to understand how natural selection has modified physiological attributes of organisms (Price 1997; Westoby et al. 1995; Muñoz-Garcia and Williams 2005). While Freckleton (2009) encourages authors to determine which model is most appropriate for their study and only report those results. Here we use both PGLS and OLS and expand our comparison to include an additional phylogenetic hypothesis using an Ornstein-Uhlenbeck transformation (RegOU, see Lavin et al. 2008), which transforms the branch lengths of the starting phylogenetic tree to allow for the simultaneous estimation of the residual error structure with the regression model parameters in which the residual error is modeled by a process similar to stabilizing selection (Hansen 1997). Thus, we have three models of evolution that range in their hierarchical structure of the residual error; OLS exhibits no hierarchal structure – a "star" phylogeny, PGLS exhibits

hierarchical structure that evolves according to a Brownian motion of evolution, and the Ornstein-Uhlenbeck transformation (RegOU), which models the residuals similar to stabilizing selection. The best fit model can be determined by a comparison of Akaike Information Criterion (AIC) values and log-likelihoods (see below) to determine if the incorporation of phylogenetic information improves the fit of the model.

We used the MATLAB program Regressionv2.m to create linear statistical models using ordinary least squares regression (OLS), phylogenetic generalized least squares (PGLS) and the RegOU model. The RegOU model alters the branch lengths of phylogenetic tree, pulling the internal nodes either towards the tips or towards the root, using an additional parameter, *d*. When $d = 1$, the tree is equal to the starting PGLS model, whereas when $d = 0$ the tree is equal to the OLS model. The RegOU model simultaneously estimates the phylogenetic signal in the residual error with the regression coefficients. Therefore, *d* provides an indicator of the amount of phylogenetic signal in the residuals of the dependent variable (Blomberg et al. 2003; Lavin et al. 2008). Furthermore, because the RegOU model contains an additional parameter, its fit can be compared with the OLS or PGLS models using a likelihood ratio test, where twice the difference in the log-likelihood is assumed to be distributed as a c^2 with 1 d.f. The critical value of a =0.05 in this distribution is 3.841 (Oufiero et al. 2011). If the RegOU model exhibits a $c^2 > 3.841$ compared with the OLS model, then the residual variation in the dependent variable exhibits statistically significant phylogenetic signal.

All data for TEWL and mass were $\log_{10}$-transformed prior to analysis to correct for deviations in normality. Habitat was coded as 0 for mesic and 1 for arid, referred to hereafter as HABITAT. The variable Q is driven by precipitation and rapidly increases as habitats become more mesic so we used $\log_{10}$-transformed Q to avoid biasing data from mesic environments (Tieleman et al. 2003). We included models with raw values for annual precipitation as well as $\log_{10}$transformed values. We tested various models using each independent variable listed above (HABITAT, P, T_{max}, T_{min}, T_{mean}, RV, VPD_{max}, VPD_{min}, VPD_{mean} and $Log_{10}Q$) to determine which was best correlated with TEWL after accounting for body mass. We tested each of these models using the three models of evolution listed above [OLS (star phylogeny), PGLS and RegOU]. We used maximum likelihood ratio tests to determine which model best fit the data as described above.

Results

We initially determined that activity time (nocturnal or diurnal), TEWL measurement method (gravimetric or hygrometric), lab status, and post absorptive state were not significant covariates in predicting TEWL and so included data for all animals in our analyses. The RegOU model had a better fit to the data than the OLS or PGLS models, based on maximum likelihood ratio tests (likelihood ratio test > 3.8414, two-tailed $p < 0.05$; Table 2.1) in all cases except for the OLS model including the predictor VPDmin. The REGOU and OLS models including this variable were not significantly different (likelihood ratio test = 3.04, two-tailed $p = 0.08$) and VPD_{min} was not a significant predictor of TEWL in any of the three models tested.

Therefore we will focus on results obtained from the RegOU evolutionary models; see tables 1.2 and 1.3 for full results of all models. Regression coefficients are presented with ± standard errors.

The equation that described our data on TEWL among mammals using the RegOU model was $Log_{10}TEWL = -0.721 + (0.693 \pm 0.03) \times Log_{10}\,Mass$ ($R^2 = 0.815$, $F_{1,108} = 590.32$, $p < 0.001$; Table 2.1, Fig. 1.1). The slope of this model was not significantly different from the OLS model (0.696; t (268) = 0.09, $p = 0.927$), but is significantly smaller than the slope reported by Chew (1965) (0.826; t (181) = 4.13, $P < 0.001$). When we included HABITAT, the regression equation between TEWL and mass was $Log_{10}TEWL = -0.619 + (0.682 \pm 0.03) \times Log_{10}Mass - (0.150 \pm 0.05) \times$ HABITAT ($R^2 = 0.833$, $F_{2,107} = 331.70$, $p < 0.001$; Table 1.1, Fig. 1.2). The partial regression coefficient for HABITAT was significantly different from zero and negative ($F = 10.42$, $p = 0.002$), indicating that species living in arid habitats have significantly lower rates of TEWL after accounting for differences in mass.

When we used a continuous variable to describe aridity, we found that the composite variable $Log_{10}Q$ along with all of its components (T_{max}, T_{min} and P) were significant predictors of TEWL (Table 1.1, Fig. 1.3). VPD_{max} also showed significant negative relationship with TEWL and is significantly correlated with $Log_{10}Q$ ($r(134) = -0.45$, $p < 0.001$). The relationships between all of these environmental variables and TEWL indicate that TEWL decreases with increasing habitat aridity. The best model predicting TEWL from environmental variables based on Akaike Information Criterion for small sample sizes (AICc) (Burnham and Anderson 2002) was the

model using habitat categorized as mesic or arid. $Log_{10}Q$ was the best continuous variable predicting TEWL described by the equation ($Log_{10}TEWL = -0.081 + (0.688 \pm 0.03) \times Log_{10}Mass - (0.081 \pm 0.04) \times Log_{10}Q$; $R^2 = 0.821$, $F_{2,107} = 305.01$, $p < 0.001$). (Table 1.1, Fig. 1.3).

Discussion

For nearly 40 years, Chew's allometric equation has been the most inclusive model available for predictions of TEWL based on body mass. These predictions are important for conservation biologists, and for modelers interested in the impact of climate change on water relations, and therefore distribution patterns of extant animals. Here we provided an allometric equation for TEWL from body mass based on data from 129 species of mammals with more stringent selection criteria than that used by Chew, and incorporation of phylogenetic relationships. Much of the data for TEWL that we included in our model was collected from relatively small mammals. Over 80% of the mammals in our analysis had a body mass of less than 1kg. The only animal in our dataset that was over 100 kg was the Asian elephant and the next largest was the Arabian oryx at 84 kg. The differences in mass distribution between our data set and that of Chew's are reflected in the predictions provided by the allometric equations. Chew's equation tends to provide higher estimates of TEWL than does our equation. The average rate of TEWL for a 100 - 200 g mammal (using values from our dataset, n = 14) is 4.9 g/day. Our allometric equation predicts the TEWL of a mammal with a mass of 100 g to be 4.6 g/day, while Chew's equation predicts 9.3 g/day for the same animal. Using our equation, a

20 kg mammal has a predicted rate of TEWL of 182.2 g/day, whereas Chew's equation predicts 735 g/day. It appears the equation we have provided is more accurate over a larger range of body size than the equation published by Chew (1965). The incorporation of phylogenetic information did not substantially change the scaling of TEWL with mass (see results).

It has long been thought that species living in extreme habitats should have adaptations to reduce rates of water loss (Schmidt-Nielsen1964). Several studies have previously investigated whether or not species of mammals from arid regions have lower rates of TEWL than mesic species (Schmidt-Nielsen and Schmidt-Nielsen 1950b; Chew 1965; Hinds and MacMillen 1985; 1986; Williams et al. 2004; Withers et al. 2006). With the exception of Chew (1965), these studies focus on a single or a few taxa. Using our more inclusive dataset we were able to provide further evidence for the relationship between TEWL and habitat while incorporating phylogenetic relationships and using a continuous variable to describe habitat aridity.

The variable Q was originally developed as an estimate of primary productivity in arid and semi-arid environments (Emberger 1955) and has since been used as an estimate of aridity based on the logic that aridity is directly related to primary productivity (Tieleman et al. 2003, Oufiero et al. 2011). Deserts are characterized by hot dry conditions and Q is calculated using both temperature and annual precipitation (see equation 1). We found that Q and all of the components were significant predictors of TEWL. We also found maximum vapor pressure

deficit to be a significant predictor of TEWL and based on the physical model of evaporation, vapor pressure deficit should be the major factor determining rates of evaporative water loss (Stoutjesdijk and Barkman 1992; Walsberg 2000). Maximum vapor pressure deficit and Q are highly correlated and both seem to be good descriptors of habitat aridity. Withers et al. (2006) suggest that it may be advantageous for animals living in areas with unpredictable and variable rainfall to conserve water by lowering TEWL and showed that rates of TEWL were negatively correlated with the variability of rainfall, however our data did not support this result.

Using continuous variables to describe habitat allows slight gradations in aridity, rather than simply grouping animals by habitat type. However, describing habitats as simply mesic or arid provided the best model for predicting TEWL based on AICc (Table 1). There are many environmental factors that make a habitat arid and it appears that the sum of their influence is more important in influencing TEWL than any single variable that we tested. In conclusion, we found that TEWL of species that live in arid environments is significantly lower than that of species that live in mesic environments, after accounting for body mass and phylogeny.

Table 1.1. Predictors of Log10 TEWL (g/day) using the REG OU model. LOGMASS is $\log_{10}$ transformed body mass (g); HABITAT is coded as 0 for mesic, 1 for arid; P = annual precipitation (mm); TMAX = mean maximum temperature of the hottest month of the year (°C); TMIN = mean minimum temperature of the coldest month of the year (°C); TMEAN = mean yearly temperature (°C); VPDMAX = mean saturation vapor pressure deficit of the driest month of the year (mbar); VPDMIN = mean saturation vapor pressure deficit of the wettest month of the year (mbar); VPDMEAN = yearly average vapor pressure deficit (mbar); RV = rainfall variability calculated as the coefficient of variation of annual rainfall; LOGQ = Log_{10} transformed values of the variable Q (measure of aridity). The RegOU models were significantly better than the OLS and PGLS models in every model except that including VPDMIN evidenced by the ln ML ratio tests at $P < 0.05$ (critical value for a c^2 distribution with one df = 3.841).

Table 1.1. Predictors of Log10 TEWL (g/day) using the REG OU model

							ln ML ratio test	
Model	Coefficient	SE	F	p-value	ln ML	AICc	RegOU vs. OLS	RegOU vs. PGLS
Intercept	-0.721	0.064	126.42	<0.001	-12.03	32.37	4.42	60.96
LOGMASS	0.693	0.029	588.53	<0.001				
Intercept	-0.619	0.067	84.21	<0.001	-6.88	24.23	5.1	60.5
LOGMASS	0.682	0.027	631.02	<0.001				
HABITAT	-0.150	0.046	10.42	0.002				
Intercept	-0.765	0.066	135.76	<0.001	-9.99	30.45	3.94	63.12
LOGMASS	0.688	0.028	616.70	<0.001				
P	0.00009	0.00004	3.99	0.048				
Intercept	-0.450	0.130	11.93	0.001	-9.50	29.45	9.3	44.02
LOGMASS	0.694	0.030	537.89	<0.001				
TMAX	-0.009	0.003	6.48	0.012				
Intercept	-0.698	0.072	94.48	<0.001	-9.91	30.28	8.66	47.8
LOGMASS	0.699	0.03	543.27	<0.001				
TMIN	-0.005	0.002	5.72	0.019				
Intercept	-0.667	0.083	63.99	<0.001	-11.53	33.52	5.34	55.58
LOGMASS	0.695	0.029	571.62	<0.001				
TMEAN	-0.003	0.003	1.21	0.274				
Intercept	-0.620	0.079	61.27	<0.001	-8.88	28.22	10.48	46.5
LOGMASS	0.690	0.030	544.29	<0.001				
VPDMAX	-0.006	0.002	7.41	0.008				
Intercept	-0.737	0.075	95.61	<0.001	-11.93	34.32	3.04	60.9
LOGMASS	0.692	0.028	592.24	<0.001				
VPDMIN	0.003	0.008	0.18	0.672				
Intercept	-0.660	0.079	69.65	<0.001	-11.05	32.56	6.22	52.3
LOGMASS	0.692	0.029	558.67	<0.001				
VPDMEAN	-0.006	0.004	2.30	0.132				
Intercept	-0.663	0.076	76.22	<0.001	-11.09	32.64	3.14	61.66
LOGMASS	0.694	0.029	603.06	<0.001				
RV	-0.072	0.054	1.80	1.83				
Intercept	-0.923	0.112	67.54	<0.001	-7.72	25.91	5.38	56.92
LOGMASS	0.688	0.028	602.53	<0.001				
LOGQ	0.081	0.035	5.36	0.023				

Table 1.2. Predictors of Log10 TEWL (g/day) using the OLS (star phylogeny) model LOGMASS is $\log_{10}$ transformed body mass (g); HABITAT is coded as 0 for mesic, 1 for desert; P = annual precipitation (mm); TMAX = mean maximum temperature of the hottest month of the year (°C); TMIN = mean minimum temperature of the coldest month of the year (°C); TMEAN = mean yearly temperature (°C); VPDMAX = mean saturation vapor pressure deficit of the driest month of the year (mbar); VPDMIN = mean saturation vapor pressure deficit of the wettest month of the year (mbar); VPDMEAN = yearly average vapor pressure deficit (mbar); RV = rainfall variability calculated as the coefficient of variation of annual rainfall; LOGQ = Log_{10} transformed values of the variable Q (measure of aridity).

Table 1.2 Predictors of Log10 TEWL (g/day) using the OLS (star phylogeny) model

Variable	Coefficient	SE	F	p-value	ln ML	AICc
Intercept	-0.733	0.53	193.86	< 0.001	-14.24	34.66
LOGMASS	0.696	0.023	889.71	<0.001		
Intercept	-0.645	0.058	122.79	<0.001	-9.43	27.17
LOGMASS	0.695	0.023	942.05	<0.001		
HABITAT	-0.143	0.046	9.75	0.002		
Intercept	-0.784	0.057	187.84	<0.001	-11.96	32.22
LOGMASS	0.699	0.023	917.26	<0.001		
P	0.00009	0.00004	4.54	0.035		
Intercept	-0.683	0.128	28.34	<0.001	-14.15	36.60
LOGMASS	0.697	0.024	876.97	<0.001		
TMAX	-0.002	0.004	0.186	0.667		
Intercept	-0.732	0.054	186.75	<0.001	-14.24	36.78
LOGMASS	0.697	0.024	862.02	<0.001		
TMIN	-0.0003	0.003	0.01	0.921		
Intercept	-0.751	0.083	81.99	<0.001	-14.20	36.71
LOGMASS	0.695	0.024	857.62	<0.001		
TMEAN	0.001	0.004	0.08	0.778		
Intercept	-0.716	-0.063	127.19	<0.001	-14.12	36.54
LOGMASS	0.698	0.024	873.81	<0.001		
VPDMAX	-0.001	0.003	0.24	0.625		
Intercept	-0.780	0.065	145.39	<0.001	-13.45	35.21
LOGMASS	0.692	0.024	863.65	<0.001		
VPDMIN	0.01	0.008	1.55	0.216		
Intercept	-0.748	0.065	131.49	<0.001	-14.16	36.63
LOGMASS	0.695	0.024	861.54	<0.001		
VPDMEAN	0.002	0.004	0.15	0.699		
Intercept	-0.664	0.065	103.60	<0.001	-12.66	33.63
LOGMASS	.699	0.023	906.84	<0.001		
RV	-0.086	0.049	3.12	0.080		
Intercept	-0.947	0.10	88.63	<0.001	-10.41	29.12
LOGMASS	0.70	0.023	904.89	<0.001		
LOGQ	0.081	0.032	6.55	0.012		

Table 1.3. Predictors of Log10 TEWL (g/day) using the PGLS model.
LOGMASS is $\log_{10}$ transformed body mass (g); HABITAT is coded as 0 for mesic, 1 for desert; P = annual precipitation (mm); TMAX = mean maximum temperature of the hottest month of the year (°C); TMIN = mean minimum temperature of the coldest month of the year (°C); TMEAN = mean yearly temperature (°C); VPDMAX = mean saturation vapor pressure deficit of the driest month of the year (mbar); VPDMIN = mean saturation vapor pressure deficit of the wettest month of the year (mbar); VPDMEAN = yearly average vapor pressure deficit (mbar); RV = rainfall variability calculated as the coefficient of variation of annual rainfall; LOGQ = Log_{10} transformed values of the variable Q (measure of aridity).

Table 1.3. Predictors of Log10 TEWL (g/day) using the PGLS model

Variable	Coefficient	SE	F	p-value	ln ML	AICc
Intercept	-0.830	0.386	4.63	0.034	-42.51	91.21
LOGMASS	0.738	0.048	233.52	<0.001		
Intercept	-0.669	0.375	3.18	0.077	-37.13	82.57
LOGMASS	0.695	0.048	206.22	<0.001		
HABITAT	-0.156	0.047	10.95	<0.001		
Intercept	-0.867	0.385	5.07	0.026	-41.55	91.40
LOGMASS	0.729	0.049	226.16	<0.001		
P	0.00007	0.00005	1.90	0.171		
Intercept	-0.254	0.38	0.46	0.499	-31.51	71.32
LOGMASS	0.681	0.046	216.75	<0.001		
TMAX	-0.015	0.003	23.61	<0.001		
Intercept	-0.642	0.366	3.08	0.082	-33.81	75.92
LOGMASS	0.686	0.047	212.69	<0.001		
TMIN	-0.007	0.002	18.16	<0.001		
Intercept	-0.651	0.385	2.86	0.094	-39.32	86.95
LOGMASS	0.709	0.049	211.50	<0.001		
TMEAN	-0.006	0.002	6.39	0.013		
Intercept	-0.534	0.364	2.15	0.146	-32.13	72.57
LOGMASS	0.672	0.047	203.91	<0.001		
VPDMAX	-0.01	0.002	21.94	<0.001		
Intercept	-0.792	0.393	4.05	0.047	-42.38	93.06
LOGMASS	0.731	0.050	211.16	<0.001		
VPDMIN	-0.004	0.007	0.27	0.604		
Intercept	-0.593	0.379	2.45	0.120	-37.20	82.71
LOGMASS	0.686	0.049	194.39	<0.001		
VPDMEAN	-0.011	0.003	10.80	<0.001		
Intercept	-0.778	0.388	4.01	0.048	-41.92	92.14
LOGMASS	0.737	0.048	232.95	<0.001		
RV	-0.061	0.06	1.17	0.282		
Intercept	-0.917	0.381	5.77	0.018	-36.18	80.66
LOGMASS	0.684	0.048	206.01	<0.001		
LOGQ	0.075	0.040	3.49	0.064		

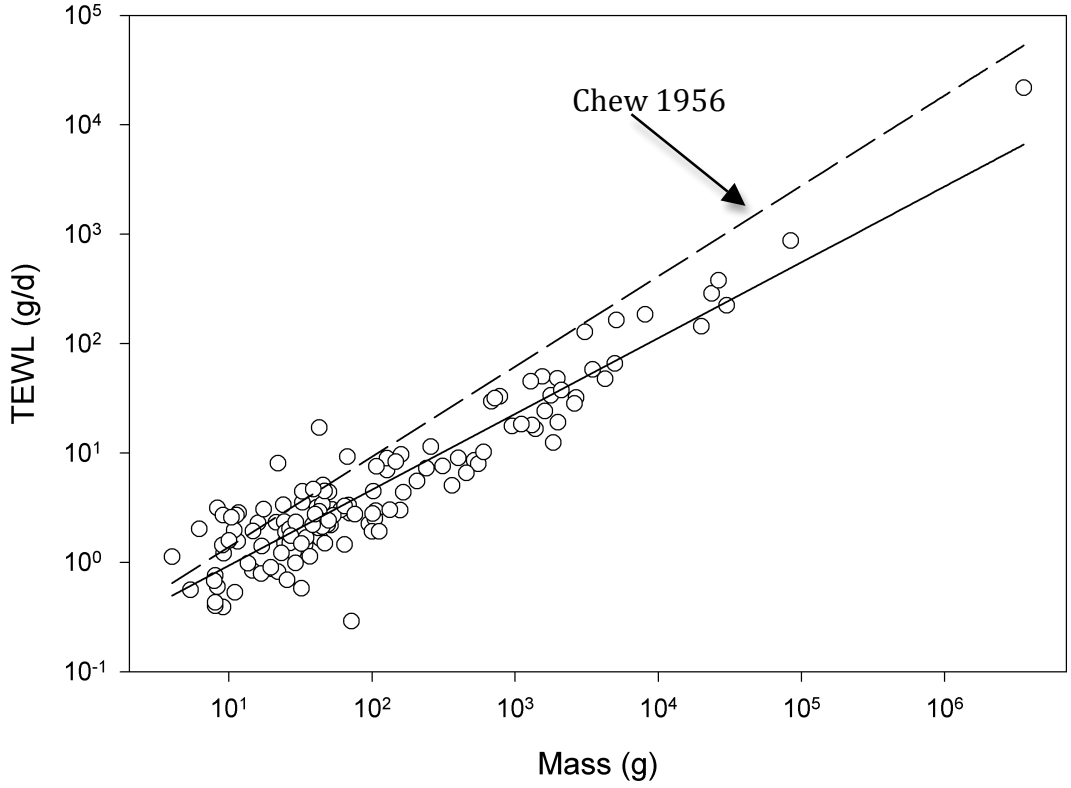

Figure 1.1. Allometric relationship of TEWL. The solid line represents the regression generated from our data using the RegOU model ($Log_{10}TEWL = -0.721 + (0.693 \pm 0.03) \times Log_{10}$ Mass ($R^2 = 0.815$, $F_{1,108} = 590.32$, $p < 0.001$). The dashed line represents the regression generated by Chew (1965).

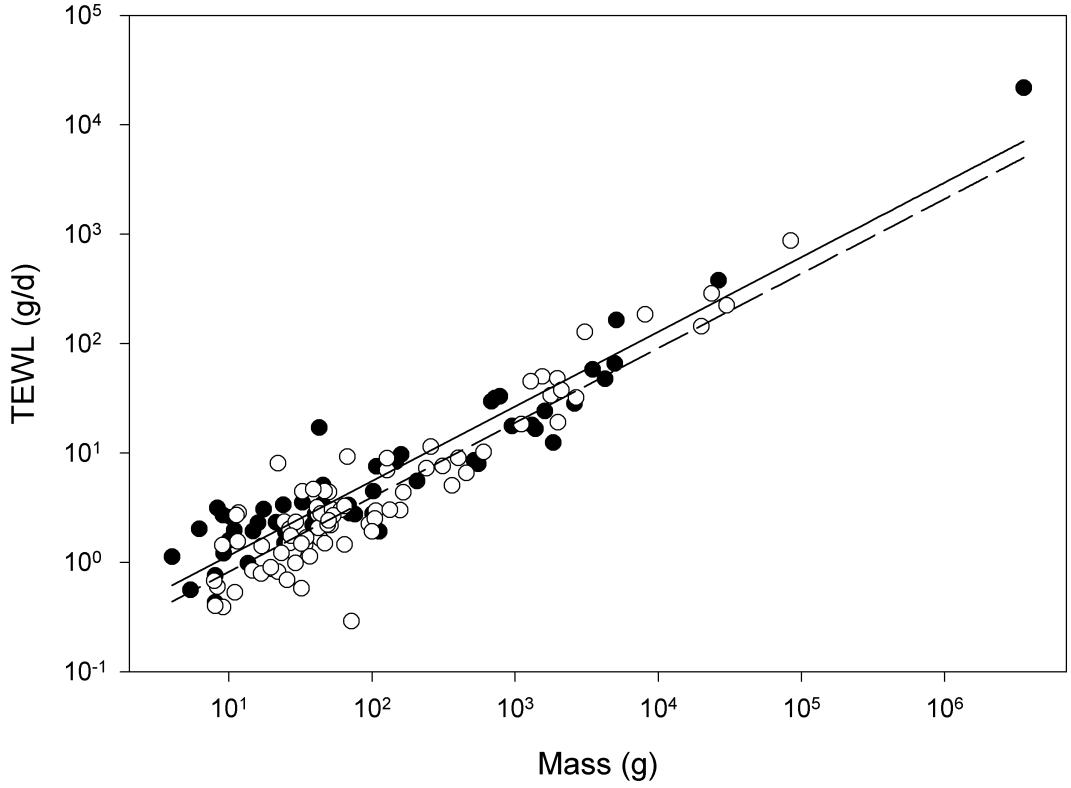

Figure 1.2. Allometric relationship of TEWL for all mammals separated by habitat using the RegOU model ($Log_{10}TEWL = -0.619 + (0.682 \pm 0.03) \times Log_{10}Mass - (0.150 \pm 0.05) \times HABITAT$ ($R^2 = 0.833$, $F_{2,107} = 331.70$, $p < 0.001$). The closed circles and solid line represent mesic species. The open circles and dashed line represents arid species.

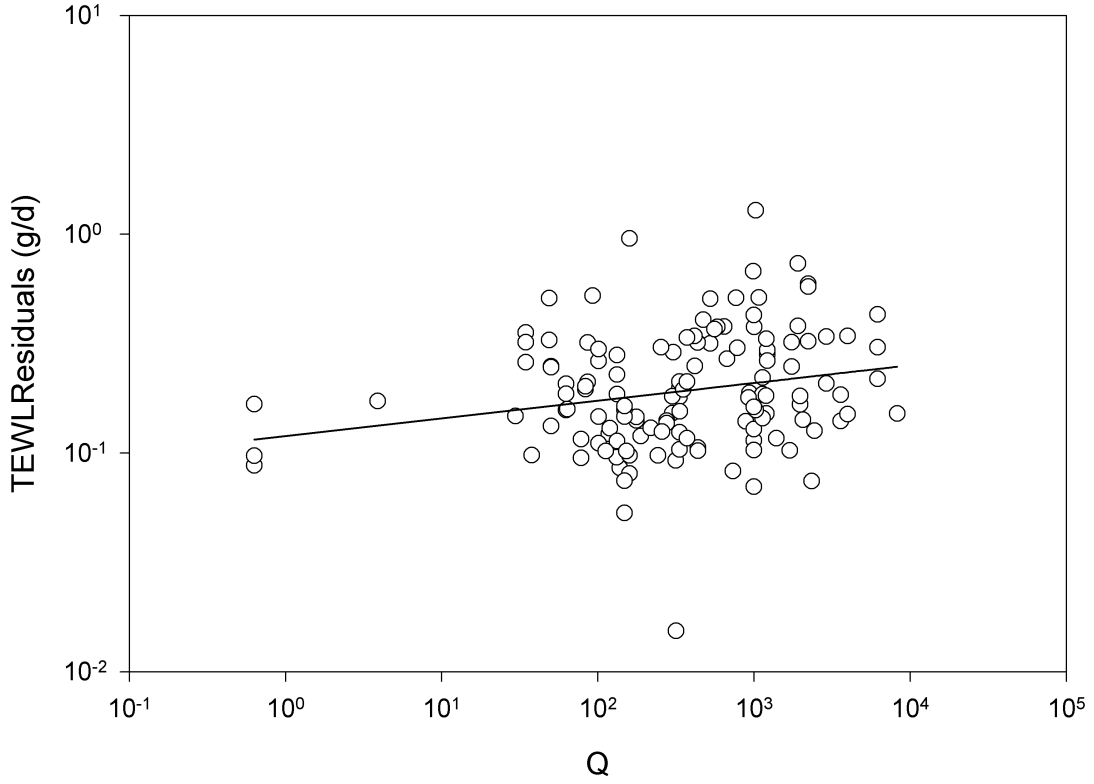

Figure 1.3 Relationship between TEWL residuals and Q using the RegOU model ($Log_{10}TEWL = -0.081 + (0.688 \pm 0.03) \times Log_{10}Mass - (0.081 \pm 0.04) \times Log_{10}Q$; $R^2 = 0.821$, $F_{2,107} = 305.01$, $p < 0.001$) indicating that rates of TEWL increase as habitats become increasingly mesic.

Appendix. List of species used in the analysis listed in taxanomic order corresponding to our phylogenetic tree. Reference refers to the original paper from which the data were obtained. The original collection location is the location where the original researchers collected the animals while weather data represents the location we used to obtain environmental data. Habitat indicates whether these animals were categorized as belonging to a mesic or arid environment.

Reference	Species	Original Collection Location	Weather Data	Habitat
Cooper and Cruz-Neto 2009	*Tarsipes rostratus*	Cataby, WA, Australia	Perth, WA, Australia	MESIC
Dawson and Degabriele 1973	*Spilocuscus maculatus*	Madang region, New Guinea	Madang, New Guinea	MESIC
Cooper and Withers 2008	*Trichosurus vulpecula*	Mt Caroline Nature Reserve, WA, Australia	Perth, WA, Australia	MESIC
Bartholomew and Hudson 1962	*Cercartetus nanus*	Hobart, TAS, Australia	Hobart, TAS, Australia	MESIC
Dawson and Bennett 1978	*Lagorchestes conspicillatus*	Barrow Island, WA, Australia	Barrow Island, WA, Australia	ARID
Dawson et al. 1969	*Macropus eugenii*	Kangaroo Island, SA, Australia	Kingscote, SA, Australia	MESIC
Dawson et al. 2000	*Macropus rufus*	Captive Reared	Broken Hill, NSW, Australia	ARID
Dawson 1973	*Macropus robustus*	Captive Reared	Broken Hill, NSW, Australia	ARID
Dawson et al. 2000	*Macropus giganteus*	Captive Reared	Roma, QLD, Australia	MESIC
Hinds and MacMillen 1986	*Antechinus stuartii*	No Mention	Brisbane, QLD, Australia	MESIC
Hinds and MacMillen 1986	*Phascogale tapoatafa*	Captive Reared	Brisbane, QLD, Australia	MESIC
Haines et al. 1974	*Dasycercus byrnei*	Birdsville Station, QLD, Australia	Brisbane, QLD, Australia	ARID
Haines et al. 1974	*Dasycercus cristicauda*	Warburton Range, NT, Australia	Warburton, NT, Australia	ARID
Schmidt et al. 2009	*Dasyurus geoffroii*	Julimar State Forest	Perth, WA, Australia	MESIC
Cooper and Withers 2010	*Dasyurus viverrinus*	Douglas Wildlife Park, Broome, WA, Australia	Broome, WA, Australia	MESIC
Cooper and Withers 2010	*Dasyurus maculatus*	Douglas Wildlife Park, Broome, WA, Australia	Broome, WA, Australia	MESIC
Cooper and Withers 2010	*Dasyurus hallucatus*	Douglas Wildlife Park, Broome, WA, Australia	Broome, WA, Australia	MESIC
Hinds and MacMillen 1986	*Pseudantechinus macdonnellensis*	No Mention	Warburton, VIC, Australia	ARID
Withers and Cooper 2009	*Sminthopsis psammophila*	Perth, WA , Australia (Zoo)	Nullarbor,SA, Australia	ARID
Cooper et al. 2005	*Sminthopsis macroura*	Lab colony	Alice Springs, NT, Australia	ARID
Haines et al. 1974	*Sminthopsis crasicaudata*	Birdsville Station, QLD, Australia	Birdsville Station, QLD, Australia	ARID
Hinds and MacMillen 1986	*Sminthopsis laniger*	No Mention	Alice Springs, NT, Australia	ARID
Warnecke et al. 2010	*Planigale gilesi*	Kinchega National Park, NSW, Australia	Menindee, NSW, Australia	ARID
Hinds and MacMillen 1986	*Planigale maculata*	No Mention	Brisbane, QLD, Australia	MESIC
Cooper and Withers 2002.	*Myrmecobius fasciatus*	Perth, WA, Australia (Zoo)	Perth, WA, Australia	MESIC
Withers et al. 2000	*Notoryctes typhlops*	Punmu, WA, Australia	Newman, WA, Australia	ARID
Withers 1992	*Isodon auratus*	Lab colony	Barrow Island, WA, Australia	ARID
Larcombe and Withers 2007	*Isodon obesulus fusciventer*	Perth, WA, Australia	Perth,WA, Australia	MESIC
Larcombe and Withers 2006	*Perameles bougainville*	Narrogin, WA, Australia	Narrogin, WA, Australia	ARID

Reference	Species	Original Collection Location	Weather Data	Habitat
Larcombe et al. 2006	*Perameles gunnii*	Hobart, TAS, Australia	Hobart, TAS, Australia	MESIC
Cooper et al. 2009	*Gracilinanus agilis*	Araraquara, Brazil	Sao Paulo, Brazil	MESIC
Cooper et al. 2010	*Micoureus paraguayanus*	Corumbatai, Brazil	Sao Paulo, Brazil	MESIC
Brower and Cade 1966	*Napaeozapus insignis*	Whiteface Mountain, NY, USA	Wilmington, NY, USA	MESIC
Shkolnik and Borut 1969	*Acomys cahirinus*	Southeast Israel Desert, Israel	Beersheba, Israel	ARID
Shkolnik and Borut 1969	*Acomys russatus*	Southeast Israel Desert, Israel	Beersheba, Israel	ARID
Zhu et al. 2008	*Apodemus chevrieri*	Jianchuan County, Yunnan Province, China	Lijiang, China	MESIC
Haines et al. 1974	*Leporillus conditor*	Franklin Island, SA, Australia	Victor Harbor, SA, Australia	ARID
Withers et al. 1979	*Notomys alexis*	Lab colony	Alice Springs, NT, Australia	ARID
Haines et al. 1974	*Notomys cervinus*	Lab colony	Birdsville, QLD, Australia	ARID
Haines et al. 1974	*Pseudomys australis*	Lab colony	Alice Springs, QLD, Australia	ARID
Haines et al. 1974	*Pseudomys desertor*	Anna Creek Station, SA, Australia	Marla, SA, Australia	ARID
Schmidt-Nielsen and Schmidt-Nielsen 1950b	*Mus musculus*	No Mention	Champaign, IL USA	MESIC
Schmidt-Nielsen and Schmidt-Nielsen 1950b	*Rattus norvegicus*	No Mention	Champaign, IL USA	MESIC
Christian 1978	*Rhabdomys pumilio*	Lab born	Windhoek, Namibia	ARID
Cortes et al. 2000a	*Akodon longipilis*	PN Fray Jorge, Chile	PN Fray Jorge/La Sarena, Chile	ARID
Cortes et al. 1990,2000a	*Akodon olivaceus*	PN Fray Jorge, Chile	PN Fray Jorge/La Sarena, Chile	ARID
Cortes et al. 2000a	*Chroeomys andinus*	Talabre, Chile	Talabre/La Sarena, Chile	ARID
Cortes et al. 1990,2000a	*Phyllotis darwini*	PN Fray Jorge, Chile	PN Fray Jorge/La Sarena, Chile	ARID
Cortes et al. 2000a	*Phyllotis magister*	Ojo Opache, Chile	Ojo Opache/Antafogasta, Chile	ARID
Cortes et al. 2000a	*Phyllotis xanthopygus*	Quebrada Jerez, Chile	Talabre/La Sarena, Chile	ARID
Cortes et al. 1990,2000a	*Oligoryzomys longicaudatus*	PN Fray Jorge, Chile	PN Fray Jorge/La Sarena, Chile	ARID
Scheck 1982	*Sigmodon hispidus*	Lab born, from College Station, TX, USA	College Station, TX, USA	MESIC
Schmidt-Nielsen and Schmidt-Nielsen 1950b	*Peromyscus crinitus*	No Mention	Las Vegas, NV, USA	ARID
Lindeborg 1955	*Peromyscus leucopus tornillo*	Alamagordo, NM, USA	Alamagordo, NM, USA	ARID
Brower and Cade 1966	*Peromyscus maniculatus*	Whiteface Mountain, NY, USA	Wilmington, NY, USA	MESIC
Coulombe 1970	*Reithrodontomys megalotis*	Ballona Creek, Southern California, USA	Culver City, CA, USA	ARID
Schmidt-Nielsen and Schmidt-Nielsen 1950b	*Mesocricetus auratus*	No Mention	Aleppo, Syria	ARID
Zhu et al. 2008	*Eothenomys miletus*	Jianchuan County, Yunnan Province, China	Lijiang, China	MESIC
Wang and Wang 2000	*Microtus oeconomus*	Menyuan County, Quinghai Province China	Xining, China	MESIC
Chew 1951	*Microtus ochrogaster*	Champaign County, IL, USA	Champaign, IL, USA	MESIC
Downs and Perrin 1994	*Desmodillus auricularis*	Colesberg, South Africa	Middelburg, South Africa	ARID

Reference	Species	Original Collection Location	Weather Data	Habitat
Christian 1978	*Gerbillurus paeba*	Gorrasis Namib Desert, Namibia	Windhoek, Namibia	ARID
Duxbury and Perrin 1992	*Tatera afra*	Nieuwoudtville, South Africa	Calvinia, South Africa	ARID
Downs and Perrin 1994	*Tatera leucogaster*	Vaalbos, South Africa	Kimberly, South Africa	ARID
Hinds and MacMillen 1985	*Perognathus flavus*	Elgin, AZ, USA	Elgin, AZ, USA	ARID
Hinds and MacMillen 1985	*Perognathus longimembris*	Joshua Tree, CA, USA	Joshua Tree, CA, USA	ARID
Lindeborg 1955	*Chaetodipus penicillatus*	Alamagordo, NM, USA	Alamagordo, NM, USA	ARID
Hinds and MacMillen 1985	*Chaetodipus fallax*	Morongo Valley, CA, USA	Morongo Valley, CA ,USA	ARID
Hinds and MacMillen 1985	*Chaetodipus baileyi*	Pima County, AZ, USA	Tucson, AZ, USA	ARID
Hinds and MacMillen 1985	*Chaetodipus hispidus*	Elgin, AZ, USA	Elgin, AZ, USA	ARID
Hinds and MacMillen 1985	*Dipodomys ordii*	Benton Valley, CA, USA	Benton Valley, CA, USA	ARID
Hinds and MacMillen 1985	*Dipodomys panaminitus*	Mono county, CA, USA	Mammoth Lakes, CA, USA	ARID
Hayes et al. 1998	*Dipodomys merriami*	No Mention	Morongo Valley, CA, USA	ARID
Hinds and MacMillen 1985	*Dipodomys deserti*	San Bernardino, CA, USA	San Bernardino, CA, ,USA	ARID
Schmidt-Nielsen and Schmidt-Nielsen 1950b	*Dipodomys spectabilis*	No Mention	Tucson, AZ, USA	ARID
Hinds and MacMillen 1985	*Microdipodops megacephalus*	Benton Valley, CA, USA	Benton Valley, CA, USA	ARID
Hinds and MacMillen 1985	*Heteromys desmarestianus*	Fina La Pacifica, Costa Rica	Nicoya, Costa Rica	MESIC
Hinds and MacMillen 1985	*Liomys salvini*	Fina La Pacifica, Costa Rica	Nicoya, Costa Rica	MESIC
Hinds and MacMillen 1985	*Liomys irroratus*	Edinburg, TX, USA	Edinburg, TX, USA	MESIC
Cortes et al. 2000a	*Octodon lunatus*	Las Chinchillas National Reserve, Chile	La Sarena, Chile	ARID
Cortes et al. 1990,2000a	*Octodon degus*	PN Fray Jorge, Chile	PN Fray Jorge/La Sarena, Chile	ARID
Cortes et al. 2000b	*Ctenomys fulvus*	Salar de Atacama, Chile	Antafogasta, Chile	ARID
Buffenstein and Yahav 1991	*Heterocephalus glaber*	Lab colony (stock from Kenya)	Nairobi, Kenya	MESIC
Cortes et al. 2000a,c	*Chinchilla lanigera*	National Park Llullaillaco (Antafogasta, Chile)	Antafogasta, Chile	ARID
Cortes et al. 2003	*Chinchilla brevicaudata*	Antafogasta, Chile	Antafogasta, Chile	ARID
Tirado et al. 2007	*Lagidium viscacia*	Almirante Latorre, Chile	La Sarena, Chile	ARID
Baudinette 1972	*Spermophilus beecheyi*	Irvine, California, USA	Irvine, CA, USA	ARID
Whittington-Jones and Brown 1999	*Graphiurus murinus*	Alexandria Forest South Africa	East London, South Africa	MESIC
Aujard et al. 1998	*Microcebus murinus*	Lab colony	Antananarivo, Madagascar	MESIC
Muller and Jaksche 1979	*Otolemur crassicaudatus*	Kenia	Nairobi, Kenya	MESIC
Zhu et al. 2010	*Tupaia belangeri*	Luquan County, Yunnan Province, China	Luquan County, China	MESIC
Williams et al. 2001	*Oryx leucoryx*	Mahazat, Saudi Arabia	Mahazat, Saudi Arabia	ARID
Whittow et al. 1976	*Tragulus javanicus*	Kuala Lumpar, Malaysia	Kuala Lumpar, Malaysia	MESIC

Reference	Species	Original Collection Location	Weather Data	Habitat
Muller and Rost 1983	*Potos flavus*	Columbia	Bogota, Columbia	MESIC
Afik and Pinshow 1993	*Canis lupis pallipes*	Lab Born from Ngev Desert parents, Israel	Negev Desert, Israel	ARID
Williams et al. 2002	*Vulpes rueppelli*	Mahazat, Saudi Arabia	Mahazat, Saudi Arabia	ARID
Williams et al. 2004	*Vulpes vulpes*	Al-Lith and Biljurshi, Saudi Arabia	Al-Lith, Saudi Arabia	ARID
Williams et al. 2004	*Vulpes vulpes*	Mahazat as-Sayd, Saudi Arabia	Mahazat, Saudi Arabia	ARID
Klir and Heath 1992	*Alopex lagopus*	No Mention	Dzalinda, Russia	MESIC
Golightly and Ohmart 1983	*Vulpes velox*	Phoenix, AZ, USA	Phoenix, AZ, USA	ARID
Williams et al. 2004	*Vulpes cana*	Al-Lith and Biljurshi, Saudi Arabia	Al-Lith, Saudi Arabia	ARID
Noll-Banholzer 1979	*Vulpes zerda*	Chott El Djerid, south Tunisia	Tozeur, Tunisia	ARID
Anderson et al. 1997	*Proteles cristatus*	De Wildt Research Centre, South Africa	Rustenburg, South Africa	ARID
Baudinette et al. 2000	*Macroderma gigas*	Pine Creek, NT, Australia	Pine Creek, Australia	MESIC
Baudinette et al. 2000	*Rhinonicteris auranita*	Litchfield Park, NT, Australia	Darwin, Australia	MESIC
Chappell and Roverud 1990	*Noctilio albiventris*	Panama	Coco Solo, Panama	MESIC
Bell et al. 1986	*Macrotus californicus*	Mountains of Colorado desert, USA	Twentynine Palms, CA, USA	ARID
Carpenter and Graham 1967	*Leptonycteris curasoae*	Rincon Mountains (near Tucson, AZ USA)	Tucson, AZ, USA	ARID
Studier 1970	*Glossophaga soricina*	Minas Armolillo, near Alamos, Mexico	Alamos, Mexico	ARID
Herreid and Schmidt-Nielsen 1966	*Tadarida brasiliensis*	Phoenix, AZ, USA	Phoenix, AZ, USA	ARID
Marom et al. 2006	*Tadarida teniotis*	Negev Desert, Israel	Beersheba, Israel	ARID
Studier 1970	*Molossus coibensis*	Barro Colorado Island, Panama	Coco Solo, Panama	MESIC
Maloney et al. 1999	*Mops condylurus*	Komatipoort, South Africa	Johannesburg, South Africa	ARID
Baudinette et al. 2000	*Miniopterus schreibersi*	Darwin, NT, Australia	Darwin, Australia	MESIC
Marom et al. 2006	*Otonycteris hemprichii*	Negev Desert, Israel	Beersheba, Israel	ARID
Hosken and Withers 1997	*Chalinolobus gouldii*	70 km south of Perth, WA, Australia	Perth, Australia	MESIC
Cryan and Wolf 2003	*Lasiurus cinereus*	Albuquerque, NM, USA	Albuquerque, New Mexico, USA	ARID
Hosken and Withers 1999	*Nyctophilus geoffroyi*	Perrup Research Centere, WA, Australia	Manjimup, WA, Australia	MESIC
Morris et al. 1994	*Nyctophilus gouldi*	Olney State Forest, NSW, Australia	Sydney, NSW, Australia	MESIC
Hosken 1997	*Nyctophilus timoriensis major*	Perth, WA, Australia	Perth, Australia	MESIC
Webb 1995	*Plecotus auritus*	North-east Scotland	Aberdeen, Scotland	MESIC
Munoz Garcia Unpublished Data	*Pipistrellus kuhlii*	Negev Desert, Israel	Beersheba, Israel	ARID
Webb 1995	*Pipistrellus pipistrellus*	North-east Scotland	Aberdeen, Scotland	MESIC
Herreid and Schmidt-Nielsen 1966	*Eptesicus fuscus*	NC USA	Raleigh, NC, USA	MESIC
Procter and Studier 1970	*Myotis lucifigus*	Montezuma, NM, USA	Montezuma, NM, USA	ARID

Reference	Species	Original Collection Location	Weather Data	Habitat
Webb 1995	*Myotis daubentonii*	North-east Scotland	Aberdeen, Scotland	MESIC
Studier 1970	*Myotis nigricans*	Barro Colorado Island, Panama	Coco Solo, Panama	MESIC
Whittow et al. 1977	*Echinosorex gymnurua*	Kuala Lumpar, Malaysia	Kuala Lumpar, Malaysia	MESIC
Downs and Perrin 1995	*Elephantulus brachyrhynchus*	Waterpoort, South Africa	Waterpoort, South Africa	MESIC
Downs and Perrinn 1995	*Elephantulus intufi*	Langjin Nature Reserve, South Africa	Langjin, South Africa	ARID
Leon et al. 1983	*Elephantulus edwardii*	Clanwilliam, South Africa	Clanwilliam, South Africa	ARID
Perrin 1995	*Elephantulus myurus*	No Mention	Pretoria, South Africa	ARID
Roxburgh and Perrin 1994	*Macroscelides proboscideus*	South-east Cape Province, South Africa	Queenstown, South Africa	ARID
Downs and Perrin 1995	*Petrodromus tetradactylus*	St. Lucia, South Africa	St. Lucia, South Africa	MESIC
Rubsamen and Kettembeil 1980	*Procavia capensis*	No Mention	Nyala, Sudan	ARID
Benedict 1936	*Elephas maximus*	No Mention	Madurai, India	MESIC

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Chapter 2

Contribution of Shivering and Nonshivering Thermogenesis to Thermogenic Capacity for the Deer Mouse (*Peromyscus maniculatus*)

Abstract

Small mammals that are active all year must develop ways to survive the cold winters. Endotherms that experience prolonged cold exposure often increase their thermogenic capacity. Thermogenic capacity is composed of basal metabolic rate (BMR), nonshivering thermogenesis (NST) and shivering thermogenesis (ST). Increasing the capacity of any of these components will result in increased thermogenic capacity. It is often thought that NST should be the most plastic component of thermogenic capacity and as such is the most likely to increase with cold acclimation. We used deer mice to test this hypothesis by acclimating 27 animals to one of two temperatures (5º or 22ºC) for eight weeks. We then measured and compared values for thermogenic capacity, BMR, ST and NST between the two groups. Thermogenic capacity and NST increased by 21% and 42%, respectively, after cold acclimation. Neither BMR nor ST showed any change after acclimation. Therefore, it appears that deer mice raise their thermogenic capacity in response to prolonged cold by altering NST only.

Introduction

One of the most difficult times for a small endotherm to survive is during the winter when ambient temperatures are low and food is scarce. Maintaining a relatively high body temperature with the amount of energy available becomes increasingly difficult. Because of these factors many small mammals enter daily to avoid the cold climate and reduce energy expenditure. However, animals that are active all year must find ways to survive in harsh conditions.

Many physiological traits are plastic and change with the changing environment. If animals living in these changing conditions have static (or non-flexible) traits they may be less likely to survive. As the ambient temperature drops, animals must make accommodations to decrease heat loss, increase heat production or both. Behaviorally, animals can alter activity patterns to avoid the coldest times of the day. Physical properties can be changed to reduce heat loss, for example animals could grow thicker pelage which may decrease thermal conductance. Physiologically, thermogenic capacity can be increased so that more heat can be produced.

Thermogenic capacity can be approximated by the maximal metabolic rate due to acute cold exposure (summit metabolism ($\dot{V}O_2$sum)) (Chappell and Hammond 2004). There are many examples of small mammals responding to cold acclimation or acclimatization by increasing $\dot{V}O_2$sum (Heimer and Morrison 1978; Hayes and Chappell 1986; Nespolo et al. 1999; Nespolo et al. 2000; Chappell and Hammond 2004; Rezende et al. 2004). $\dot{V}O_2$sum can be increased by increasing any

of it components which are shivering thermogenesis (ST), non-shivering thermogenesis (NST) and basal metabolic rate (BMR) (Wunder and Gettinger 1996).

Non-shivering thermogenesis has received a lot of attention and is very important for the survival of small mammals during the winter (Wunder 1985). Many species do not even use ST unless the capacity of NST is insufficient at maintaining body temperature (Brück 1960; Heldmaier 1972; Lilly and Wunder 1979). Non-shivering thermogenesis occurs in brown adipose tissue through the function of an uncoupling protein, UCP1. Proton fluxes across the mitochondria inner membrane are uncoupled from ATP production so that the net result is heat (Cannon and Nedergaard 2003). This process is stimulated by norepinephrine (NE). Several studies in multiple species have shown that NST increases after small mammals are exposed to prolonged cold exposure and/or decreased photoperiod (Lilly and Wunder 1979; Zegers and Merritt 1988; Wiesinger et al. 1989; Nespolo et al. 1999, 2000; Wang et al. 1999). With the exception of Nespolo (1999, 2000), all of these studies only investigated changes in NST while it is $\dot{V}O_2$sum that is the measure of an animal's thermogenic capacity (the ecologically important variable).

Heat production by NST is regarded as more efficient than ST as both heat and muscle movement result from ST (Nespolo et al. 1999). Therefore, it is commonly assumed that NST is the most likely component of $\dot{V}O_2$sum to be changed in response to cold exposure and it is often assumed that changes in $\dot{V}O_2$sum are due to changes in NST. However, Nespolo (1999) demonstrated that this is not necessarily a safe assumption to make. *Phyllotis xanthopygus* acclimated to 15ºC for

one month showed a 94% higher $\dot{V}O_2$sum than animals acclimated to 30ºC. This increase in VO_2sum was due to a 76% increase in NST and a 200% increase ST suggesting that both NST and ST are important for maintaining body temperature. It is likely that this relationship exists in other species, but may have been overlooked. Many researchers interested in $\dot{V}O_2$sum do not look at its components and many researchers interested in the components do not compare these values with VO_2sum. We had reason to believe that the deer mouse (*Peromyscus maniculatus*) increases $\dot{V}O_2$sum by increasing NST as well as ST.

The deer mouse has one of the largest ranges of any rodent in North America and occurs at altitudes from below sea level in Death Valley to over 4300 m in the Sierra Nevada Mountains (Hayes 1989). This species is also active throughout the coldest times of the year. Therefore, increasing thermogenic capacity with the onset of winter is extremely important for survival. Hayes (1989) showed that deer mice had higher rates of $\dot{V}O_2$sum during the winter than mice tested during the summer. Several studies have demonstrated that $\dot{V}O_2$sum increases after cold acclimation (Heimer and Morrison 1978; Hayes and Chappell 1986; Chappell and Hammond 2004; Rezende et al. 2004). All of these studies were only interested in thermogenic capacity and not its components.

It is important to note that cold acclimation not only increases $\dot{V}O_2$sum, but also increases exercise induced maximal oxygen consumption ($\dot{V}O_2$max). Hayes and Chappell (1986) found that cold acclimation increased $\dot{V}O_2$sum by 31% and increased $\dot{V}O_2$max by 9%. These results led the authors to suggest that the oxygen

delivery system may be limiting. However, the simplest version of the oxygen delivery-limitation hypothesis dictates that the change in $\dot{V}O_2$sum and $\dot{V}O_2$max should be equal. One explanation offered by the authors is that cold acclimation may affect skeletal muscle as well as brown adipose tissue and it is likely that changes in muscle tissue that help to increase ST may also lead to increases in $\dot{V}O_2$max.

Chappell and Hammond (2004) were interested in the combined effects of cold exposure and exercise in deer mice. They found that cold acclimated mice had improved thermogenic capacity, presumably due to increased NST. Since, brown adipose tissue and skeletal muscle can function independently and simultaneously, Chappell and Hammond assumed that cold acclimated animals would exhibit higher VO_2max when exercised at lower temperatures compared with room temperatures. They found, however, no difference between the $\dot{V}O_2$max at cold and warm temperatures. The authors suggest that during intense exercise blood is routed away from brown adipose tissue to ensure adequate perfusion in the skeletal muscle. Vasoconstriction during exercise would also suppress the metabolism of brown adipose tissue. These conclusions are based on the assumption that VO_2sum increases primarily as a result of increased NST. This assumption has never been tested in deer mice. The results of Nespolo et al. (1999) provide evidence that VO_2sum can be increased by altering ST and the results of Hayes and Chappell (1986) suggest that this may be true for deer mice. We used deer mice to test the

null hypothesis that increases in VO_2sum for this species are due solely to increases in NST.

Materials and Methods

Animals and acclimation

We used 27 *Peromyscus maniculatus sorniensis* (14 males and 13 females) five to six generations removed from the wild. The initial mice in this breeding colony were captured in the White Mountains of eastern California. The breeding program maximized outcrossing and there was no intentional selection, except that the founding population was serologically tested to insure that none carried Sin Nombre virus (a variant of Hantavirus). Animals were housed individually in standard mouse cages with a photoperiod of 14L: 10D. Each animal was provided with bedding (wood shavings and cotton) and ad libitum water and rodent chow (LabDiet 5001 Rodent Diet; 23% protein, 4.5% fat, 6% fiber, 8% ash, and 2.5% minerals). All housing and measurement protocols were approved by the IACUC of the University of California-Riverside (protocol # A-0606017). Fifteen animals were acclimated to 20-22ºC for eight weeks and will be referred to as "warm acclimated". Twelve animals were acclimated to 5ºC for eight weeks and will be referred to as "cold acclimated". Eight weeks was chosen as this period of time is sufficient for $\dot{V}O_2$sum to fully acclimate (Rezende et al. 2004).

The sample sizes for each analysis vary as it was difficult to keep some mice still enough to obtain BMR measurements. After acclimation, the animals were tested for NST capacity, BMR and $\dot{V}O_2$sum. BMR and NST could be measured during

the same trial, but measurements of $\dot{V}O_2$sum required a separate test. The tests were arranged so that some animals received the NST test first while others received the $\dot{V}O_2$sum test first. Animals were given approximately two days to rest between tests.

Non-shivering thermogenesis

Non-shivering thermogenesis capacity was measured closely following the procedures of Nespolo et al. (1999). We measured rates of oxygen consumption using open-flow respirometry. Metabolic chambers had a volume of 525 ml and contained bedding, but no food or water. The metabolic chambers were maintained at 25ºC by placing the chambers in a temperature controlled cabinet. Dry air flow to the chambers was regulated at 630-670 ml/min STP ± 1% with Porter (Hatfield, PA, USA) mass flow controllers upstream from the metabolic chambers. Approximately 100 ml/min of excurrent gas was scrubbed of CO_2 and water vapor using soda lime and Drierite, respectively then routed to our oxygen sensor. Changes in oxygen concentrations were measured with an Applied Electrochemistry (Pittsburgh, PA, USA) S-3A analyzer and recorded along with flow rates on a Macintosh computer equipped with a National Instruments A-D converter and custom software ("LabHelper", www.warthog.ucr.edu). We calculated oxygen consumption ($\dot{V}O_2$, ml/min) as:

$$\dot{V}O_2 = \dot{V} \times (F_iO_2 - F_eO_2)/(1 - F_iO_2) \quad (1)$$

Where V is the flow rate (ml/min; STP) and F_iO_2 and F_eO_2 are the fractional oxygen concentrations of incurrent and excurrent gases, respectively.

Animals were fasted for 4-8 hours prior to testing. The cold acclimated animals could not sustain much more than a 4 hour fast. Animals were weighed and then placed into the chambers. Oxygen consumption was recorded for two hours then the animal was removed and given an intramuscular injection of saline. After the saline injection, animals were returned to the chambers and oxygen consumption was recorded for 45 min. The animals were then removed from the chambers and given an intramuscular injection of norepinephrine (NE) followed by 45 min of metabolic measurements. After the final measurement, the animals were returned to their cages. NE doses were calculated from the equation by Wunder and Gettinger (1996) and ranged from 16-20μg

$$\text{NE dose} = 2.53 \times M^{-0.4} \quad (2)$$

where NE dose is in mg/kg and M is the mass of the animal (g).

BMR was considered the lowest 5 min average of oxygen consumption during the first two hours of testing. NST was considered the highest 60 second average after NE injection.

Summit metabolism

Summit metabolism ($\dot{V}O_2$sum) was measured in an atmosphere of heliox (21% O_2, 79% He) at continuously decreasing temperatures from 5 to -10ºC until metabolic rate no longer increased with decreasing temperature. This gas mixture causes heat loss rates several times higher than air (Rosenmann and Morrison 1974) allowing us to elicit maximal oxygen consumption at only moderately low temperatures greatly reducing the risk of frostbite. These measurements typically

lasted from 7-10 min. We placed animals in the metabolic chambers with a small amount of wood shavings. The metabolic chamber was housed in an environmental cabinet maintained at a temperature 5º and 0ºC at the start of the run. Heliox was pumped into the chamber at a flow rate of 1700 ml/min STP regulated with a Porter mass flow controller. We decreased the temperature of the chamber at approximately 0.5-1.0ºC/min while monitoring oxygen consumption. When oxygen consumption declined or no longer increased after a 3ºC drop in temperature the test was ended and body temperature was measured with a 36 gauge type T thermocouple (all animals were hypothermic).

Shivering thermogenesis

We calculated shivering thermogenesis (ST) using our measurements of $\dot{V}O_2$sum and NST, obtained as described above, and following the equation of Wunder and Gettinger (1996)

$$ST = \dot{V}O_2\text{sum} - NST \quad (3)$$

where NST includes BMR (see results). NST should be measured within the thermoneutral zone (Böckler et al. 1982; Nespolo et al. 1999). The lower critical temperature of deer mice is 25-30ºC (Chappell 1985; Chappell and Hammond 2004). We measured NST at 25ºC rather than 30ºC to prevent hyperthermia after NE injections. However, $\dot{V}O_2$sum must be measured at low temperatures that thermally challenge the animals (Rosenmann and Morrison 1974).

Statistics

Statistical analyses were performed using SAS 9.1(SAS Institute Inc., Cary, NC, USA) and SYSTAT 10.0 (Systat Software Inc., San Jose, CA, USA). We used ANCOVA (with body mass and age as covariates) to determine if sex or acclimation temperature (5ºC or 20-22ºC) had any effect on BMR, NST or ST. We also used ANOVA to test whether body mass changed with acclimation temperature. We used a match-pairs t-test to verify that oxygen consumption after saline injection was less than after NE injection. Results are reported as least squared means ± S.D. unless otherwise noted. Treatment and error degrees of freedom are shown as subscripts for F-values. Error degrees of freedom vary because some mice were restless and BMR was unobtainable for these animals.

Results

The BMR of cold and warm acclimated mice were not different (0.9±0.17 versus 0.8±0.18 ml O_2/min, respectively, $F = 2.10_{2,19}$, $p = 0.164$; Fig. 2.1). Based on our results and the results of Russell and Chappell (2007), which also showed no change in BMR due to acclimation temperature, for deer mice from our colony we decided not to subtract BMR from our measurements of NST.

At the time of our $\dot{V}O_2$sum measurements the average mass of our mice was 24.0±4.39 g and there was no difference between the mass of the cold and warm acclimated groups ($p = 0.992$). Acclimation temperature did have an affect on $\dot{V}O_2$sum, with cold acclimated animals having a 21% higher $\dot{V}O_2$sum than warm

acclimated animals (6.4±0.82 versus 5.3±0.82 mlO_2/min, respectively, F = $8.68_{2,22}$, p = 0.002; Fig. 2.2).

At the time of our NST measurements the average mass of our mice was 23.4±4.35 g and there was no difference between the mass of the cold and warm acclimated groups (p = 0.321). Our control injection of saline had a significantly smaller effect on oxygen consumption than the NE injections (means = 2.3±0.52 versus 3.3±1.07 ml O_2/min, respectively, t = -5.356, df = 26, p = <0.001). Acclimation temperature did have an affect on NST, with cold acclimated animals having a 42% higher NST (includes BMR) than warm acclimated animals (4.0±0.72 versus 2.8±0.73 ml O_2/min, respectively, F = $8.96_{4,22}$, p < 0.001; Fig. 2.3). Sex did not have an effect on NST, however there was a slight interaction between sex and acclimation temperature as males exhibited more of an increase in NST when acclimated at 5ºC than females (Fig. 2.4). There was no difference between ST of cold acclimated and warm acclimated mice (2.4±0.96 versus 2.5±0.96 ml O_2/min, respectively, F = $0.05_{2,22}$, p = 0.955; Fig. 2.5).

Discussion

Acclimation to cold temperatures can cause many physiological changes in endotherms to increase survival. However, the magnitude of change caused by acclimation varies on a species by species basis. The end result is typically an increase in thermogenic capacity (Hayes and Chappell 1986; Zegers and Merritt 1988; Nespolo et al. 1999; Wang et al. 1999; Nespolo et al. 2000; Chappell and Hammond 2004). Changes in any of the components of thermogenic capacity can

produce changes in $\dot{V}O_2$sum. We will discuss the various components of thermogenic capacity and how they were affected by cold acclimation in this study as well as others.

The $\dot{V}O_2$sum of cold acclimated animals in this study was 21% higher than that of the warm acclimated animals. This is a common response for *P. maniculatus* after prolonged cold acclimation. The magnitude of the change in $\dot{V}O_2$sum in many small mammals varies between studies depending on methods used and acclimation temperature/light cycle. Increases in $\dot{V}$O2sum after cold acclimation range from about 25 to 94% (Hayes and Chappell 1986; Zegers and Merritt 1988; Nespolo et al. 1999; Wang et al. 1999; Nespolo et al. 2000; Chappell and Hammond 2004). The 23% increase in $\dot{V}O_2$sum found in this study is on the low end of the range, but within normal values nonetheless. Therefore, since $\dot{V}O_2$sum is changing, one of its components must also be changing.

BMR is a plastic trait in both mammals and birds. Changes in BMR can be elicited by diet (Koteja 1996), photoperiod (Zhao and Wang 2005) or acclimation temperature. The result of cold acclimation on BMR is quite species specific. Some studies report no change (Koteja 1996; Nespolo et al. 1999; Russell and Chappell 2007), while others report an increase in BMR after cold acclimation (Dawson and Olson 1988; Nespolo et al. 2000; Nespolo et al. 2002; Li and Wang 2005; Novoa et al. 2005). It is not known for certain if increased BMR contributes to improved cold tolerance in endotherms (Vézina et al. 2006). Elevated BMR after cold exposure may be a consequence of the physiological upregulation necessary to survive long

term cold exposure. Cold acclimation may cause organs involved in heat production to increase in size or cause a mass-independent change in tissue metabolic activity leading to increased BMR (Piersma 2002; Vézina et al. 2006). The energy demand hypothesis suggests that organs involved in digestion may increase in size in response to increased daily energy intake resulting in elevated BMR (Williams and Tieleman 2000).

Varying photoperiods have been shown to cause changes in BMR as well (Zhao and Wang 2005). As the seasons change in nature, animals experience both changing temperature and photoperiods; however, neither acclimatization nor acclimation seem to affect the BMR of deer mice (Hayes 1989, Russell and Chappell 2007 and this study). So, regardless of whether or not higher BMR can increase cold tolerance, this is not the mechanism used by the deer mouse in response to prolonged cold exposure.

Brown adipose tissue is the site of non-shivering thermogenesis in small mammals. Brown adipose tissue is responsive to changing seasons and by default so is NST. Both cold acclimation and shortening day length have been shown to either independently have an effect on NST or have compounding effects on NST when applied together (Heldmaier 1975; Sheffield and Andrews 1980; Feist and Morrison 1981; Zegers and Merritt 1988; Wang et al. 1999; Zhao and Wang 2005). The magnitude of change depends on the species being investigated and the acclimation conditions. Zegers and Merritt (1988) found 13-19% increase in NST of *P. maniculatus* acclimated to 5ºC on a 16L: 8D photoperiod compared with animals

acclimated to 25ºC on the same photoperiod. While they found increases of 25-29% in NST of *P. maniculatus* acclimated to the same temperatures above with a shorter photoperiod, 8L: 16D. We found a larger increase for NST (42%) after cold acclimation.

Altering shivering thermogenesis as a way to increase $\dot{V}O_2$sum is often overlooked in many studies. NST is considered a more efficient method of thermogenesis than ST and as such should be the most likely way to change $\dot{V}O_2$sum (Nespolo et al. 1999, Cannon and Nedergaard 2003). However, it is possible that chronic shivering of animals maintained in a cold environment may lead to muscle training resulting in increased ST capacity (Cannon and Nedergaard 2003). Nespolo et al. (1999) showed that Phyllottis *xanthopygus* increased its ST by 200% in response to cold acclimation while their NST increased by only 50%. This is the only example we know of where ST has been shown to increase after cold acclimation, but as mentioned before; it is one of few studies to carefully investigate this topic. There may be more exceptions to the standard assumption that $\dot{V}O_2$sum increases due to increasing NST and so the contribution of ST to $\dot{V}O_2$sum should not be overlooked. The deer mice in this study, however, showed no change in ST after cold acclimation.

Previous work on deer mice has shown that acclimation to cold temperatures results in an increase in their $\dot{V}O_2$sum and, to a lesser degree, $\dot{V}O_2$max (Hayes and Chappell 1986). The authors suggested that any changes in the skeletal muscle that increased $\dot{V}O_2$ during thermogenesis would also increase $\dot{V}O_2$ during exercise,

providing similar results to what the authors found in their study. We thought that chronic shivering during cold acclimation could alter muscle tissue producing a higher ST capacity. The results of this study show that this is not true. *P. maniculatus* did not alter ST in response to cold acclimation. The differences between the magnitude of changes in $\dot{V}O_2$sum and $\dot{V}O_2$max after cold acclimation seem to be most consistent with the oxygen delivery-limitation hypothesis as originally stated Hayes and Chappell (1986). Chappell and Hammond 2004 assumed that increased $\dot{V}O_2$sum after cold acclimation in deer mice was due primarily to changes in NST. This assumption was not tested prior to this study; however we can now verify that changes in VO_2sum can be accounted for by increased NST.

Most researchers interested in NST do not look at ST or $\dot{V}O_2$sum. This is unfortunate as VO_2sum is an estimate of the thermogenic capacity of an animal. An animal's thermogenic capacity has a very large ecological importance. NST is only one component of an animal's thermogenic capacity. Much more information can be gathered if NST measurements are looked at in the context of thermogenic capacity as a whole. Also, assuming that ST is static is also a mistake. ST has been overlooked in the majority of studies interested in partitioning $\dot{V}O_2$sum into its components. Although this study shows that this assumption is true for deer mice, Nespolo et al. (1999) showed that this assumption needs to be investigated on a species by species basis.

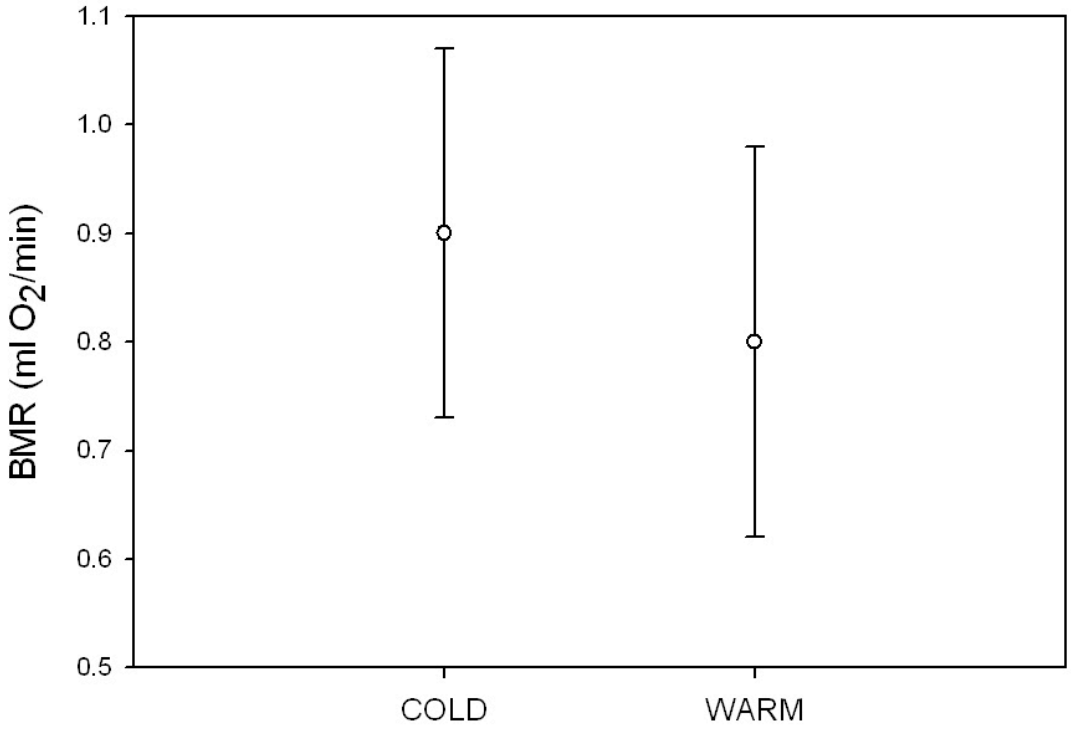

Figure 2.1. Least squares means of BMR for cold and warm acclimated mice ± 1 S.D. ($F = 2.10_{2,19}$, $p = 0.164$). Animals acclimated to 5ºC are listed as Cold and animals acclimated to 22ºC are listed as warm.

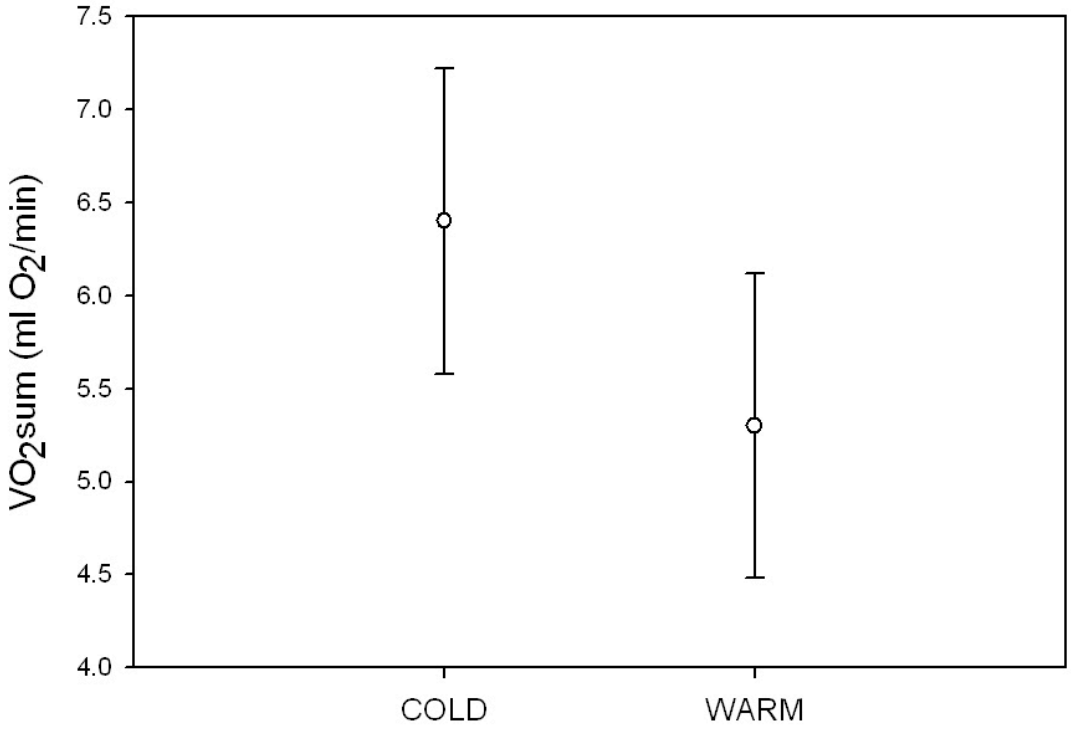

Figure 2.2. Least squares means of *V*O2sum for cold and warm acclimated mice ± 1 S.D. ($F = 8.68_{2,22}$, $p = 0.002$). Animals acclimated to 5ºC are listed as Cold and animals acclimated to 22ºC are listed as warm.

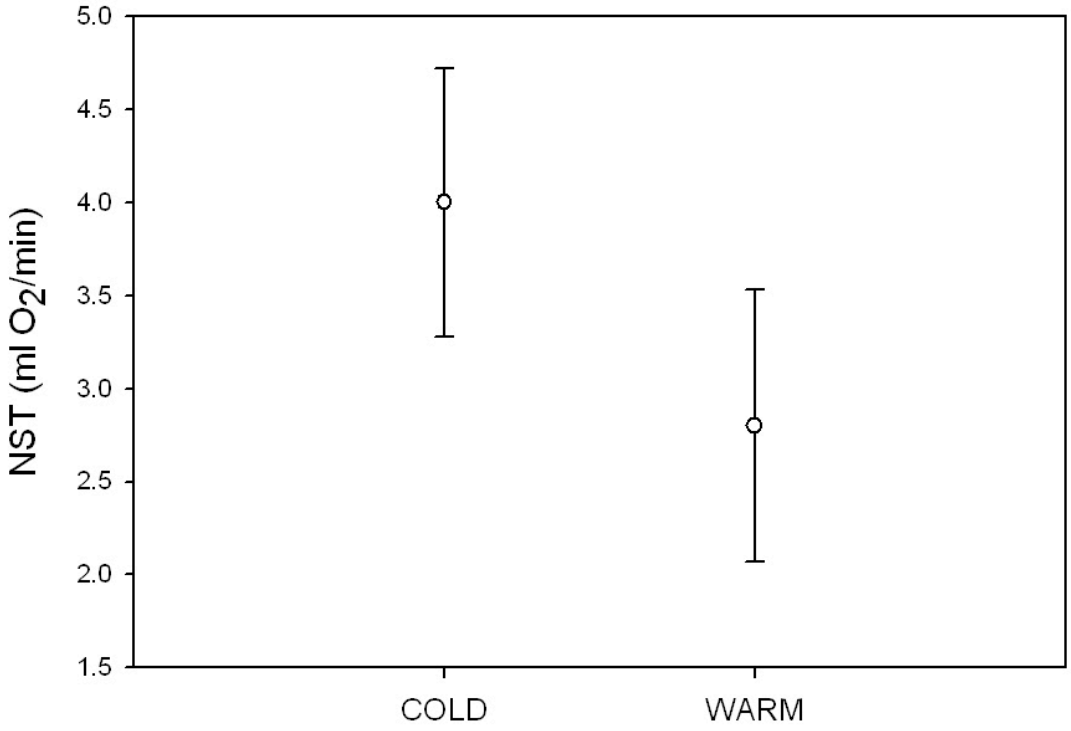

Figure 2.3. Least squares means of NST for cold and warm acclimated mice ± 1 S.D. ($F = 8.96_{4,24}$, $p < 0.001$). Animals acclimated to 5ºC are listed as Cold and animals acclimated to 22ºC are listed as warm.

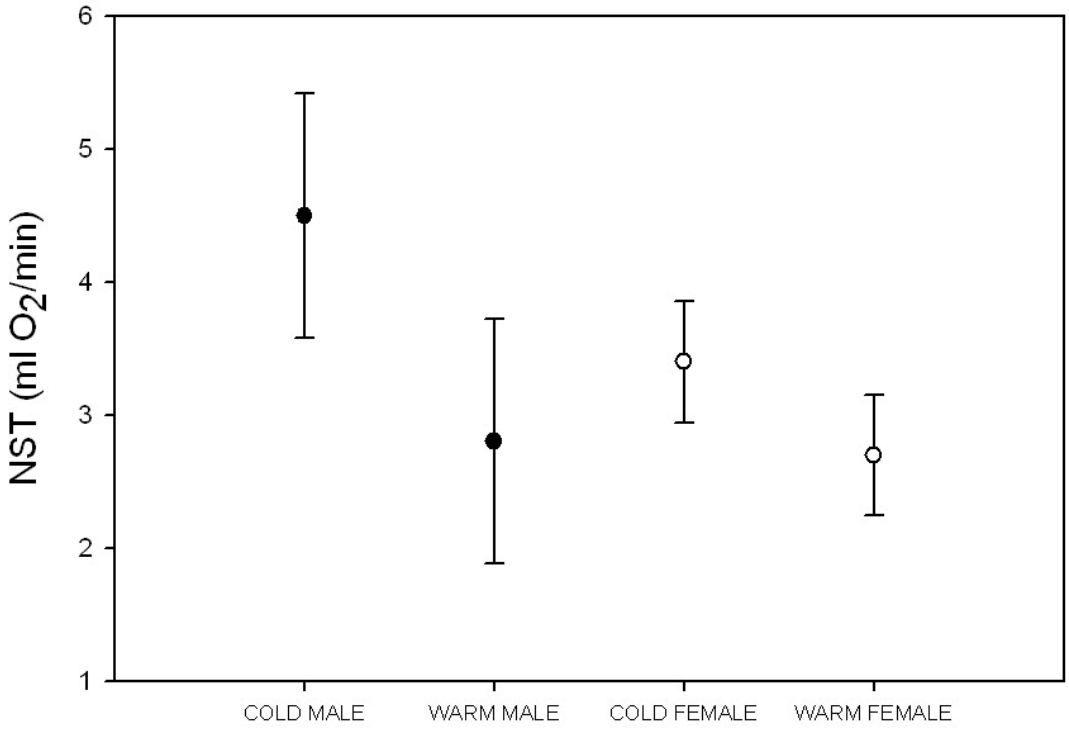

Figure 2.4. The interaction of NST and acclimation temperature is shown with the least squares means of NST for both male and female mice plotted (closed circles representing males and open circles representing females). Animals acclimated to 5ºC are listed as Cold and animals acclimated to 22ºC are listed as warm.

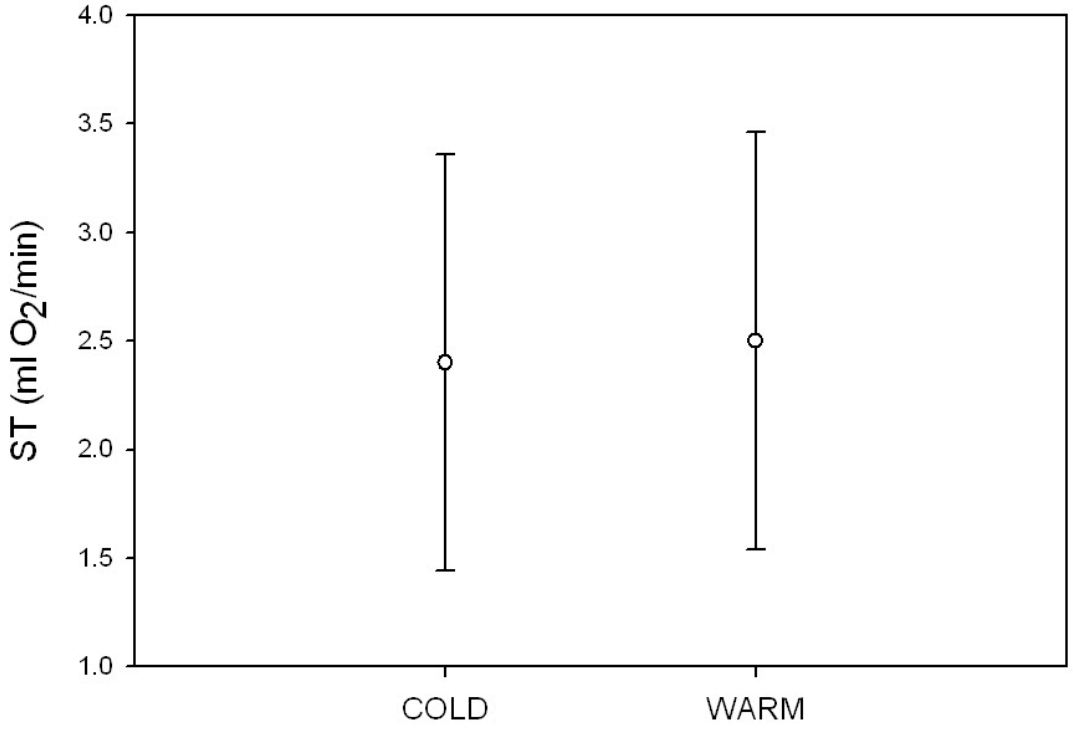

Figure 2.5. Least squares means of ST for cold and warm acclimated mice ± 1 S.D. ($F = 0.05_{2,22}$, $p = 0.955$). Animals acclimated to 5ºC are listed as Cold and animals acclimated to 22ºC are listed as warm.

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Chapter 3

Limits in the blood – oxygen carrying capacity in the deer mouse (*Peromyscus maniculatus*)

Abstract

The maximal rate of oxygen consumption that can be attained during exercise (VO_2max) sets the upper limit of aerobic activities such as running and consequently has likely been a target of natural selection because. Many researchers have investigated what sets the upper limit to VO_2max. It has previously been suggested that the circulatory delivery of oxygen is one of the factors that limit VO_2max. Physiological systems are often matched to meet the functional requirements imposed by the environment, while some systems have been shown to be in excess of what the environment typically requires. Maintaining an excess capacity comes at an energetic cost, but could be very beneficial for organisms to deal with sudden changes in physiological demand. In this study we investigated whether or not the deer mouse (*Peromyscus maniculatus*) has an excess capacity for circulatory delivery of oxygen by monitoring changes in VO_2max over the course of several days after taking blood. We found that red blood cell number returned to baseline values seven days after taking blood. VO_2max also returned required seven days to return to baseline values after blood removal. Additionally, we found a direct relationship between the amount of blood removed and the decrease in VO_2max, indicating that the circulatory delivery of oxygen limits VO_2max in these animals and the deer mice does not have an excess capacity for carrying oxygen in the blood.

Introduction

Maintaining an adequate supply of oxygen (O_2) to the cells is crucial for sustained aerobic activity. Without sufficient O_2, animals cannot produce enough energy to support muscles and activity is restricted. At the alveolar surfaces O_2 is diffused from inhaled air into the blood. Most of that O_2 is bound to the respiratory pigment, hemoglobin, and transported to cells in the body through the bloodstream. Each molecule of hemoglobin can carry four molecules of O_2 and so the amount of O_2 that can be transported throughout the body is limited by the number of blood cells and ultimately the amount of hemoglobin contained within these cells. During exercise respiration increases, as does heart rate in an effort to deliver O_2 to muscles at a faster rate. As exercise intensity increases, the amount of O_2 consumed (and delivered to tissues) increases. Due to the limitations of the circulatory and respiratory systems there is an upper limit to the intensity of exercise that can be maintained for prolonged periods. The maximal rate of O_2 consumption that can be attained during exercise is known as $\dot{V}O_2max$ and it is likely that this has been the target of natural selection (Garland and Huey 1987; Noakes et al. 2001) because it sets the upper limit of aerobic activities such as running.

There have been several attempts to determine what sets the upper limit to $\dot{V}O_2max$ (see Lindstedt and Conley 2001; Noakes et al. 2001). The flow of O_2 at each level of the respiratory system must equal the flow of O_2 into the mitochondria in order to maintain steady state conditions; therefore each of these steps could potentially limit O_2 flow (Taylor and Wiebel 1981). Limitations could lie within the

uptake of O_2 in the lungs, O_2 delivery by the circulatory system, or O_2 uptake at the mitochondria (Lindstedt and Conley 2001). The concept of symmorphosis, originally proposed by Taylor and Wiebel (1981), suggests that the structural design of the respiratory system should be matched to the functional requirements (load) such that the structural elements satisfy the requirements of the system without being in excess. This idea was motivated by the belief that animals are built reasonably and structural design should be optimized, as maintaining structures in excess of functional needs is wasteful (Taylor and Weibel 1981). The results of Taylor and Wiebel (1981) and Garland and Huey (1987) show that the allometric relationship for the slope of pulmonary diffusing capacity is greater than that of $\dot{V}O_2$max, thus contradicting the principles of symmorphosis. The principle of symmorphosis has been criticized as an unlikely evolutionary outcome because organisms are not designed and selection typically leads to adequacy, not optimality (Garland 1998). Nevertheless, the idea of load/capacity matching has led to an enormous amount of research and discussion. Animals evolve by natural selection and so theoretically their biological capacities have evolved to meet the loads that are experienced in nature. However, the load placed on a system varies greatly depending on the situation, age and state of health of the animal. It would be beneficial for an animal to have systems with an "excess capacity" for dealing with higher loads that are encountered during emergency situations such as escaping from a predator. However, obviously there is a biosynthetic and an energetic cost to maintaining and using excess capacities. Despite this, numerous examples of

animals having excess capacities exist (Toloza et al. 1991; Diamond and Hammond 1992; Hammond and Diamond 1992; McWilliams and Karasov 2005).

When animals are at rest there is more than enough O_2 carried in the blood to support tissues. In humans, for example, only about 25% of the oxygen in the blood is needed for maintenance at rest (Hill et al. 2008). The remaining O_2 can then be used to fuel tissues as needed if activity is increased and the blood never completely releases all of its O_2. It seems there is an excess blood–O_2–carrying capacity; during most of the time more O_2 is carried in the blood than is needed by an animal. This excess capacity may be increasingly important when an animal is faced with extreme aerobic demands, such as running from a predator. Additionally if an animal encounters an O_2–limiting environment, such as high altitude, the availability of excess O_2 in the blood will become increasingly important during the erythropoietic phase of acclimating to low O_2 conditions. Exactly how much of an excess O_2-carrying capacity vertebrates have is not known. Previous studies in our laboratory on the deer mouse (*Peromyscus maniculatus*) have shown a decrease in $\dot{V}O_2max$ after drawing blood. In these studies the amount of blood removed was unknown and so we were not able to determine the relationship between the amount of blood removed and the change in $\dot{V}O_2max$. However, this finding encouraged us to test whether the circulatory delivery of O_2 was limiting $\dot{V}O_2max$ in these animals or if deer mice possess an excess blood–O_2–carrying capacity.

The deer mouse (*Peromyscus maniculatus*) has one of the largest ranges of any rodent in North America, occurring from Central America to northern Canada

and inhabiting altitudes from below sea level in Death Valley to more than 4,300 m in the Sierra Nevada Mountains (Hayes 1989). Deer mice living at high altitudes are faced with the combined problems of colder environmental temperatures and reduced partial pressure of O_2 with respect to low altitudes. Given these conditions deer mice at high altitudes have higher energetic demands and caloric intake and likely experience limitations to highly aerobic such as exercise and heat production (Lenfant 1973; Snyder 1981; Chappell et al. 1988). Therefore, animals at high altitudes must expend energy at higher rates than those at lower altitudes, but must do so under hypoxic conditions. Despite these challenges, deer mice at high altitudes are highly successful and live a productive and highly aerobic life.

There are several critically important and documented genetic adaptations that allow deer mice to do well at altitudes above 3000m. Early work on deer mice revealed altitude-dependent hemoglobin α-chain polymorphisms that improved blood- O_2 affinity and aerobic performance (Chappell et al. 1988; Chappell and Snyder 1984; Snyder 1981; Snyder et al. 1988). More recent studies on deer mice hemoglobin genes show additional adaptations that also potentially increase aerobic performance by increasing O_2 binding affinity for hemoglobin under hypoxic conditions, potentially allowing more loading of O_2 at the lungs (Storz et al. 2007; Storz and Moriyama 2008; Storz et al. 2009; Storz et al. 2010). However, the O_2 binding affinity of mice living at high altitude is only modestly better than the O_2 binding affinity in low altitude populations (Storz et al. 2010). Due to the close proximity of low and highland populations of deer mice in California and their large

nightly altitudinal range, the populations of deer mice in California likely interbreed so that there is very little difference in the hemoglobin–O_2 affinity of the hemoglobin in high and low populations (Storz *pers. comm.* to K Hammond). Therefore, the California *P. maniculatus* populations may have developed other mechanisms of dealing with low partial pressures of O_2. In this study we test whether or not the deer mouse has any excess blood–O_2–carrying capacity.

Materials and Methods

Animals

We used *Peromyscus maniculatus sonoriensis* that were eight to ten generations removed from the wild. The initial mice in this breeding colony were captured in the White Mountains of eastern California. The breeding program maximized outcrossing, and there was no intentional selection, except that the founding population was serologically tested to ensure that no mice carried the Sin Nombre virus (a variant of hantavirus). Animals were housed individually in standard mouse cages with a photoperiod of 14L: 10D. Each animal was provided with bedding (wood shavings and cotton), and *ad libitum* water and rodent chow (LabDiet 5001 Rodent Diet; 23% protein, 4.5% fat, 6% fiber, 8% ash, and 2.5% minerals). All housing and measurement protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside (protocol A-0606017).

General procedure

Our goal was to determine whether deer mice have an excess blood–O_2–carrying capacity by monitoring changes in $\dot{V}O_2max$ associated with changes in red blood cell number. The majority of O_2 carried in the blood is bound to hemoglobin contained within red blood cells. The blood's potential for carrying O_2 is largely dependent on the number of red blood cells. We used hematocrit (HCT, a measure of the proportion of red blood cells to total blood volume) as a proxy for the blood's potential to carry O_2. We first determined the rate of red blood cell recovery in deer mice after taking blood samples from mice. We then used a different set of mice to determine how changes in red blood cell number affected $\dot{V}O_2max$. To do this, we first obtained a baseline $\dot{V}O_2max$ value for all of the animals in this treatment group. Once baseline values were obtained we removed a known amount of blood from each animal and measured their $\dot{V}O_2max$ on subsequent days. We then determined the time it took for $\dot{V}O_2max$ to return to baseline values compared with the time it took red blood cell number to return to baseline values. In order to determine whether or not repeated $\dot{V}O_2max$ measurements would affect our results we measured the $\dot{V}O_2max$ of a control group of mice following the same schedule as the treatment group, however no blood was taken from these mice.

Hematocrit recovery

We used 70 deer mice to determine the rate of red blood cell recovery. Mice were weighed and then placed in a jar that contained cotton balls soaked with liquid isoflurane until the mice were lightly anesthetized. Then a blood sample was drawn

with a heparanized capillary tube using a retro-orbital puncture. It was common for mice to continue bleeding after removal of the capillary tube. The normal practice in this situation would be to use a cloth to put pressure on the eye until the bleeding stopped. We wanted to know how much blood was removed during our blood draws so we did not blot excess blood. We made every effort to collect excess blood in additional capillary tubes so that we could obtain an accurate measure of total blood removed. Blood was then dispensed into a pre-weighed microcentrifuge tube and re-weighed to determine the mass of blood removed from the mouse. We estimated the total blood in each mouse based on body mass assuming that total blood in each animal was 8% of total body mass (Riches et al. 1973). We were then able to calculate the percent of total blood that was removed from each mouse. Blood was then re-drawn into capillary tubes and centrifuged for 10 minutes. HCT was calculated as the proportion of packed cells as a percent of total volume of blood in the tube. HCT was measured in duplicate fore each animal using the average value in subsequent analyses. We measured the HCT of all mice on day 0 to obtain a baseline value. We then measured the HCT of a subset of mice on a day that was either 1,3,5,7,or 11 days after the initial measurement so that each mouse was only measured twice (Table 1).

Day 0	Day 1	Day 3	Day 5	Day 7	Day 11
HCT (70)	HCT (14)	HCT (12)	HCT (11)	HCT (16)	HCT (17)

Table 3.1. HCT testing procedure. Day 0 was the initial measurement of hematocrit (HCT) of all mice to determine baseline values. Day 1,3,5,7,11 correspond to the number of days after initial HCT measurement on which a second measurement was made on a subset of mice. Numbers in parentheses are sample sizes of mice measured on that day. Each mouse was only measured twice.

VO_2max measurements

We determined rates of O_2 consumption ($\dot{V}O_2$) with open-flow respirometry. Changes in O_2 concentration were measured with an Applied Electrochemistry S-3A analyzer and recorded on a Macintosh computer equipped with a National Instruments A-D converter and custom software (''LabHelper'',www.warthog.ucr.edu). Gas flow was regulated with Tylan mass flow controllers upstream from the metabolism chambers. About 100 ml/min of sample gas was scrubbed of CO2 and water vapor (soda lime and drierite) and routed through the O_2 sensor. We calculated $\dot{V}O_2$ as:

$$\dot{V}O_2 \text{ (in ml/min)} = \dot{V} * (F_iO_2 - F_eO_2)/(1 - F_iO_2) \quad (1)$$

Where $\dot{V}$ is flow rate (ml/min STP) and F_iO_2 and F_eO_2 are the fractional O_2 concentrations in incurrent and excurrent gas, respectively (F_iO_2 was 0.2095).

$\dot{V}O_2$ max was determined by running mice in an enclosed motorized treadmill (Chappell 1984; Chappell and Snyder 1984; Hayes and Chappell 1990; Chappell and Hammond 2004). The treadmill's working section was 6 cm wide, 7 cm high, and 13.5 cm long; the total enclosed gas volume was about 970 ml. We

used a flow rate of 2100 ml/min STP of dry air. To begin a test, we placed a mouse in the treadmill's working section and allowed an adjustment period for $\dot{V}O_2$ to become stable (2–4 minutes) before starting the tread at low speed (approximately 0.15 m/s). We increased speed in increments of about 0.1 m/s every 45–60 seconds until the mouse could no longer maintain position and $\dot{V}O_2$ did not increase with increasing speed, at which time the tread was stopped. After the end of exercise, we continued to monitor metabolism until $\dot{V}O_2$ had begun to decrease, and then removed the animal. Tests typically lasted about 5–12 minutes. Reference readings of incurrent gas were obtained before and after each run. $\dot{V}O_2$max was calculated as the highest one-minute average of $\dot{V}O_2$ over the course of a trial.

Treatment and control groups

We used 51 deer mice in the treatment group to determine the effect of reduced blood volume on repeated measures of $\dot{V}O_2$max. $\dot{V}O_2$max was first determined for all mice as described above to establish baseline values. After initial measurements mice were given one day to recover. Following the recovery period HCT was measured and the volume of blood removed was calculated as described above. The maximum amount of blood recommended to be collected at one time is 10-15% of total blood volume (Hoft 2000). Because of the bleeding procedure we employed (see above) we often inadvertently exceeded this suggested limit. We wanted to determine if there were differential effects on $\dot{V}O_2$max depending on whether the amount of blood taken was within or in excess of the suggested limit. Therefore, in our analysis of the treatment group we separated the mice into low

blood (<15%) and high blood (15–24%) groups. $V\dot{O}_2$max was then measured for all treatment mice on subsequent days corresponding to days 1,3,5,7,and 11 days after HCT measurement. We used 28 control mice to test the effects of repeated $V\dot{O}_2$max measurement. A baseline value of $V\dot{O}_2$max was measured for these mice followed by one day of rest. $V\dot{O}_2$max was measured for all control mice on subsequent days corresponding to days 1,3,5,7,and 11 days after the day of rest.

Predictions

We predicted that mice would initially respond to blood removal by increasing plasma volume so as to maintain total blood volume and that red blood cell volume would be replaced over several days (Hoft 2000). Therefore, we expected to see reduced HCT on days closely following baseline HCT measurements. We predicted that the treatment mice would have decreased $V\dot{O}_2$max on days closely following blood removal and that the days necessary for $V\dot{O}_2$max to return to baseline values would be similar to the number of days required for HCT recovery if mice had no excess blood–O_2–carrying capacity. Similarly, if there is no excess capacity, we expected that the percent of red blood cells removed would closely match percent changes in $V\dot{O}_2$max. If mice do in fact have an excess blood–O_2–carrying capacity then we predicted that changes in $V\dot{O}_2$max and the return to baseline values would not match changes in red blood cell volume or the time necessary for HCT recovery. We predicted that the $V\dot{O}_2$max of control mice would not significantly vary between repeated measurements.

Statistical analysis

All statistical analyses were completed using SPSS 19. We used repeated measures general linear models to test for differences in HCT and $\dot{V}O_2$max measured on multiple days. The assumptions of sphericity necessary for repeated measures analysis were violated in the analysis of the treatment group and so we used the output from the more conservative multivariate ANOVA. We used post–hoc tests from a one–way ANOVA to determine how many days between HCT measurements were necessary for HCT to return to baseline values. We used HCT and the percent of total blood removed to calculate the percent of red blood cells removed. We then used a one–way ANOVA to test if the amount of red blood cells removed from mice in the control group during HCT measurement was significantly different from the percent change in $\dot{V}O_2$max one day after HCT measurement and used linear regression to test for a relationship between the amount of blood removed and the percent change in $\dot{V}O_2$max. All post–hoc tests were evaluated using LSD corrections for alpha inflation. We included age, sex and mass as covariates in all analyses and removed them when they were not significant. All means are presented as mean value ± standard error.

Results

Hematocrit recovery

The average mass of the mice used in these tests were 22.8 ± 0.3 g and the average age was 138 ± 7 days. The percentage of total blood taken ranged from 13.4 – 28.1% with an average amount of 19.7 ± 0.4%. Initial HCT readings averaged 46.7

± 0.3% and decreased an average of 10.8 ± 0.6% one day following blood drawing. There was no effect of age, mass or sex on the change in HCT from time 1 to time 2. HCT measured at time 1 and time 2 were significantly different ($F_{1,65} = 149.32$, $p < 0.001$) and there was a significant interaction between HCT and the number of days between measurements ($F_{4,65} = 39.21$, $p < 0.001$). HCT did not return to baseline values until seven days after the initial measurement (Fig. 3.1).

VO_2max treatment group

Mice in the treatment group had an average mass of 23.7 ± 0.6 g and an average age of 95 ± 2 days old at the start of the experiment. The average baseline value for $V\text{O}_2$max was 3.51 ± 0.1 mlO_2/min. There was no effect of age or sex on $V\text{O}_2$max. Repeated measures of $V\text{O}_2$max differed significantly between days ($F_{5,45} = 2.72$, $p = 0.031$). Following initial $V\text{O}_2$max measurements, the average HCT of mice was 46.5 ± 0.3%. The amount of blood taken from mice during HCT measurement ranged from 7.2 – 23.7% of total blood volume with an average value of 14.2 ± 0.6%. This resulted in an average reduction in red blood cell volume of 6.6 ± 0.3% which corresponded to an average decrease in $V\text{O}_2$max of 7.94 ± 1.6% on the day following HCT measurement. There was not a significant difference between the reduction of red blood cell volume and the decrease in $V\text{O}_2$max ($F_{1,49} = 5.1$, $p = 0.34$). The recovery of $V\text{O}_2$max occurred gradually and did not reach baseline values until seven days had passed since taking blood (Fig. 3.2). The percent of total blood taken (%Blood) had a significant effect on the percent change in $V\text{O}_2$max ($\Delta V\text{O}_2$max = 3.34

– 0.793 * %Blood, $F_{1,49} = 5.03$, $P = 0.029$). Whether mice were in the high blood group (%Blood 15 – 24%, n = 24) or low blood group (<15%, n = 27) had a significant effect on repeated measures of VO_2max (Blood group, $F_{1,48} = 7.25$, $P = 0.10$). Both groups showed decreases in VO_2max after drawing blood, but this effect was more pronounced in the high blood group (Fig. 3.3).

VO_2max control group

Mice in the control group had an average mass of 22.1 ± 0.5 g and were 99 ± 3 days old at the start of the experiment. The average baseline measurement for VO_2max was 3.17 ± 0.1 mlO_2/min. Sex was not a significant predictor of VO_2max, but there was a significant interaction of age on VO_2max and so was left in the model. Repeated measurements did have a significant effect on VO_2max of the control mice ($F_{5,21} = 2.977$, $p = 0.014$). The second measurement of VO_2max was on average 3.56 ± 2.7% lower than baseline measurements. The effect of repeated measurements on the control group was similar to that of the treatment group although the observed pattern was different (Fig. 3.4).

Discussion

When the idea of symmorphosis was originally proposed and tested in the mammalian respiratory system it was found that the diffusive capacity of the lungs did not hold with the tenets of symmorphosis (Taylor and Weibel 1981; Weibel et al. 1981). This same result has been demonstrated multiple times since and it is generally accepted that the lungs operate in excess. Many biologists now agree that organisms have capacities in excess of what is typically needed (Garland 1998).

Additionally, it has been suggested that capacities should exceed the peak load experienced in nature, for capacities and loads to be matched perfectly would be too dangerous (Diamond and Hammond 1992). In the present study we were testing whether or not deer mice have an excess blood–O_2–carrying capacity. If deer mice have an excess capacity, we expected the mice to be able to perform at their normal maximum aerobic level with a reduced number of red blood cells. Deer mice occupy a large altitudinal gradient and are extremely active at high altitudes, traversing longer distances than mice at lower altitudes (Hayes 1989). Having the ability to carry excess oxygen may be beneficial to the mice, especially when they are active at high altitudes. However, our data do not support the predictions we expected that would provide support for excess oxygen–carrying capacity.

We predicted that HCT would be lower on days immediately following blood removal due to the differential time required for replacing red blood cells and that required for replacing plasma volume (Hoft 2000). Our data support this prediction, with HCT not returning to baseline values until seven days after initial measurement. We also predicted that the decrease in $\dot{V}O_2max$ and the amount of blood removed would be disproportionate, additionally we expected $\dot{V}O_2max$ to return to baseline values before the time necessary for HCT to fully recover if deer mice had an excess blood–O_2–carrying capacity; our data did not support these predictions. Taking blood from mice had an effect on $\dot{V}O_2max$ as average values of $\dot{V}O_2max$ decreased the day after drawing blood. However, the amount of red blood

cells removed corresponded with a proportionate percent decrease in $V\dot{O}_2$max. Therefore, it makes sense that there was a differential decreases in $V\dot{O}_2$max for mice in the high and low blood groups, although it is important to note that the $V\dot{O}_2$max of both groups decreased below baseline values following HCT measurement suggesting that small decreases in red blood cell number can affect $V\dot{O}_2$max. Our repeated measurements of $V\dot{O}_2$max indicate that mice did not fully return to baseline values until seven days after taking blood (Fig 4.2), the same amount of time required for HCT to return to baseline values. As a whole, these results suggest that deer mice have no significant excess blood–O_2–carrying capacity indicating that transport of O_2 through the blood could be a limiting factor for $V\dot{O}_2$max.

There is much support for the idea that circulatory delivery of O_2 is a major factor limiting $V\dot{O}_2$max. Studies on humans have demonstrated that, whether O_2 delivery is reduced by anemia or hypoxia, $V\dot{O}_2$max declines in direct proportion to O_2 delivery (Lindstedt et al. 1988; Lindstedt and Conley 2001). Results such as these suggest a direct relationship between O_2 in the blood and $V\dot{O}_2$max, tempting highly competitive endurance athletes to take exogenous erythropoietin to increase red blood cell production. However, increasing O_2 in the blood results in different effects than decreasing O_2. Spriet et al. (1986) gave trained endurance runners up to three units of additional blood increasing O_2 delivery by up to 30%, but this was only accompanied by a 7% increase in $V\dot{O}_2$max. Rezende et al. (2006) also found a disproportionate increase in $V\dot{O}_2$max with increased O_2 delivery in mice. These

results imply that O_2 delivery exceeded the capacity of the mitochondria and so no benefit could be gained by the additional O_2 (Lindstedt and Conley 2001). However, the small increases in $\dot{V}O_2$max associated with increased O_2 delivery suggests that peripheral muscles can increase aerobic metabolism if more O_2 is available (Rezende et al. 2006). Circulatory delivery of O_2 is dependent on cardiac output and hemoglobin, but based on the relationship between hemoglobin and $\dot{V}O_2$max in humans, Lindstedt et al. (1988) determined that hemoglobin was not a limiting factor of $\dot{V}O_2$max. This suggests that cardiac output is the factor most limiting O_2 circulation in humans, but different results have been found in other mammals. Goats were able to maintain their normoxic (21% O_2) $\dot{V}O_2$max while breathing air that containing 15% O_2 by increasing cardiac output, so it seems goats are clearly not limited cardiac output in normoxia (Karas et al. 1987). Horses were able to increase their $\dot{V}O_2$max by 11% when breathing air containing 25% O_2 without increasing cardiac output; their oxygen saturation is only 77% in normoxia and was increased by hyperoxia (Jones et al. 1992; Jones and Lindstedt 1983). Additionally, horses were able to increase their $\dot{V}O_2$max by 19% when breathing air containing 35% O_2 with only a 10% increase in cardiac output; stroke volume increased during hyperoxia, but heart rate never exceeded normoxic values (Jones et al. 1992; Jones and Lindstedt 1993). While it appears that maximal aerobic performance of humans may be in large part limited by O_2 circulation, that is definitely not true for the animal kingdom as a whole and it is worth while to investigate these hypotheses in multiple species of varying athleticism.

Possessing an excess blood–O_2–carrying capacity could potentially beneficial for deer mice that are active at high altitudes, but other aspects of their physiology may make this an unnecessary adaptation. In addition to the potential for increased O_2 loading at the lungs due to hemoglobin adaptations, high altitude deer mice have other physiological adaptations that may be beneficial for O_2 distribution. Wild deer mice from high altitudes have larger lungs and hearts than mice from nearby lower altitudes (Hock 1961; Hock 1964). Wyckoff and Frase (1990) found that deer mice from high altitudes had higher hematocrit, hemoglobin content, and mean red cell volume than white–footed mice (*Peromyscus leucopus*) from low altitudes. Hammond et al. (2001) showed that deer mice acclimated to high altitudes increased hematocrit and heart and lung mass. All of these phenotypically plastic traits would likely increase the circulatory delivery of O_2 and might make the observance of an excess blood–O_2–carrying capacity in the deer mouse unlikely as other researchers have indicated that structures lacking a high capacity for phenotypic plasticity are likely to exhibit larger excess capacities than those that can be easily modified (Lindstedt and Conley 2001). It may be more economical for animals to up regulate systems that can be easily altered when the load placed on them increases rather than maintaining these systems in excess continuously.

The results from repeated measures of $\dot{V}O_2max$ in our control group further confound the interpretation of our data. Control mice, like the treatment group, suffered a substantial decrease in $\dot{V}O_2max$ following baseline measurements and did

not return to baseline values until seven days after baseline measurments (Fig 4.4). There was a large increase in $\dot{V}O_2$max between days 5 and 7 in the control group that was unobserved in the treatment group. It is important to note that $\dot{V}O_2$max is forced exercise and in our experiment the willingness to run was solely up to the motivation of the mouse. We did not use a treadmill with a shock grid and none of the mice we used had been "trained" to run on a treadmill. The figures for $\dot{V}O_2$max show average values for all of the mice, but looking at individual values reveals much variation in $\dot{V}O_2$max. While, the average values decrease between day 0 and day 1 this pattern was not observed in each individual. Some mice, in both groups, increased their $\dot{V}O_2$max from day 0 to day 1. The reason for this is unknown to us, but it could be due to the mouse's willingness to run or it may have taken some mice more time to become accustomed to running on a treadmill. The sample sizes between the groups also differed (51 for treatment and 28 for control). Increasing the sample size might smooth out the averages and better account for individual variation, but we were limited in the number of animals available. It is possible that deer mice do in fact have an excess O_2–carrying capacity in the blood that could not be revealed due to the confounding effects of repeated measurement and blood drawing on $\dot{V}O_2$max, however this seems unlikely due to the close relationship between amount of red blood cells removed and reduction in $\dot{V}O_2$max.

This study also allows us to issue some cautionary statements that others may find useful. In physiological studies $\dot{V}O_2$max along with HCT are often

measured. Many studies, especially field studies, are limited in the number of animals available and so multiple measurements must be made on the same individual. Researchers should be aware that the measurement of one physiological trait might impact other traits in ways that are not necessarily obvious. It is important to know how measuring any trait will affect an animal's physiology and the time course necessary for animals to make a full recovery. While this may seem obvious to some, we feel it is important to mention and keep in mind when designing experiments.

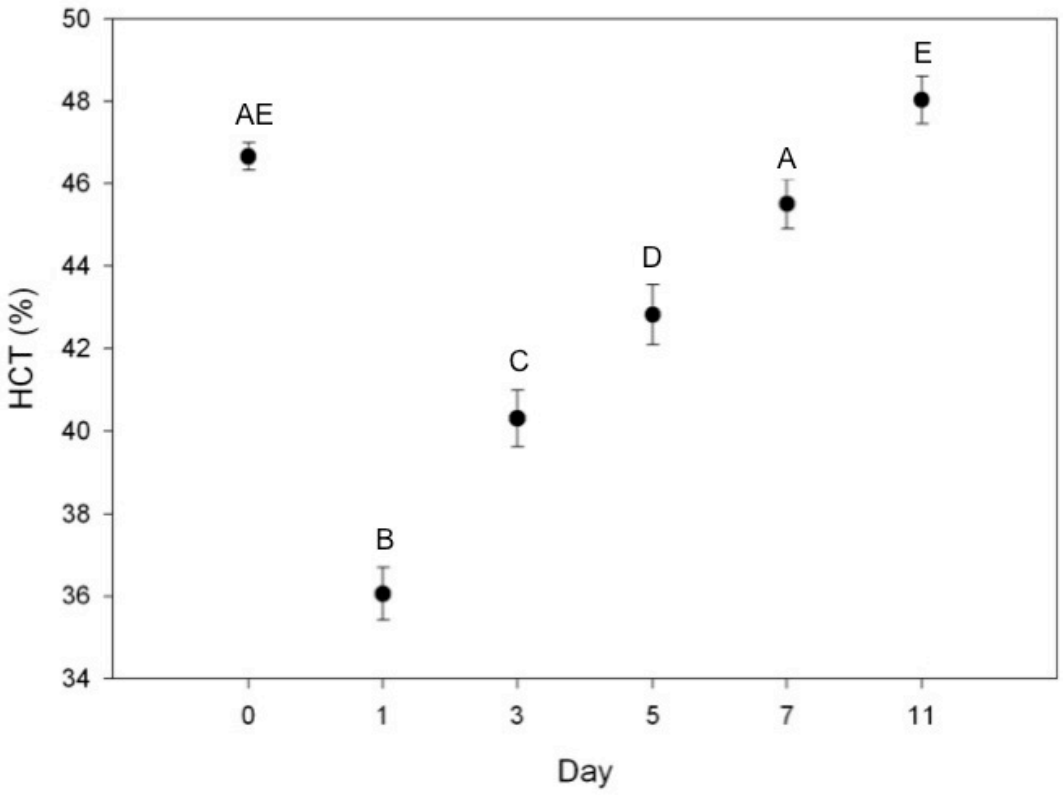

Figure 3.1. Plot of hematocrit (HCT) across treatment days. Day 0 represents baseline measurements of HCT for all mice. Days 1–11 represent the number of days between the first and second measurement of HCT. Points bearing the same letters are not significantly different from each other. HCT returned to baseline values seven days after initial measurement.

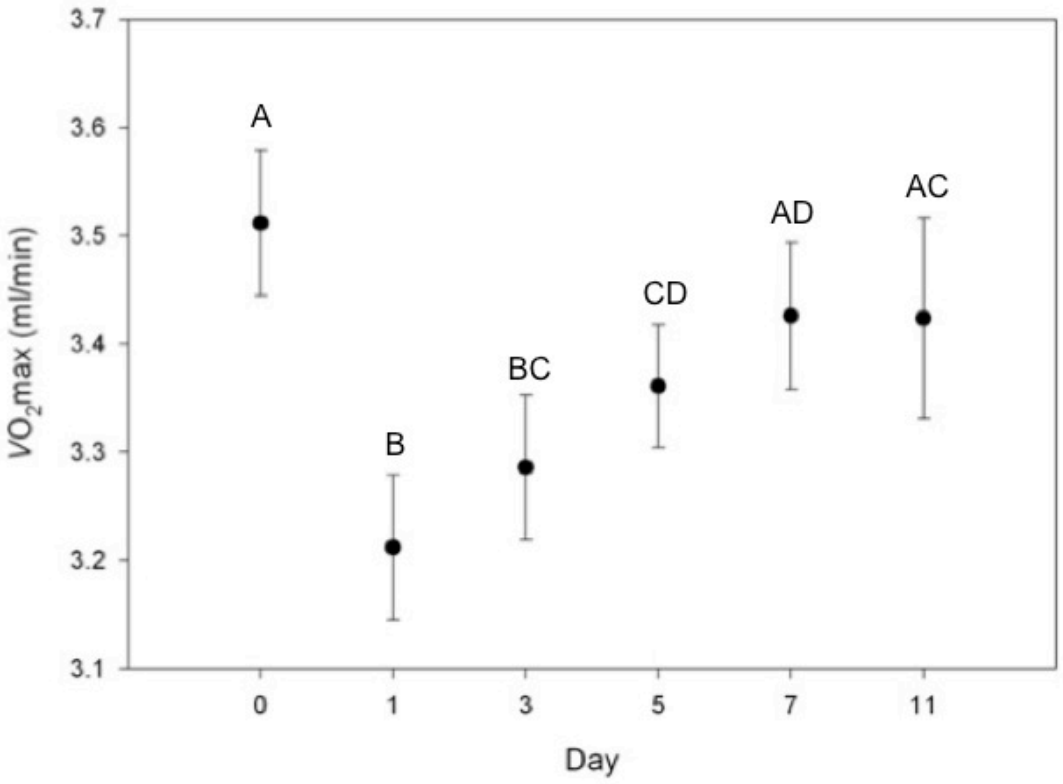

Figure 3.2. Plot of $V\mathrm{O}_2$max of treatment mice across days. Day 0 represents baseline measurements of $V\mathrm{O}_2$max for all mice. Days 1–11 represent the number of days after blood was taken for HCT measurement. Points bearing the same letters are not significantly different from each other. $V\mathrm{O}_2$max returned to baseline values seven days after HCT measurement.

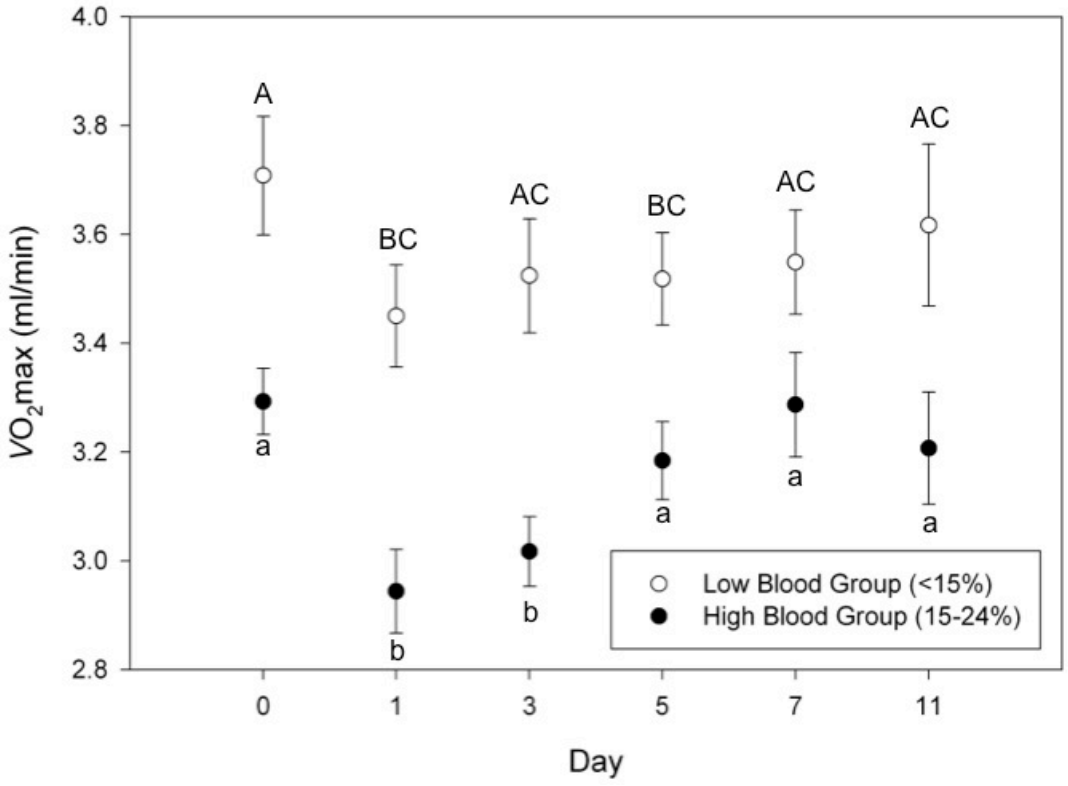

Figure 3.3. Plot of VO_2max across treatment days for mice separated into groups based on total amount of blood taken during HCT measurements. Low blood group mice had less than 15% of total blood volume removed and high blood group mice had between 15 and 24% of total blood volume removed. Day 0 represents baseline measurements of VO_2max for all mice. Days 1–11 represent the number of days after blood was taken for HCT measurement. Points bearing the same letters are not significantly different from each other. VO_2max of mice from both groups decreased on the day after blood was taken and gradually returned to baseline values.

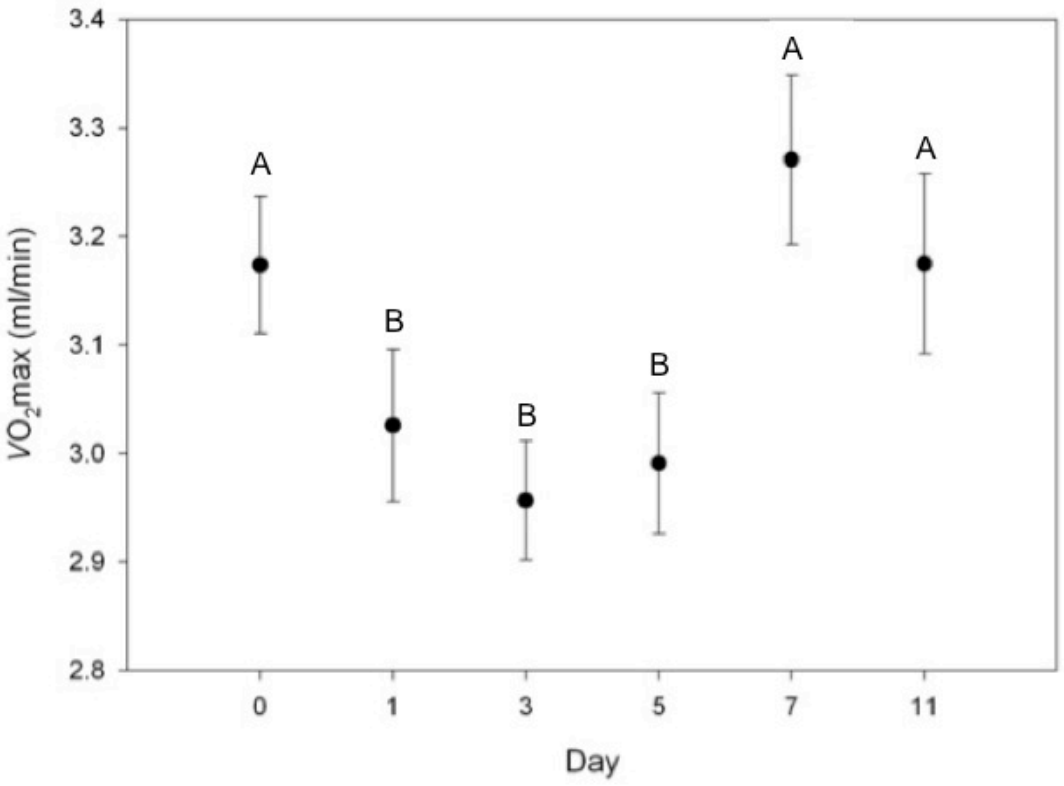

Figure 3.4. Plot of VO_2max of control mice across days. Day 0 represents baseline measurements of VO_2max for all mice. Days 1–11 represent the number of days after a day of rest following baseline measurements. Points bearing the same letters are not significantly different from each other. Despite no blood being taken, VO_2max was still affected by multiple measurements and returned to baseline values seven days after initial measurement.

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Conclusions

In this dissertation I have investigated some of the physiological adaptations that allow mammals to thrive in environmentally challenging habitats. I first investigated whether mammals inhabiting arid regions have evolved mechanisms to reduce rates of total evaporative water loss (TEWL). Indeed, mammals living in arid regions have lower rates of TEWL compared with mammals from more mesic regions. I found this result when using both a categorical descriptor of habitat as well as when using continuous variables to describe habitat. The mechanism by which these mammals reduce TEWL is not known and should be an area of future research. There is evidence to suggest that cutaneous evaporative water loss (CEWL) may contribute larger to TEWL than previously thought and mammals may be able to reduce TEWL by altering CEWL.

The importance of thermogenic capacity cannot be understated for mammals living in cold environments and there has been evidence of directional selection for increased thermogenic capacity in these environments. Thermogenic capacity is the sum of basal metabolic rate, shivering thermogenesis and nonshivering thermogenesis. Mammals that can increase any of these components can increase their overall thermogenic capacity. All of these components have been shown to increase with cold acclimation, but results are not consistent between species. I have shown that the deer mouse increases thermogenic capacity primarily by increasing the capacity for nonshivering thermogenesis. This has often been thought of as the main mechanism for small mammals to increase thermogenic

capacity. However, this result, while true for some species, has not been found for all species of mammals studied. The exact mechanism for increased thermogeneic capacity after cold acclimation should not be assumed without measuring the individual components, as this result seems to be species specific.

Mammals living at high altitude face the combined challenges of cold temperatures, reduced oxygen availability and decreased primary productivity. Despite all of these challenges, many species of mammals live a successful, highly aerobic lifestyle at high altitudes. The ability of the muscles to acquire sufficient oxygen is crucial for heat production and aerobic activity. Having an excess ability to carry oxygen in the blood could potentially be extremely advantageous for mammals living at high altitudes. I tested whether or not the deer mouse has an excess carrying capacity of oxygen in the blood and found that this was not likely the case. The circulatory distribution of oxygen seems to, at least in part, set an upper limit for sustained aerobic activity in these animals. While the ability to carry excess oxygen would be beneficial for the deer mouse, they have other adaptations that may make this unnecessary. For example, the lung size, heart size and hematocrit of these animals are highly plastic and can quickly be adjusted to meet the increased demands of oxygen delivery at high altitudes. It may be more economical for these animals to adjust their physiology when needed rather than to maintain excess capacities at all times.