Matching oxygen supply and oxygen demand: do heterothermic rodents tolerate cold and hypoxia through the retention of neonatal traits?

by

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Abstract

There are striking similarities between newborn mammals and adult mammals capable of hibernation (heterotherms) that are not present in adult mammals that do not hibernate (homeotherms). Unlike most mammals, adult heterotherms and newborns are exceptionally tolerant of cold and hypoxia. However, the basis of this enhanced tolerance remains largely unknown. In this thesis I determined how newborn and adult rodents that vary in heterothermic expression match O_2 supply and O_2 demand when exposed to cold and hypoxia, either alone, or in combination. I hypothesized that this enhanced tolerance reflects the retention of newborn responses in adult heterotherms that are not present in adult homeotherms.

This thesis has shown that the responses rodents use to match O_2 supply and O_2 demand vary with age, O_2 level, ambient temperature, and degree of heterothermic expression. When exposed to cold alone all newborns significantly decreased their body temperature, O_2 consumption rate, and ventilation. However, as adults all rodents maintained a high body temperature by increasing or maintaining their O_2 consumption rate and ventilation.

When exposed to hypoxia alone, I found that all rodents used similar responses to match O_2 supply and O_2 demand. Newborn and adult, homeotherms and heterotherms alike all reduced their metabolic demand for O_2, and increased O_2 supply by increasing their ventilation.

When exposed to both hypoxia and cold, however, adult heterotherms exhibited a greater reduction in O_2 consumption rates, and a reduced ventilatory response compared to adult homeotherms, responses more similar to those of newborns when they were exposed to hypoxia and cold.

My thesis supports the hypothesis that heterotherms match O_2 supply and O_2 demand through the retention newborn responses, but only when exposed to hypoxia in the cold, as: (1) homeotherms and heterotherms both differed in their responses to cold as newborns and adults; and (2) all rodents used similar responses when exposed to hypoxia alone. These data provide insight into the basis of enhanced cold and hypoxia tolerance of adult heterotherms and newborns.

Lay Summary

Unlike most mammals, adult hibernators are exceptionally tolerant of low O_2 and cold. It is conceivable that this enhanced tolerance reflects the retention of newborn responses in adult hibernators, and that these responses are different from that of adult non-hibernators. To test this, I exposed newborn and adult hibernators and non-hibernators to low O_2 and cold, alone and together, and measured how they matched O_2 supply and O_2 demand. I found that hibernators do retain newborn responses to low O_2, with both groups reducing their metabolic demand for O_2 more than adult non-hibernators, but only when also exposed to cold. In addition to reducing their metabolic demand for O_2, adult non-hibernators increased ventilation (O_2 supply). Thus, enhanced tolerance in adult hibernators may have evolved through the retention of newborn responses. These data provide insight into the basis of enhanced tolerance to low O_2 and cold of adult hibernators and newborns.

Preface

A version of Chapter 1 has been published as part of a larger review as Dzal, Y.A., Jenkin, S.E.M., Laguë, S.L., Reichert, M.N., York, J.M., and Pamenter, M.E. (2015). Oxygen in demand: How oxygen has shaped vertebrate physiology. *Comparative Biochemistry and physiology Part A: Molecular and Integrative Physiology*. **186:** 4-26. I wrote the section entitled "Reducing oxygen demand in hypoxia: ventilatory, metabolic, and thermoregulatory strategies of small neonatal and adult mammals". All authors contributed equally to the manuscript and provided editorial feedback under the supervision of Dr. Matthew E. Pamenter.

Chapters 2 to 5 have been written as separate manuscripts and are ready for submission with Dzal, Y.A. and Milsom, W.K. listed as authors. For each chapter I was the primary contributor to the experimental design, data collection, data analysis, and manuscript preparation under the supervision of Dr. W.K. Milsom.

Ground squirrels used in this thesis were trapped with the approval of Manitoba Conservation and Water Stewardship, under the wildlife scientific permit WB15027. All experimental procedures in this thesis conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the UBC Committee on Animal Care (under protocol A13-0091).

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List of Symbols

FiO_2	fractional incurrent O_2 concentration (dry gas)
$FiCO_2$	fractional incurrent CO_2 concentration (dry gas)
FeO_2	fractional excurrent O_2 concentration (dry gas)
$FeCO_2$	fractional excurrent CO_2 concentration (dry gas)
pHa	arterial pH
P_{50}	partial pressure at which 50% of the blood is saturated with O_2
PO_2	partial pressure of O_2
PCO_2	partial pressure of CO_2
Q_{10} effect	the rate of change of a biological or chemical system as a consequence of a change in body temperature of 10°C
$\dot{V}$i	incurrent flow rate
$\dot{V}O_2$	O_2 consumption rate

List of Abbreviations

BTPS	body temperature and pressure, saturated
HMR	hypoxic metabolic response
HVR	hypoxic ventilatory response
LCT	lower critical temperature
STPD	standard temperature and pressure, dry
Tb-Ta differential	body temperature-ambient temperature differential
TNZ	thermoneutral zone

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Chapter 1: General Introduction

1.1 The "hibernator as a neonate" hypothesis

Most mammals are homeotherms, pursuing a strategy in which body temperature is maintained relatively constant independent of ambient temperature. For example, when homeotherms such as rats (*Rattus norvegicus*) are exposed to low ambient temperatures, they maintain a high body temperature and O_2 consumption rate independent of season and environmental conditions (Yoda et al. 2000). Homeothermy, however, does not exclude the possibility that body temperature may vary. All homeotherms reduce body temperature (by < 3°C) and O_2 consumption rate to some extent during sleep, as well as when energy stores fall (Challet et al. 1997; Mortola and Seifert 2000; Heller and Ruby 2004; Refinetti and Piccione 2005). Among mammals, heterothermy is also common, with heterotherms being widely distributed within all three mammalian lineages (monotremes, marsupials, and placentals; Lyman 1982; Lovegrove 2011; Lovegrove 2012). Unlike homeotherms, heterotherms are capable of extreme and controlled reductions in body temperature and O_2 consumption rate. The extent to which they reduce their body temperature and O_2 consumption rate depends on ambient temperature and season.

Facultative heterotherms, such as mice (*Mus musculus*) and hamsters (*Mesocricetus auratus*), reduce their body temperature and O_2 consumption rate (i.e. use torpor) at any time of the year when confronted with energetic stressors such as low ambient temperature (Ellison and Skinner 1992; Heldmaier et al. 1999) or limitations in food or energy reserves (Lyman 1948; Hudson and Scott 1979). Mice undergo relatively short (less than 24 hours) and shallow torpor bouts, (Hudson and Scott 1979; Geiser and Ruf 1995; Geiser 2004; Heldmaier et al. 2004; Wang and Lee 2011), while hamsters may undergo longer (a few days) and deeper torpor bouts (Lyman 1948). Torpor in obligate heterotherms, such as ground squirrels (*Ictidomys tridecemlineatus*), is usually seasonal. When exposed to cold during the active, non-hibernating season, obligate heterotherms generally increase heat production to maintain body temperature, similar to the response observed in homeothermic species (Wang and Lee 2011). However, during the hibernating season, ground squirrels exhibit repetitive and deep torpor bouts that last several days or weeks (Snapp and Heller 1981; Wang 1989; Geiser and Ruf 1995; Geiser 2004).

There are two ongoing hypotheses to explain the evolution of heterothermy in mammals. The first suggests that heterothermy evolved independently in multiple lineages of monotremes, marsupials, and placental mammals, and is the result of convergent evolution (Geiser 2008). The second hypothesis proposes that the common ancestor of all mammals was a heterotherm, and that heterothermy was independently lost in multiple lineages (Robertson et al. 2004; Kikuchi and Vanneste 2010; Lovegrove 2011; Dausmann et al. 2012; Lovegrove 2012). In support of the second hypothesis, heterothermy in mammals has not been associated with novel genes (Srere et al. 1992; Carey et al., 2003). It is conceivable that the genetic basis of heterothermy is common to the mammalian genome. Thus, variation in the expression of such genes may provide the basis for the degree of heterothermic expression observed among mammals, spanning from homeothermy at one extreme, to obligate heterothermy at the other (Srere et al. 1992; Boyer and Barnes 1999; Lovegrove et al. 1999; O'Hara et al. 1999; Eddy and Storey 2002; Carey et al. 2003; Brauch et al. 2005; Williams et al. 2005; Yan et al. 2006; Crawford et al. 2007; Yan et al. 2008; Morin and Storey 2009).

In addition to evolutionary and genetic evidence suggesting that the common ancestor of all mammals was a heterotherm, newborn mammals and heterothermic mammals share numerous physiological traits (for a list see Harris et al. 2004). Collectively, this evidence led Harris et al. (2004) to propose the "hibernator as neonate" hypothesis, which postulates that heterothermy stems from the retention of traits that are common to all newborn mammals. This hypothesis contends that the genetic potential for heterothermy is expressed, to some extent, in all newborn mammals and that heterothermy in some species of adults results from the continued expression of such pathways. Among the striking parallels between newborn mammals and adult heterotherms is their remarkable tolerance to extreme reductions in body temperature (i.e. cold) and to hypoxia. However, our current understanding of how newborn and adult rodents match O_2 supply and O_2 demand when they are exposed to thermal and hypoxic challenges is limited, as is our knowledge of how these responses change during postnatal development.

What little data there are indicate that most newborn mammals reduce O_2 demand (i.e. reduce body temperature and O_2 consumption rate) when exposed to a reduction in ambient temperature and O_2 availability (Mortola et al. 1989; Mortola 1991; Mortola and Dotta 1992; Frappell and Mortola 1994; Hill 2000; Mortola 2004). As homeotherms develop from newborns into adults they respond to cold and hypoxia by maintaining body temperature, and in order to do

so they increase or maintain their O_2 consumption rate, and increase O_2 supply primarily by an increase in ventilation (Mortola et al. 1989; Mortola 1991; Frappell et al. 1991; Chappell 1992; Frappell et al. 1992; Mortola and Dotta 1992; Frappell and Mortola 1994; Mortola 2004). Conversely, adult heterotherms continue to respond to cold and hypoxia by significantly decreasing O_2 demand just like newborns (McArthur and Milsom 1991; Mortola 1991; Frappell et al. 1992; Osborne and Milsom 1993; Frappell and Mortola 1994; Barros et al. 2001; Tattersall and Milsom 2009). However, these observations are based on data from only a few studies. Thus, my thesis focuses on testing the "hibernator as a neonate" hypothesis by determining how newborn and adult homeothermic and heterothermic rodents match O_2 supply and O_2 demand in the cold and in hypoxia. I hypothesized that newborn homeothermic and heterothermic rodents would be indistinguishable in how they matched O_2 supply and O_2 demand under thermal and hypoxic challenges; primarily reducing body temperature and their O_2 consumption rate. Furthermore, I hypothesized that differences in cold and hypoxia tolerance in adult homeothermic and heterothermic rodents reflect different developmental trajectories in the way in which O_2 supply and O_2 demand are matched under thermal and hypoxic challenges; adult homeotherms primarily increasing O_2 supply, and adult heterotherms responding more like newborns and relying on a decrease in O_2 demand. Within each of the data chapters to follow I examine the thermoregulatory, metabolic, and ventilatory responses to decreases in ambient temperature and/or O_2 in newborn and adult rodents ranging in their degree of heterothermic expression, from true homeothermy (Sprague Dawley rat: *Rattus norvegicus*), to facultative heterothermy (common mouse: *Mus musculus*, and golden hamster: *Mesocricetus auratus*), and obligate heterothermy (13-lined ground squirrel: *Ictidomys tridecemlineatus*)). The remainder of this introduction will review what is known about how rodents that vary in heterothermic expression match O_2 supply and O_2 demand in hypoxia, and how their responses vary with age, heterothermic expression, ambient temperature, and severity of hypoxic exposure. Lastly, my introduction will finish with an overview of my thesis objectives and overarching hypotheses.

1.2 The effects of progressive cooling on matching O_2 supply and O_2 demand in adult rodents in normoxia and hypoxia

The passive thermoregulatory capacity of a mammal is reflected in its thermoneutral zone

(TNZ), defined as the range of ambient temperatures over which body temperature can be held constant, while their O_2 consumption rate is at a minimal and steady value (Scholander et al. 1950; McNab 1980; Fig. 1.1). The TNZ generally coincides with the animal's preferred range of ambient temperatures (Nagy 1993). Mammals passively maintain their body temperature within the TNZ by altering heat dissipation via regulation of insulation through passive changes in conduction, convection, and radiation (Scholander et al. 1950; McNab 1980; Fig. 1.1). The lowest ambient temperature of the TNZ is the lower critical temperature (LCT; Scholander et al. 1950; McNab 1980; Fig. 1.1). Below the LCT reducing heat dissipation by passive means is no longer sufficient to maintain body temperature (Scholander et al. 1950; McNab 1980; Fig. 1.1). Instead, mammals rely on their capacity to produce heat endogenously to maintain their body temperature via thermogenesis, which significantly increases their O_2 consumption rate (Janský 1973; Hohtola 2004; Haman 2006; Fig. 1.1). Matching the high energetic requirement for thermogenesis requires a proportional increase in O_2 supply and thus, it is often accompanied by an increase in ventilation or lung O_2 extraction efficiency (Chappell 1992). Eventually, as ambient temperature falls, thermogenesis is insufficient to compensate for the increasing thermal gradient between body temperature and ambient temperature, and body temperature begins to fall (i.e. hypothermia sets in; Fig. 1.1). An alternative strategy used by some mammals when ambient temperature falls below the LCT is to reduce O_2 demand by a controlled reduction in body temperature, which is an advantageous strategy during energetic shortfalls (Barros et al. 2001).

Thermal conductance dictates the ease with which heat enters or leaves an animal's body, and is reflected in the slope of the relationship between O_2 consumption rate and ambient temperature below the LCT. The thermal conductivity of an animal is highly dependent on the presence of an insulating layer, such as fur or subcutaneous fat, and can be physiologically manipulated through vasomotor control of blood flow, as well as sweat production (McNab 1980; Rowell 2011). Both adult homeotherms and heterotherms are effective thermoregulators and actively control their body temperature (Heller and Colliver 1974; Snapp and Heller 1981; Heldmaier and Ruf 1992; Geiser 2004). In preparation for the non-active, hibernating season, heterotherms increase their fat stores and insulating layer thickness, resulting in a wider TNZ, and a reduced LCT and thermal conductance in the fall (Scholander et al. 1950; Fig 1.2B) than in the summer (Fig. 1.2A). Homeotherms also increase their fat stores and insulation in preparation

for the winter, albeit not to the same extent as heterotherms (Scholander et al. 1950).

To maintain an aerobic energy balance, changes in O_2 demand are supported by equivalent changes in O_2 supply through the O_2 transport pathway (Ingram and Legge 1970; Chappell and Roverud 1990; Chappell 1992; Barros et al. 2001; Larcombe 2002; for a review see Mortola and Frappell 2000). While any given step in the O_2 transport pathway may be modified, the O_2 supply to arterial blood can be regulated in two main ways. The first is by changing ventilation, achieved through modifications in either breathing frequency or the volume of air that is moved per breath (i.e. tidal volume) (Casey et al. 1979; Chappell and Roverud 1990; Chappell and Dawson 1994; Larcombe 2002). The second is by altering the efficiency with which O_2 is extracted from each breath in the lungs (i.e. lung O_2 extraction efficiency), which can be adjusted by modifying ventilation-perfusion matching, the diffusion distance between alveolar air and pulmonary capillary blood, or the O_2 partial pressure gradient for pulmonary diffusion (Weibel 1984).

Adult mammals exhibit a variety of responses to match O_2 supply and O_2 demand in the cold, with no clear distinction between homeothermic and heterothermic species. To match the increased O_2 demand required for thermogenesis, some species increase their ventilation, some increase lung O_2 extraction efficiency, and some employ a mix of the two responses (Chappell and Dawson 1994; Barros et al. 2001). When living in a hypoxic environment, supplying enough O_2 to sustain thermogenesis is even more challenging (Rosenmann and Morrison 1975; Hayes 1989; Lui et al. 2015). In environmental hypoxia most small mammals reduce the temperature at which they regulate their body temperature, their thermoregulatory set-point (Barros et al. 2001; Tattersall and Milsom 2009). This hypoxia-induced reduction in the thermoregulatory set-point results in an inhibition of thermogenesis and a transient increase in heat loss, which leads to a rapid reduction in body temperature (Barros et al. 2001; Tattersall and Milsom 2009). This lowering of the thermoregulatory set-point in hypoxia is beneficial because it reduces the energetic demands that accompany the recruitment of thermoregulatory mechanisms outside of the TNZ. Furthermore, the reduction of body temperature during exposure to hypoxia is protective because a drop in body temperature increases the affinity of hemoglobin for O_2, which results in improved extraction of O_2 at the lungs, a desirable response in conditions of low alveolar O_2 pressure.

In adult homeothermic and heterothermic rodents, it appears that the reduction of the thermoregulatory set-point in hypoxia is dependent on both the ambient temperature at which hypoxic exposure occurs, as well as the level of hypoxic exposure; with the reduction in the thermoregulatory set-point, O_2 consumption rate, and body temperature being greater at lower ambient temperatures and lower environmental O_2 levels (Hill 1959; Barros et al. 2001). However, to my knowledge, in adult rodents, the extent to which the relationship between O_2 consumption rate and ambient temperature is modified by hypoxia has only been addressed in ground squirrels at a single level of hypoxia (7% O_2) (Barros et al. 2001). It remains unknown whether adult homeothermic and heterothermic rodents exposed to environmental hypoxia and then progressively cooled: 1) conform to their external environment by reducing O_2 demand through a reduction in their thermoregulatory set-point, O_2 consumption rate, and body temperature; 2) maintain their thermoregulatory set-point and body temperature by increasing thermogenesis and increasing O_2 supply; or 3) some combination of these strategies. In Chapter 2 I address these questions. I hypothesized that the enhanced cold and hypoxia tolerance of adult heterotherms stems from their remarkable ability to reduce their thermoregulatory set-point, O_2 consumption rate, and body temperature in the cold and hypoxia compared to adult homeotherms. I predicted that in all species of adult rodents within their TNZ, environmental hypoxia would reduce their O_2 consumption rate (the hypoxic metabolic response; HMR) and body temperature of all species of adult rodents. Furthermore, I predicted that in all species of adult rodents exposed to environmental hypoxia, progressive cooling would be associated with a drop in body temperature due to the reduced ability to mount a thermogenic response (as indicated by a reduction in the slope of the relationship between O_2 consumption rate and ambient temperature, i.e. thermal conductance). Finally, I predicted that adult heterotherms exposed to environmental hypoxia would reduce their thermoregulatory set-point, O_2 consumption rate, and thus body temperature beyond that of adult homeotherms. Obligate heterotherms are capable of even greater reductions in their O_2 consumption rate and body temperature than facultative heterotherms (Ruf and Geiser 1995; Geiser 2004). Thus, I predicted that in environmental hypoxia, the increase in O_2 consumption rate below the LCT would be reduced most in the obligate heterotherm, and least in the homeotherm, with facultative heterotherms falling somewhere in between.

1.3 The effects of progressive cooling on matching O_2 supply and O_2 demand in newborn rodents in normoxia and hypoxia

Both homeothermic and heterothermic newborn mammals are more tolerant of cold and hypoxia than their adult counterparts (Avery and Johlin 1932; Kabat 1940; Fazekas et al. 1941; Glass et al. 1944; Adolph 1948a; Adolph 1948b; Hiestand et al. 1953; Adolph 1969; Singer 1999; Hill 2000; Fong 2010). However, unlike adults, newborns are poor thermoregulators (Adolph 1957; Alexander 1975; Hill 1983; McClure and Porter 1983; Spiers and Adair 1986; Knight 1987; Newkirk et al. 1995). With their large surface area relative to their volume, and with their hairless skin, newborns have a high thermal conductance and are susceptible to rapid heat loss (Fig. 1.3). While some newborn mammals are capable of limited heat production through shivering thermogenesis (e.g. piglets; LeBlanc and Mount 1969), most newborn rodents can only mount a limited thermogenic response based on heat produced via non-shivering thermogenesis (e.g. rats (Moore and Underwood 1963), hamsters (Hissa 1968; Newkirk et al. 1995), and ground squirrels (Maxwell and Morton 1975; for a review see Brück and Hinckel 1996 and references within)). As a result of their poor thermogenic capacity, their TNZ is often undefinable (Taylor 1960; Hissa and Lagerspetz 1964; Hissa 1968; Alexander 1975; Mortola and Dotta 1992; Fig. 1.3), and body temperature simply tracks ambient temperature (Taylor 1960; Mortola and Dotta 1992; Fig. 1.3). Thus, body temperature regulation in newborns is primarily a result of behavioural regulation, or of parental care, rather than of physiological thermoregulation (Hissa and Lagerspetz 1964; Hissa 1968). As rodents develop, insulation increases, and the relative surface area to volume ratio decreases, resulting in a reduction in thermal conductance, which combined with an increase in thermoregulatory capacity results in a widening of the TNZ (Adolph 1957; Alexander 1975; McClure and Porter 1983; Hill 1983; Spiers and Adair 1986; Knight 1987; Newkirk et al. 1995).

When environmental O_2 is low, newborn rodents, like adults, reduce the temperature at which they attempt to regulate their body temperature (Mortola et al. 1989). For example, when newborn rats are exposed to hypoxia they alter their behavioural responses to cold, leading to a reduction in inter-animal huddling and an increase in the body surface area available for heat dissipation (Mortola and Feher 1998). However, to my knowledge, the effects of hypoxia on the physiological thermoregulatory response of newborn rodents exposed to progressive cooling has

only been addressed in rats, at a single level of hypoxia (10% O_2) (Mortola and Dotta 1992). Whether newborn homeothermic and heterothermic rodents are similar in how they match O_2 supply and O_2 demand when they are exposed to hypoxia during progressive cooling remains unknown.

In Chapter 3 I examine how newborn homeothermic and heterothermic rodents match O_2 supply and O_2 demand in response to cold and hypoxia, either alone or in combination. I hypothesized that there would be no difference in the thermoregulatory, metabolic, or ventilatory responses between newborn homeotherms, facultative heterotherms, and obligate heterotherms when exposed to progressive cooling in normoxia or hypoxia. Since newborn rodents have a poor thermoregulatory capacity, I predicted that when exposed to progressive cooling in normoxia, newborns would conform to their external environment by reducing O_2 demand. Thus, unlike adults, their TNZ would be narrow and undefinable, and body temperature, O_2 consumption rate, and ventilation would fall with decreases in ambient temperature. Furthermore, I predicted that like in normoxia, when exposed to progressive cooling in hypoxia all newborn species would conform to their external environment by reducing O_2 demand. However, the reduction in body temperature, O_2 consumption rate, and ventilation with progressive cooling would be even greater in hypoxia than in normoxia. This hypoxia-induced reduction in the O_2 consumption rate would have to be temperature independent.

1.4 The effects of progressive hypoxia on matching O_2 supply and O_2 demand in rodents within their TNZ

It is the role of the cardio-respiratory system to regulate the respiratory gases, O_2 and CO_2, and exchange them with the environment according to the animal's metabolic demands (Dejours 1975; Taylor and Weibel 1981). Oxygen from the environment is carried into the lungs by inspiration, where it diffuses into the red blood cells, is transported to the tissues through blood circulation, and finally diffuses from the blood to the mitochondria of working cells in a pathway referred to as the O_2 transport pathway (for a review see Taylor and Weibel 1981; Weibel 1984). The total amount of O_2 stored in the body is relatively low, thus, animals depend on a continuous supply of environmental O_2 to support fluctuations in their O_2 consumption rate, and maintain cellular function. Energy balance is disrupted when O_2 supplies fail to meet O_2

demands, and the mismatch ultimately leads to organ failure and death (Boutilier 2001).

Generally, when mammals are exposed to low O_2 environments, their immediate compensatory response is to hyperventilate (i.e. the hypoxic ventilatory response; HVR) (Powell et al. 1998). This response is often biphasic (Powell et al. 1998). The first phase of the HVR is an immediate increase in ventilation within one breath of a change in arterial levels of O_2, mediated by the stimulation of the peripheral chemoreceptors (Eldridge and Millhorn 1986; Bisgard and Neubauer 1995). The second phase of the HVR begins with a slow decline in ventilation to a steady lower level, mediated by: 1) central mechanisms, such as the reduction in temperature at which they regulate their body temperature (their thermoregulatory set-point) and O_2 consumption rate (the HMR); as well as 2) peripheral mechanisms, due to the time dependent decline in the sensitivity of the carotid bodies to hypoxic stimuli (Bisgard and Neubauer 1995). Although the biphasic response to hypoxia is typical of most mammals, the relative magnitude of the response both within and between individuals can vary depending on many factors. These factors include the level of hypoxia, chemoreceptor sensitivity, levels of CO_2, pH, time course of hypoxic exposure, pattern of repeated exposure, O_2 consumption rate, body temperature, and developmental stage (Powell et al. 1998).

Adult homeotherms and heterotherms also vary in the factors that may affect their hypoxic responses. For example, in normoxia, adult heterotherms exhibit a lower level of ventilation and a lower O_2 consumption rate than adult homeotherms (Mortola 1991; Frappell et al. 1992; Osborne and Milsom 1993; Barros et al. 2001). The ventilatory equivalent (i.e. the ratio between ventilation and O_2 consumption rate) of adult heterotherms is also typically lower than that of adult homeotherms (Mortola 1991; Frappell et al. 1992; Osborne and Milsom 1993; Barros et al. 2001). As a direct result of this reduced ventilatory equivalent, the arterial PO_2 is reduced and PCO_2 is elevated compared to adult homeotherms. However, adult heterothermic blood typically has a greater O_2 carrying capacity than adult homeothermic blood (Deveci et al. 2001). In adult heterotherms hemoglobin concentration, hematocrit levels, and erythrocyte counts are all elevated relative to that of adult homeotherms (Musacchia and Volkert 1971; Maginniss and Milsom 1994; Deveci et al. 2001). Additionally, red blood cell organic phosphate levels in their blood are reduced, causing a left-shifted O_2 equilibrium curve that further contributes to their enhanced O_2 carrying capacity in hypoxia (Musacchia and Volkert 1971; Maginniss and Milsom 1994; Deveci et al. 2001). A high hemoglobin-O_2 affinity corresponds

with a low P_{50} (partial pressure at which 50% of the blood is saturated with O_2). Lowered P_{50} levels are often reported in adult heterotherms, with levels as low as 18 torr; half of that typical of most mammals (measured at pHa 7.49 and 37°C; Maginniss and Milsom 1994). While an elevated hemoglobin-O_2 affinity promotes O_2 loading in hypoxic conditions, it often does so at the expense of O_2 unloading at the tissues. However, in adult heterotherms, high CO_2 levels improve O_2 unloading at the tissues by an enhanced Bohr effect (Boggs et al. 1984). These hematological adaptations are also common in newborns (Mortola 2001). At birth, hemoglobin-O_2 affinity is high (Mortola 2001). However, during postnatal development the gradual replacement of fetal type hemoglobin with adult hemoglobin results in reduced hemoglobin-O_2 affinity and thus reduced blood-O_2 carrying capacity (Mortola 2001). An increase in organic phosphates and a reduction in hematocrit and hemoglobin concentrations also contribute to a lower O_2 carrying capacity during postnatal development (Mortola 2001).

Collectively, these hematological traits suggest that a larger reduction in environmental O_2 may be required to elicit ventilatory and metabolic responses in newborn and adult heterotherms (i.e. they will exhibit a reduced O_2 response threshold and hypoxic sensitivity) than in adult homeotherms. While the O_2 response threshold and hypoxia sensitivity of the adult facultative heterotherm, the golden hamster (*Mesocricetus auratus*), has been found to be slightly lower than that of most adult homeotherms (Mortola 1991), other studies have found that the O_2 response threshold and hypoxia sensitivity of adult heterotherms do not differ from typical mammalian values (Boggs et al. 1984; Mortola et al. 1989; McArthur and Milsom 1991; Frappell et al. 1992; Osborne and Milsom 1993; Barros et al. 2001).

Newborns, on the other hand, have a drastically reduced HVR compared to their adult counterparts (Bureau et al. 1984; Mortola et al. 1989; Bavis et al. 2010). In fact, in many newborns ventilation may actually decrease to below normoxic levels within minutes of hypoxic exposure (Haddad et al. 1982; Bonora et al. 1984; McCooke and Hanson 1985; Bonora and Gautier 1987; Saetta and Mortola 1987; Martin-Body and Johnston 1988; Mortola and Rezzonico 1988; Rigatto et al. 1988; Mortola et al. 1989; Wangsnes and Koos 1991; Moss 2000). This time-dependent reduction in ventilation is not due to a reduced O_2-chemosensitivity, but rather reflects a fall in the rate of O_2 consumption (Cross et al. 1958; Hill 1959; Taylor 1960; Mortola et al. 1989; Clark and Fewell 1996; Rohlicek et al. 1996; Mortola and Feher 1998). As a result, ventilation tracks the fall in O_2 consumption rate and the ventilatory equivalent remains

relatively constant.

The limited data that exist indicate that a reliance on the HVR to match O_2 supply and O_2 demand appears progressively with postnatal development (Sankaran et al. 1979; Bonora et al. 1984; Bonora and Gautier 1987; Wangsnes and Koos 1991). Most adult homeotherms do still suppress their O_2 consumption rate and body temperature in hypoxia; however, available data indicate that this is more common in small mammals, and they do so to a much lesser extent than newborns (Challet et al. 1997; Mortola and Seifert 2000; Refinetti and Piccione 2005). Interestingly, the homeotherm's developmental transition from decreasing O_2 demand as a newborn, to increasing O_2 supply as an adult appears to coincide with their loss of hypoxia tolerance (Avery and Johlin 1932; Kabat 1940; Glass et al. 1944; Britton and Kline 1945; Adolph 1969; Lister 1993; for a review see Singer 1999 and references within). This suggests that the ability to reduce O_2 demand in hypoxia may explain the basis of the enhanced hypoxia tolerance seen in newborns and adult heterotherms. However, these data are based on only a few studies comparing the thermoregulatory, metabolic, and ventilatory responses of only a handful of species briefly exposed to one arbitrary level of hypoxia.

Chapter 4 of my thesis examines how rodents that range in their degree of heterothermic expression match O_2 supply and O_2 demand during progressive hypoxia as newborns and as adults to elucidate: (1) whether adult homeothermic and heterothermic rodents differ in how they match O_2 supply and O_2 demand in hypoxia; and if so, (2) whether their responses reflect different developmental trajectories. If heterotherms retain physiological traits common to all newborns, I hypothesized that they would not change how they match O_2 supply and O_2 demand in hypoxia during postnatal development. I predicted that in response to progressive hypoxia homeothermic rodents would go from decreasing O_2 demand (i.e. suppressing their rate of O_2 consumption) as newborns, to increasing O_2 supply as adults, and that facultative and obligate heterotherms would be similar as newborns and as adults, responding to hypoxia primarily with a decrease in O_2 demand. Because heterotherms are capable of greater reductions in body temperature and O_2 consumption rate than homeotherms, I predicted that newborn and adult heterotherms would exhibit a blunted HVR and an enhanced HMR compared to adult homeotherms. Coincident with this, I hypothesized that hypoxia tolerance would be associated with a lower O_2 response threshold, and that the level of inspired O_2 at which a significant HVR was mounted, and the relative magnitude of this HVR, would be lowest in newborn mammals <

obligate heterotherms < facultative heterotherms < homeotherms.

1.5 The effects of progressive hypoxia on matching O_2 supply and O_2 demand in rodents in the cold

When mammals are exposed to ambient temperatures below their LCT during the non-hibernating, euthermic season, they increase thermogenesis, O_2 consumption rate, and ventilation, to maintain a constant body temperature (Lim 1960; Ingram and Legge 1970; Gautier et al. 1992; Barros et al. 2001). With extreme cold exposure, however, the gradient between body temperature and ambient temperature becomes large, thermogenesis is not sufficient to maintain body temperature, and eventually hypothermia ensues (Saiki and Mortola 1996; Tattersall and Milsom 2003a).

Newborns drop their body temperature more rapidly than adults in the cold as a result of their poor thermoregulatory capacity, yet their ability to tolerate cold (i.e. low body temperatures) is about equal to adult heterotherms, and significantly greater than that of adult homeotherms (Adolph 1948a; Adolph 1948b; Fairfield 1948; Adolph 1951; Hill 2000; for a review see Fong 2010). Decreasing the body temperature of newborns and adults in hypoxia significantly extends the amount of time they can survive low O_2 availability. Thus, newborns exposed to cold have increased survival rates in hypoxia compared to newborns held at ambient temperatures within their TNZ (Miller and Miller 1966). This is also true of adult homeotherms, who have an increased survival time in 5% O_2, when their body temperature is reduced from 37°C to 35°C, and decreased survival time when body temperature is elevated to 40°C (Artu and Michenfelder 1981). Similarly, adult heterotherms have been found to tolerate anoxia for up to two hours when their body temperatures are low during hibernation (3-10°C), compared to less than 5 minutes when they are euthermic (37°C; Biörck et al. 1956).

Exposure to hypoxia produces two opposing effects on the drive to breathe, reflecting a balance between: 1) an increase in chemosensitive drive, due to the stimulation of the peripheral chemoreceptors; and 2) a reduced metabolic drive, linked to the depression of body temperature and O_2 consumption rate. Available evidence suggests that the relative magnitude of the hypoxia-induced reduction in body temperature is greater at ambient temperatures below the TNZ than within the TNZ (Gautier and Bonora 1992; Barros et al. 2001). Thus, within the TNZ

the reduction of body temperature and O_2 consumption rate of adult homeotherms in hypoxia is minimal, and they must increase ventilation substantially in order to match O_2 supply and O_2 demand (Saiki et al. 1994). In the cold, however, adult homeotherms reduce body temperature and O_2 consumption rate slightly more, and increase ventilation slightly less (Saiki et al. 1994). Adult heterotherms, on the other hand, lower O_2 consumption rate significantly more and increase ventilation less than homeotherms within their TNZ. In the cold adult heterotherms respond with even greater reductions in O_2 consumption rate, and increase ventilation even less than at thermoneutrality. In fact, in the cold, adult heterotherms may have a lower level of ventilation in hypoxia than in normoxia (Barros et al. 2001).

Collectively, these data indicate that in both homeotherms and heterotherms the relative magnitude of the HMR is enhanced, and the HVR is reduced at ambient temperatures below their TNZ compared to those at ambient temperatures within their TNZ. Although the evidence is sparse, newborns appear to respond to hypoxia within their TNZ and in the cold solely by suppressing their O_2 consumption rate (Mortola and Dotta 1992). Simultaneous measurements of O_2 consumption rate and ventilation in the cold are needed, however, to establish how widespread these similarities and differences are between newborns and adults of homeothermic and heterothermic species. This is the goal of Chapter 5 of this thesis, in which I examine the thermoregulatory, metabolic, and ventilatory responses of newborn and adult rodents to progressive decreases in inspired O_2 at an ambient temperature well below the animal's TNZ, or preferred ambient temperature. I hypothesized that exposure to hypoxia and cold would result in a greater HMR and a blunted HVR than exposure to hypoxia within the animal's TNZ, or at their preferred ambient temperature (see Chapter 4). Thus, the O_2 response threshold and sensitivity to hypoxia would be lower in the cold than at thermoneutrality. I predicted that because heterotherms are capable of more extreme reductions in O_2 consumption rate and body temperature than homeotherms, that the combined effects of cold and hypoxia on the HMR and the HVR would be greater in heterotherms (facultative and obligate) than in homeotherms, and owing to their poor thermogenic capacity, greater still in newborns of both groups.

1.6 Thesis approach

The overarching objective of this thesis is to elucidate how newborn and adult rodents

that range in their degree of heterothermic expression match O_2 supply and O_2 demand when ambient temperature and/or environmental O_2 are reduced separately or in combination. Despite the few seminal studies documenting that adult heterotherms and newborns are more tolerant of extreme reductions in body temperature and hypoxia than adult homeotherms (Avery and Johlin 1932; Kabat 1940; Glass et al. 1944; Hiestand et al. 1950; Bullard et al. 1960; Adolph 1969), our current understanding of how the mammalian hypoxic response changes with postnatal development is limited. Unlike adult homeotherms, adult heterotherms and newborn mammals can survive extreme reductions in body temperature and hypoxia for extended periods of time. Therefore, it seems possible that differences in cold and hypoxia tolerance among homeothermic and heterothermic rodents may reflect developmental changes in their thermoregulatory, metabolic, and ventilatory responses to decreases in ambient temperature and O_2. Accordingly, I hypothesized that heterothermic rodents match O_2 supply and O_2 demand through the retention of traits common to all newborn mammals, and that homeothermic rodents lose these traits during development. In the preceding sections I provide background for the four data chapters that follow (Chapters 2-5). Chapter 6 concludes my thesis and synthesizes the results of the four data chapters. Ultimately, this concluding chapter examines the evidence for (or against), the "hibernator as a neonate" hypothesis.

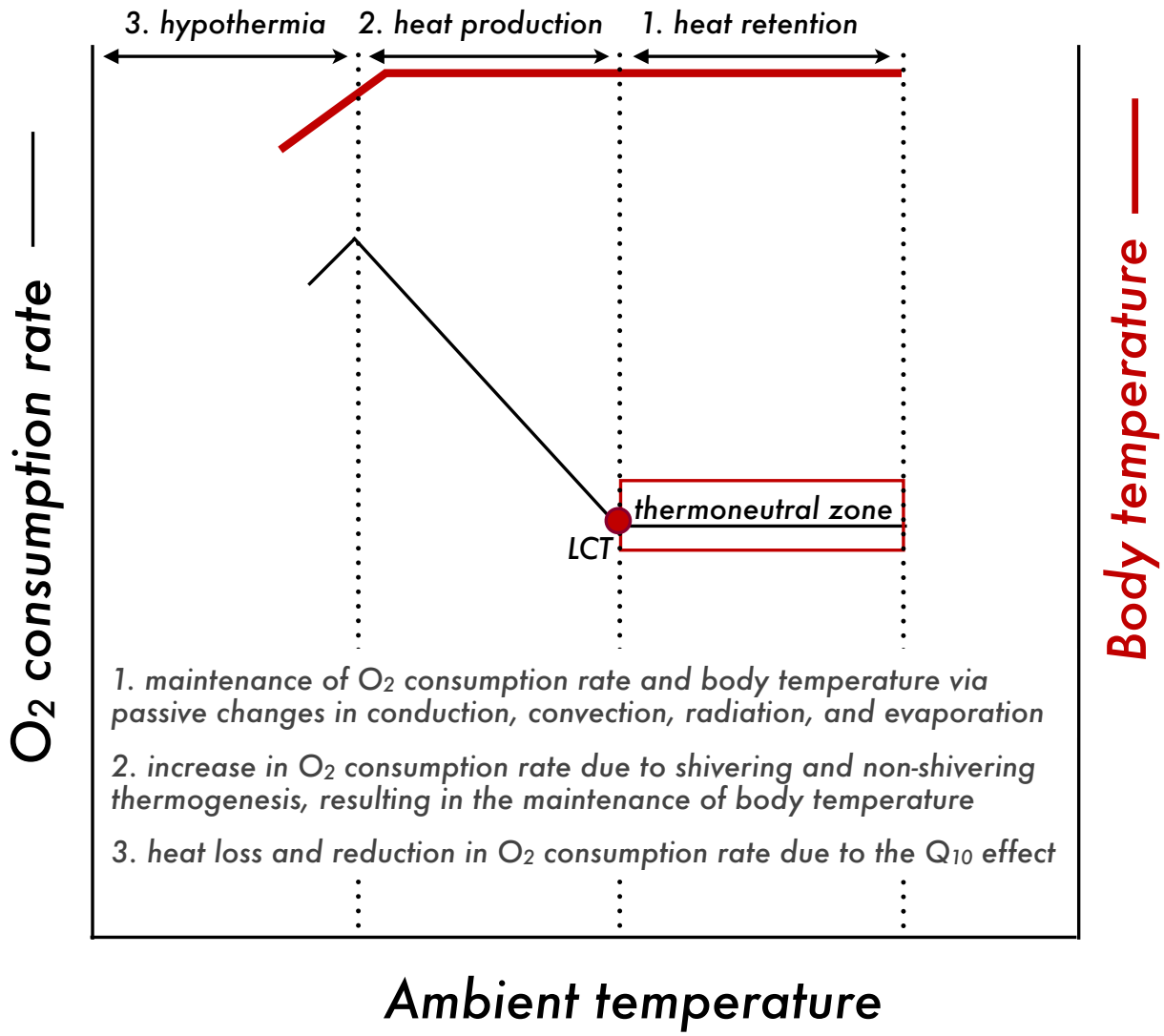

Figure 1.1. Schematic representation of changes in O_2 consumption rate (solid black line) and body temperature (solid red line) as a function of ambient temperature. The thermoneutral zone (TNZ) is indicated by the box outlined in red. The lower critical temperature (LCT) is indicated by the red circle and defines the ambient temperature at which reducing heat dissipation by passive means is no longer sufficient to maintain body temperature. Changes in ambient temperature below the TNZ result in an increase in the rate of O_2 consumption. The slope of this line is proportional to the thermal conductance of the animal. Oxygen consumption rate and body temperature are displayed in arbitrary units. Figure modified from Mortola (2001).

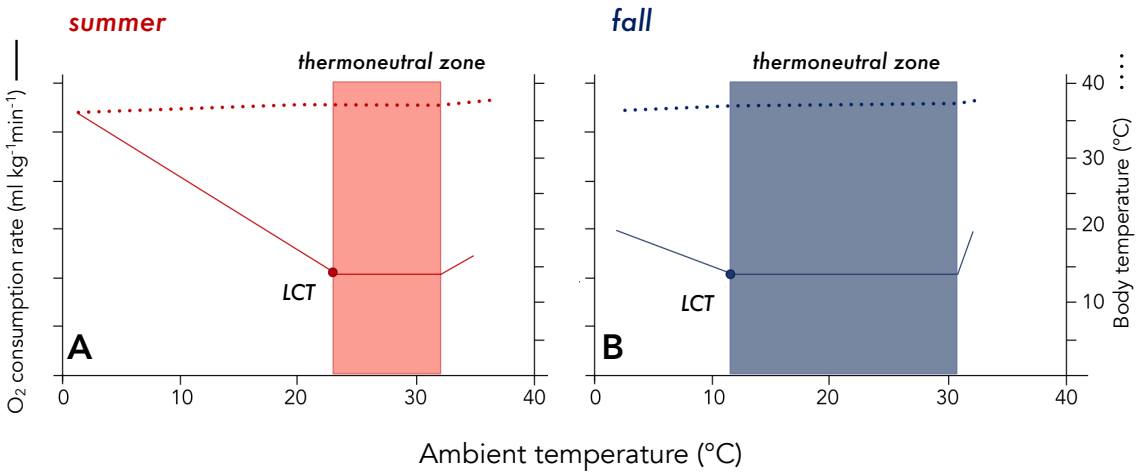

Figure 1.2. Schematic representation of the changes in O_2 consumption rate (solid lines) and body temperature (dashed lines) as a function of ambient temperature between **(A)** summer and **(B)** fall adult rodents. Oxygen consumption rate is displayed in arbitrary units. The thermoneutral zone (TNZ) is indicated by the shaded boxes. The lower critical temperature (LCT) is indicated by the filled circles and defines the ambient temperature at which reducing heat dissipation by passive means is no longer sufficient to maintain body temperature. Changes in ambient temperature below the TNZ result in an increase in the rate of O_2 consumption. The slope of this line is proportional to the thermal conductance of the animal. **(A)** In the summer, rodents are less insulated and have a higher thermal conductance, rightward shift in their TNZ (and thus higher LCT), and a larger increase in their O_2 consumption rate at lower ambient temperatures than rodents in the fall, which are insulated by thicker fur and more subcutaneous fat in preparation for the winter **(B).**

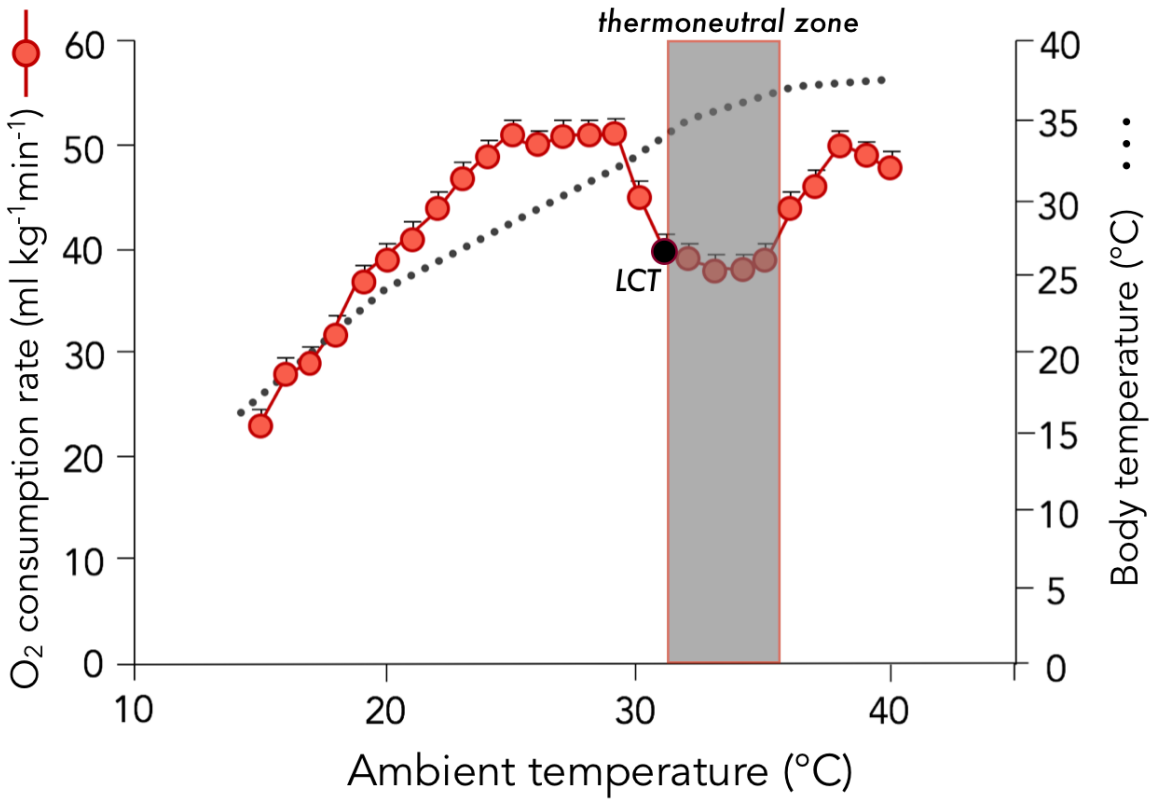

Figure 1.3. Oxygen consumption rate (red circles) and body temperature (dashed line) at different ambient temperatures in newborn rats. The thermoneutral zone (TNZ) is indicated by the shaded grey box. The lower critical temperature (LCT) is indicated by the black circle and defines the ambient temperature at which reducing heat dissipation by passive means is no longer sufficient to maintain body temperature. Changes in ambient temperature below the TNZ result in an increase in the rate of O_2 consumption. The slope of this line is proportional to the thermal conductance of the animal. The drop in body temperature with the decrease in ambient temperature occurs despite the newborn's thermogenic response to cold. Because of their poor thermogenic capacity, at lower ambient temperatures their O_2 consumption rate also drops. Figure modified from Mortola (2004).

Chapter 2: The effects of progressive cooling on matching O_2 supply and O_2 demand in adult rodents in normoxia and hypoxia

2.1 Introduction

When ambient temperatures fall, adult mammals elicit a thermogenic response, significantly increasing their O_2 consumption rate and ventilation to meet the associated increase in O_2 requirement (Hill 1959; Dupré et al. 1988; Saiki and Mortola 1996; Barros et al. 2001). However, when living in an environment where atmospheric O_2 is low, supplying enough O_2 to sustain thermogenesis can be challenging (Rosenmann and Morrison 1975; Hayes 1989; Lui et al. 2015). While most adult mammals increase ventilation under cold and hypoxic conditions in an attempt to maintain O_2 supply and support thermogenesis (Kottke et al. 1948; Kuhnen et al. 1987; Dupré et al. 1988; Maskrey 1990; Barros et al. 2001), some adult mammals exhibit a hypoxia-induced reduction in O_2 consumption rate and body temperature (the HMR), and inhibit thermogenesis (Frappell et al. 1992; Barros et al. 2001; Tattersall and Milsom 2009). The relative magnitude of the HMR is greater in smaller mammals than in larger mammals, and is also greater in the cold than within the TNZ in all species studied to date (Saiki et al. 1994; Barros et al. 2001). This inhibition of thermogenesis does not result from an inability to thermoregulate, but rather represents a defensive response in which adult mammals adjust and regulate their body temperature at a new and lower level (i.e. lower their thermoregulatory set-point), reducing the need to increase O_2 supply when O_2 availability is limited (Barros et al. 2001; Tattersall and Milsom 2009).

The relative magnitude of the HMR also appears to vary with the degree of heterothermic expression, with adult heterotherms exhibiting a greater reduction in body temperature and O_2 consumption rate in hypoxia than adult homeotherms (Frappell et al. 1992). Thus, it is conceivable that the heterotherm's inherent ability to reduce body temperature and metabolic demand for O_2 during energetic shortfalls may explain the enhanced cold and hypoxia tolerance observed in this group. However, the extent to which environmental hypoxia affects the metabolic, thermoregulatory, and ventilatory responses of rodents to progressive cooling has only been addressed in the heterothermic golden-mantled ground squirrel (*Spermophilus lateralis*), at one level of hypoxia (7% O_2) (Barros et al. 2001). It remains unknown whether

adult homeothermic and heterothermic rodents exposed to environmental hypoxia and then progressively cooled: 1) conform to their external environment by reducing O_2 demand through a reduction in their thermoregulatory set-point, O_2 consumption rate, and body temperature; 2) maintain their thermoregulatory set-point and body temperature by increasing thermogenesis and increasing O_2 supply; or 3) some combination of these strategies.

The aims of this study were to determine whether adult rodents that vary in their degree heterothermic expression differ in how they match O_2 supply and O_2 demand when exposed to: (i) falling ambient temperature in normoxia; (ii) hypoxia at an ambient temperature within their TNZ (their LCT); and (iii) hypoxia at ambient temperatures below their TNZ. I hypothesized that enhanced cold and hypoxia tolerance of adult heterotherms stems from their remarkable ability to reduce their thermoregulatory set-point, O_2 consumption rate, and body temperature in the cold and hypoxia compared to adult homeotherms. To test this hypothesis I investigated the metabolic, thermoregulatory, and ventilatory responses of adult rats (*Rattus norvegicus*), mice (*Mus musculus*), hamsters (*Mesocricetus auratus*), and ground squirrels (*Ictidomys tridecemlineatus*) during the non-hibernating season, when the rodents were euthermic and active. I selected these species as they are all small, altricial mammals, belonging to the order Rodentia, and range in their degree of heterothermic expression (Table 2.1). The rat is a true homeotherm. It is not known to use torpor, and maintains a high body temperature and O_2 consumption rate independent of season and environmental conditions (Yoda et al. 2000). Mice and hamsters are facultative, non-seasonal heterotherms, they use torpor any time of the year when confronted with energetic stressors such as low ambient temperature (Ellison and Skinner 1992; Heldmaier et al. 1999), or limitations in food or energy reserves (Lyman 1948; Hudson and Scott 1979). The ground squirrel is an obligate heterotherm, and generally uses torpor seasonally, only during the winter (Wang and Lee 2011). I predicted that: (i) in normoxia, all species would behave in a similar fashion given the fact that I conducted all experiments during the active, euthermic season of the heterotherms; (ii) within their TNZ, hypoxia would cause a modest reduction in O_2 consumption rate and body temperature of all species accompanied by an HVR; and (iii) hypoxia would cause a reduction in the ability of all species to mount a thermogenic response to progressive cooling, as indicated by a reduction in the LCT, a widening of the TNZ, a reduction in thermal conductance, and a drop in body temperature with ambient temperature. Because facultative and obligate heterotherms are capable of reductions in O_2

consumption rate and body temperature when faced with energetic shortfalls (Ruf and Geiser 1995; Geiser 2004), I predicted that they would reduce their O_2 consumption rate and body temperature in hypoxia more than homeotherms. Furthermore, obligate heterotherms are capable of even greater reductions in O_2 consumption rate and body temperature than facultative heterotherms (Ruf and Geiser 1995; Geiser 2004), albeit only during the non-active, hibernating season. However, thermoregulatory mechanisms that decrease O_2 demand in hypoxia in obligate heterotherms, appear to be fully functional even during the active euthermic season (Barros et al. 2001; Tattersall and Milsom 2003b; Tattersall and Milsom 2009). Thus, I predicted that in hypoxia the increase in O_2 consumption rate below the LCT would be reduced most in the obligate heterotherm, and least in the homeotherm, with facultative heterotherms falling somewhere in between.

2.2 Methods

2.2.1 Experimental animals and housing

Rats, mice, and hamsters were purchased from Charles River Laboratories (Portage, MI, USA), while ground squirrels were live-trapped in Carman, MB, Canada (49°30′ N, 98°01′ W) and transferred to an animal care holding facility at the University of British Columbia (UBC) in Vancouver, BC, Canada. Ground squirrels were trapped with the approval of Manitoba Conservation and Water Stewardship, under the wildlife scientific permit WB15027. Animals were housed in an environmental chamber, held under a 07:00 to 19:00 photoperiod cycle, at an ambient temperature of 21 to 23°C, and 45-55% humidity. All animals were provided with food and water *ad libitum*. Rats, mice, and hamsters were fed Purina Rodent Chow (no. 5001, Purina LabDiet, St. Louis, MO), while ground squirrels were given 1:1 IAMS dog food (Mars Incorporated, Mount Olive, NJ) and Purina Rodent Chow supplemented with sunflower seeds, fruit, and vegetables. Animals were not fasted prior to experiments. Each species was split into two groups: a normoxic group (21% O_2), and a hypoxic group (7% O_2) (Table 2.1). All experimental procedures in this study conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the UBC Committee on Animal Care (under protocol A13-0091).

2.2.2 Experimental protocol

On the day of the experiment an adult was instrumented with a thermal probe and given ~2 hours to to fully recover from anaesthesia before being placed within an animal chamber inside a temperature-controlled environmental chamber set to 24°C. The experimental apparatus consisted of two identical 200 to 1000 ml Plexiglass chambers depending on the size of the animal, one being the designated animal chamber, and the other the designated reference chamber. Ambient temperature was continuously recorded throughout the experiment using a temperature datalogger (iButton Maxim Integrated, Chandler, CA, USA) placed inside the animal chamber. Animals were given 30 minutes to acclimate to the experimental apparatus before beginning Series I (normoxia) or Series III (hypoxia) trials. A total of 66 adults across the 4 species were used in these experiments (35 normoxia (Series I and II), and 31 hypoxia (Series II and III); Table 2.1).

2.2.2.1 Series I- Responses to progressive cooling in normoxia

To determine how adult rodents match O_2 supply and O_2 demand during progressive cooling in normoxia (Series I), following acclimation and baseline recordings of O_2 consumption rate, body temperature, and ventilation at 24°C, ambient temperature was gradually increased up to 38°C, and then reduced down to 7°C at an average rate of 0.15 ± 0.01°C min^{-1}. This temperature range was chosen to simulate situations that rodents are exposed to naturally in the wild, but more importantly to define the TNZ of each species. All variables were continuously recorded as ambient temperature was manipulated.

2.2.2.2 Series II- Effects of hypoxia at each species' LCT (within the TNZ)

To determine how adult rodents match O_2 supply and O_2 demand in normoxia (Series I) and hypoxia (Series III) within their TNZ, body temperature, O_2 consumption rate, and ventilation were compared under each condition (normoxia and hypoxia) at each species' normoxic LCT (rat- 22°C; mouse- 26°C; hamster- 29°C; and ground squirrel- 30°C). These data were calculated once all data for Series I and III for each species were collected. The LCT

represents the threshold ambient temperature for thermogenesis, and for each species was defined as the ambient temperature at which O_2 consumption rate increased as ambient temperature was reduced, and was determined from the species' mean. Because the range of ambient temperatures that encompass the TNZ varies between species, the LCT was selected as a standardized temperature at which to make species comparisons of the effects of hypoxia within the TNZ.

2.2.2.3 Series III- Responses to progressive cooling in hypoxia

To determine how adult rodents match O_2 supply and O_2 demand during progressive cooling in hypoxia (Series III), following acclimation in normoxic air (30 minutes) at 24°C, each animal was exposed to hypoxic gas (7% O_2) for 3 hours to ensure O_2 consumption rate, body temperature, and ventilation reached steady state. I selected this level of inspired O_2 as it is: (1) low enough to desaturate the arterial blood of all species investigated in this study (Schmidt-Nielsen and Larimer 1958; Maginniss and Milsom 1994); (2) within the range of environmental O_2 levels that some of these species would face in the wild (Studier and Proctor 1971; Maclean 1981; Kuhnen 1986); and (3) identical to levels used in comparable studies (Barros et al. 2001; Tattersall and Milsom 2009). Because the same level of inspired O_2 results in different degrees of blood and tissue hypoxia (hypoxemia) among species, the results of the present study should be examined in the context of the experimental conditions in which they have been obtained. Different levels and durations of environmental hypoxia may modify species relationships reported here.

An identical protocol was followed as that in Series I. Once hypoxic steady-state values at 24°C were recorded for 30 minutes, ambient temperature was gradually increased up to 35°C, and then reduced down to 5°C, at an average rate of 0.12 ± 0.01°C min^{-1}. All variables were continuously recorded as ambient temperature was manipulated. Normoxic (Series I) and hypoxic (Series III) experiments were performed on different animals. Each experimental trial lasted ~ 6.5 hours (326 ± 31 minutes for normoxia; 482 ± 24 minutes for hypoxia).

2.2.3 Body temperature measurements

Prior to being placed into the animal chamber, each animal was instrumented with a sterile, 0.64 mm diameter copper-constantan thermal probe (Physitemp IT-18, Clifton, NJ, USA). The thermal probe was calibrated with a National Bureau of Standards certified thermometer, and was used to measure the body temperature of animals in normoxia and hypoxia with changes in ambient temperature. Each animal was anaesthetized by gaseous inhalation of isoflurane (1-5% isoflurane in a 21% O_2 mixture; flow rate = 1-5 L min^{-1}). Once the animal was anaesthetized, the fur over the abdomen and between the shoulder blades was cropped, treated with a depilatory cream, and the skin cleaned with distilled water, and disinfected with an antiseptic surgical scrub (Betadine®, Purdue Pharma LP, Stamford, CT, USA), followed by 95% ethanol. Two small subcutaneous incisions (< 0.5 cm) were made, one in the cleaned abdominal area, and another in between the animal's shoulder blades. A 16.5 G needle was inserted into the peritoneal cavity through the incision site in the cleaned abdominal area of the animal. The thermal probe was fed through the 16.5 G needle, and once ~1 cm of the thermal probe was within the peritoneal cavity, the 16.5 G needle was removed and the thermal probe was sutured to the skin using prolene sutures. The thermal probe was fed through to the shoulder blades via a 30 cm long trocar needle. The trocar needle ran subcutaneously from the incision site in the abdominal area, through the back of the animal, and exited at the cleaned incision site between the shoulder blades. Once the thermal probe was fed through to the shoulder blades, the trocar needle was removed, and both incision sites were sutured shut. Placement of the thermal probe in this location minimized the likelihood of the animal tugging, chewing, or removing the thermal probe during the experiment. The procedure took less than 20 minutes to complete. Surgical analgesic (Metacam (Boehringer Ingelheim Vetmedica Inc., Duluth, Georgia, USA: 1-5 mg kg^{-1}) was administered subcutaneously, pre-operatively. All animals were fully recovered from anesthesia (~2 hours) before initiating experiments.

2.2.4 O_2 consumption rate measurements

Oxygen consumption rate was determined using open-flow respirometry. Animals were placed in individual chambers, with incurrent airflow pushed through the animal chamber at a flow rate of 150 to 1000 ml min^{-1} using calibrated flowmeters (PRSFM4302-1; Praxair Technology Inc., Danbury, CT, USA). The incurrent airflow was adjusted for each individual to

ensure that neither the O_2 concentration, nor CO_2 concentration of the air leaving the animal chamber were altered more than 1% by the animal's metabolism. Hypoxia was achieved by mixing compressed dry air and nitrogen in appropriate ratios using the calibrated flowmeters. The incurrent and excurrent airflow, as well as the composition of the gas mixture were continuously monitored using a Field Metabolic System (Sable Systems, Las Vegas, NV, USA) with a built in flowmeter, and O_2 and CO_2 gas analyzers. The gas analyzers were calibrated for O_2 and CO_2 before and after each experiment with dry commercial air (Praxair Technology Inc., Danbury, CT, USA), and a premixed gas mixture (1.55% CO_2 balanced with N_2; Praxair Technology Inc., Danbury, CT, USA), respectively. A subsample of the excurrent gas was passed through a desiccant media (DM-060-24; Perma Pure LLC, Toms River NJ, USA) before entering the cells of the CO_2 and O_2 analyzers. Incurrent gas concentrations and gas flow were checked every 15 minutes throughout the experiment. Oxygen consumption rate was calculated using equation 10.6 in Lighton (2008):

$$\dot{V}O_2 = \dot{V}i\,[(FiO_2 - FeO_2) - FeO_2\,(FeCO_2 - FiCO_2)]/(1 - FeO_2) \qquad (1)$$

Where $\dot{V}O_2$ is O_2 consumption rate (ml min^{-1}), $\dot{V}i$ is incurrent flow rate (ml min^{-1}), FiO_2 and $FiCO_2$ are fractional concentrations of incurrent O_2 and CO_2 of dry gas, respectively, and FeO_2 and $FeCO_2$ are fractional concentrations of excurrent O_2 and CO_2 of dry gas, respectively. Barometric pressure and ambient temperature were used to convert O_2 consumption rate measurements to standard temperature and pressure, dry (STPD).

2.2.5 Ventilatory measurements

Ventilatory measurements were acquired using barometric whole-body plethysmography, in an open-flow system (e.g. Chapin 1954; Drorbaugh and Fenn 1955; Jacky 1978; Jacky 1980; Mortola and Frappell 2013). Ventilation-induced pressure oscillations were detected with a differential pressure transducer (DP103-18; Validyne, Northridge, CA, USA) connected between the animal and reference chamber. As the animal breathed within its chamber it resulted in pressure oscillations induced by the warming and humidification of inspired air, these pressure oscillations were compared to the pressure of the reference chamber. To determine tidal volume,

the system was calibrated with the animal present in the chamber, as described by McArthur and Milsom (1991). To determine tidal volume from pressure oscillations, the system was calibrated prior to each experiment by injecting and withdrawing known volumes of air (0.2 to 2.0 ml) into the animal chamber at a rate similar to the animal's breathing frequency to produce pressure oscillations at least 10 times as great as that of the animal's breathing. Tidal volume was calculated by integrating expiratory ventilatory flow, and using the equation of Drorbaugh and Fenn (1955) modified for flow-through plethysmography by Jacky (1978). In both newborn and adult rodents, breathing frequency was calculated directly from the ventilation-induced pressure oscillations, while ventilation was calculated from the product of tidal volume and breathing frequency. All ventilatory measurements are reported at body temperature and pressure, saturated (BTPS).

2.2.6 Calculation of ventilatory equivalent and lung O_2 extraction efficiency

The ventilatory equivalent (the quotient of ventilation and O_2 consumption rate), and lung O_2 extraction efficiency were calculated from O_2 consumption rate and ventilatory measurements. The percent of O_2 extracted from each breath was calculated by dividing O_2 consumption by ventilation multiplied by the fractional concentration of O_2 in inspired air (O_2 delivery to the lungs), and multiplying that by 100.

2.2.7 Data collection and analysis

All signals (body temperature, incurrent and excurrent O_2 concentration, and the ventilation-induced pressure signal) were amplified, filtered, and recorded to a PowerLab 8/35 data acquisition system (ADInstruments Pty Ltd., Colorado Springs, CO, USA). From the recorded signals and calculated dependent variables I determined average: O_2 consumption rate, body temperature, breathing frequency, tidal volume, ventilation, the ventilatory equivalent, and lung O_2 extraction efficiency for the entire period the animal was exposed to each ambient temperature.

Statistical analyses were performed using R (R Core Team 2017, Vienna, AT, EUR). For Series I and Series III results, I used linear mixed effects models (lme4 package in R; Bates et al.

2015) to account for any effects that body mass and sex may have had on the dependent variables, and to account for repeated sampling of the same individual with changes in ambient temperature, with individual treated as a random effect. For Series II, I ran a general linear model to account for any effects that body mass and sex may have had on the dependent variables. When visual inspection of residuals, and q-q plots revealed deviations from the assumptions of general linear and linear mixed effects models (normality, homogeneity of variances, linearity, and independence), I log transformed the dependent variable. For each series, ambient temperature, level of inspired O_2, species, body mass, and sex were fixed effects in my initial models. I tested all 2- and 3-way interactions of ambient temperature, level of inspired O_2, and species. When body mass or sex did not have a significant effect on the dependent variables, they were removed from the model. I did not remove any other terms from my models given the importance of all independent variables and interactions to my research objectives. I used lmerTest in R to obtain F-statistics and *P*-values for each model (Kuznetsova 2016). Following the methods of Fangue et al. (2009), when interaction terms were significant the data were separated and analyzed independently using a one-way ANOVA, followed by a Tukey-Holm post-hoc analysis to determine differences among ambient temperatures, O_2 level, and species, and to correct for multiple pairwise comparisons. All results are presented as mean ± s.e.m., with statistical significance set as $P<0.05$.

2.3 Results

2.3.1 Series I- Responses to progressive cooling in normoxia

2.3.1.1 All adult rodents exhibit a significant increase in O_2 consumption rate below their TNZ in normoxia, accompanied by a slight decrease in body temperature

The LCT for each species in normoxia (defined as the ambient temperature at which the relationship between O_2 consumption rate and ambient temperature began to rise) was: rat= 22°C; mouse= 26°C; hamster= 29°C; and ground squirrel= 30°C. These increases became significant at 15°C in the rat, 17°C in the mouse, 22°C in the hamster, and 19°C in the ground squirrel ($F_{1,34}=122.3$, $P<0.0001$; Fig. 2.1A). Despite the increase in O_2 consumption rate, body

temperature fell slightly in all species (Fig. 2.1B). However, the manner in which body temperature fell varied among species, as exhibited by a significant interaction between ambient temperature and species ($F_{3,26}$=4.9, P<0.01; Fig. 2.1B). The reduction in body temperature with progressive cooling did not reach significance in the rat, and only became significant at an ambient temperature of 14°C in the mouse, 22°C in the hamster, and 21°C in the ground squirrel (Fig. 2.1B).

2.3.1.2 Adult rodents increase ventilation below their TNZ by increasing both breathing frequency and tidal volume

As ambient temperature was reduced below the TNZ in normoxia, ventilation increased ($F_{1,65}$=5.5, P<0.05; Fig. 2.2A), and did not vary significantly among species (P=0.55; Fig. 2.2A). The cold-induced increase in ventilation was due to an increase in both breathing frequency ($F_{1,37}$=16.5, P<0.001; Fig. 2.2B), and tidal volume ($F_{1,34}$=19.6, P<0.0001; Fig. 2.2C) in all rodents.

2.3.1.3 Adult rodents are similar in how they match O_2 supply and O_2 demand below their TNZ

While there was a significant interaction between ambient temperature and species on the ventilatory equivalent of rodents in normoxia ($F_{3,31}$=4.2, P<0.05; Fig. 2.3A), in all species increases in ventilation mirrored increases in O_2 consumption rate with progressive cooling, and thus each species maintained a constant ventilatory equivalent (Fig. 2.3A), and lung O_2 extraction efficiency (P= 0.30; Fig. 2.3B).

2.3.2 Series II- Effects of hypoxia at each species' LCT (within the TNZ)

2.3.2.1 Adult rodents vary in their metabolic and thermoregulatory responses when exposed to hypoxia within their TNZ

Rodents varied in their metabolic responses to hypoxia within their TNZ, as exhibited by a significant interaction between O_2 level and species ($F_{3,46}$=11.8, P<0.0001; Fig. 2.4A). When

exposed to 7% O_2 within their TNZ, mice reduced their O_2 consumption rate by 60% (P<0.001; Fig. 2.4A). All other species maintained their O_2 consumption rate in hypoxia within their TNZ, (Fig. 2.4A).

Although body temperature dropped in hypoxia in all species ($F_{1,38}$=10.2, P<0.01), mice (P<0.01) and hamsters (P<0.01) were the only species in which the drop was significant (Fig. 2.4B). The relative magnitude of the hypoxic decrease in body temperature was lowest in ground squirrels, which decreased their body temperature by less than 1%, and greatest in mice, which decreased their body temperature by 10%, from normoxic values (Fig. 2.4B). There was no significant interaction between O_2 level and species on the thermoregulatory response of rodents within their TNZ (P=0.13; Fig. 2.4B).

2.3.2.2 Adult rodents increase ventilation when exposed to hypoxia within their TNZ

All rodents increased ventilation when exposed to 7% O_2 within their TNZ ($F_{1,48}$=5.5, P<0.05; Fig. 2.4C). Although there was no significant interaction between O_2 level and species (P= 0.10; Fig. 2.4C), the relative magnitude of the increase in ventilation in hypoxia was least in rats and ground squirrels (30%), modest in the mouse (50%), and greatest in the hamster (130%) (Fig. 2.4C). In all species, the HVR was primarily due to an increase in breathing frequency ($F_{1,49}$=26.8, P<0.0001; Fig. 2.4D), as there was no significant effect of O_2 level on tidal volume (P= 0.42; Fig. 2.4E).

2.3.2.3 Adult rodents differ in how they match O_2 supply and O_2 demand in hypoxia within their TNZ

All rodents increased ventilation relative to their O_2 consumption rate when exposed to 7% O_2 within their TNZ, with a significant interaction between O_2 level and species ($F_{3,45}$=27.1, P<0.0001; Fig. 2.4F). Mice exhibited the greatest increase in ventilation relative to O_2 consumption rate, which resulted in an increase in their ventilatory equivalent of 280% from normoxic levels (P<0.0001; Fig. 2.4F). Whereas, all other species exhibited a modest increase in their ventilatory equivalent of 50-125%, this increase was not statistically significant for any species other than the mice (Fig. 2.4F). When exposed to hypoxia within their TNZ, all species

but the mice increased lung O_2 extraction efficiency, ($F_{1,45}$=9.0, P<0.05; Fig. 2.4G). To sustain O_2 demand under hypoxic conditions, rats (P<0.05), hamsters (P=0.11), and ground squirrels (P=0.12) increased their lung O_2 extraction efficiency by 25-90%, compared to normoxic levels, however, this hypoxia-induced increase in lung O_2 extraction efficiency was not significant in hamsters, nor ground squirrels.

2.3.3 Series III- Effects of hypoxia on the response to progressive cooling

2.3.3.1 Adult rodents exhibit significant reductions in their O_2 consumption rate and body temperature when challenged with progressive cooling in hypoxia

There was no significant 3-way interaction between ambient temperature, O_2 level, and species on O_2 consumption rate (P=0.62; Fig. 2.1A). However, there were differences in the effects of progressive cooling in hypoxia on O_2 consumption rate among species, as reflected by a significant interaction between ambient temperature and species ($F_{3,62}$=3.0, P<0.05; Fig. 2.1A). While all species increased O_2 consumption rate by 75-175% when ambient temperature was reduced from their TNZ to 12°C in normoxia (Fig. 2.1A), in hypoxia O_2 consumption rate increased by only 20-75% when ambient temperature was reduced over this same range in all species but the rat (Fig. 2.1A). The increase in O_2 consumption rate during progressive cooling only reached significance in the ground squirrel (Fig. 2.1A). Conversely, when the rat was exposed to hypoxia, O_2 consumption rate decreased with ambient temperature (Fig. 2.1A). All species had lower levels of O_2 consumption rate in hypoxia than in normoxia, as reflected by a significant 2-way interaction between ambient temperature and O_2 level ($F_{1, 62}$=62.9, P<0.0001; Fig. 2.1A).

As in normoxia, the body temperature of all rodents decreased as ambient temperature fell below the normoxic TNZ in hypoxia, and there was a significant interaction between ambient temperature, O_2 level, and species ($F_{3,52}$=9.8, P<0.0001; Fig. 2.1B). Whereas mice (P<0.0001) and hamsters (P<0.0001) had lower body temperatures in hypoxia than in normoxia at ambient temperatures below the TNZ, rats (P=0.12) and ground squirrels (P=0.35) did not (Fig. 2.1B).

2.3.3.2 All adult rodents exhibit a blunted HVR at ambient temperatures below their TNZ

Ventilation was significantly affected by interactions between ambient temperature, O_2 level, and species ($F_{3,58}$ =4.5, P<0.01; Fig. 2.2A). As ambient temperatures fell below their TNZ in normoxia, all rodents increased ventilation (Fig. 2.2A). However, as ambient temperature fell in hypoxia, all species, except for ground squirrels decreased ventilation (Fig. 2.2A). In rats and mice, the reduction in ventilation was due to a decrease in breathing frequency, as tidal volume remained constant as ambient temperature fell below the TNZ (Fig. 2.2B; Fig. 2.2C). Hamsters, on the other hand, increased breathing frequency but decreased tidal volume (Fig. 2.2B&C). Unlike the other species, ground squirrels, maintained ventilation in hypoxia as ambient temperature fell below the TNZ (Fig. 2.2A). These results are reflected by a significant interaction between ambient temperature, O_2 level, and species on breathing frequency ($F_{3,63}$ =12.1, P<0.0001; Fig. 2.2B), and a significant interaction between ambient temperature and O_2 level ($F_{1,63}$ =19.0, P<0.0001), as well as O_2 level and species ($F_{3,60}$ =3.2, P<0.05) on tidal volume (Fig. 2.2C).

All species exhibited an HVR, increasing ventilation above normoxic levels within their TNZ (Fig. 2.2A). However, as ambient temperature fell below 20°C, ventilation fell below normoxic values (i.e. the HVR was abolished; Fig. 2.2A). This was primarily due to a drop in breathing frequency in all species, except for hamsters, in which it was due to a decrease in tidal volume (Fig. 2.2B&C).

2.3.3.3 Adult homeothermic and heterothermic rodents differ in how they match O_2 supply and O_2 demand when challenged with hypoxia below their TNZ

The ventilatory equivalent of rodents was significantly affected by the interaction between ambient temperature, O_2 level, and species ($F_{3,58}$ =4.5, *P*<0.01; Fig. 2.3A). Unlike in normoxia, where all species generally matched changes in ventilation to changes in O_2 consumption rate as ambient temperature fell below the TNZ, in hypoxia, all species but the rat decreased their ventilatory equivalent (Fig. 2.3A). Unlike the other species, rats increased their ventilatory equivalent as ambient temperature was reduced below the TNZ in hypoxia (Fig. 2.3A). However, due to increases in ventilation and/or decreases in O_2 consumption rate in

hypoxia, all species exhibited a significantly higher ventilatory equivalent when challenged with hypoxia during progressive cooling than in normoxia (Fig. 2.3A). In rats, as ambient temperature fell below the TNZ, the relative difference between the normoxic and hypoxic ventilatory equivalent progressively increased (Fig. 2.3A) whereas in the other species, the relative difference between normoxic and hypoxic ventilatory equivalent progressively decreased with ambient temperature (Fig. 2.3A).

Lung O_2 extraction efficiency was also significantly affected by the interaction between ambient temperature, O_2 level, and species ($F_{3,54}$ =2.8, P<0.05; Fig. 2.3B). In normoxia, all species maintained a constant level of lung O_2 extraction as ambient temperature fell below the TNZ (Fig. 2.3A; Fig. 2.3B). In hypoxia, however, the decrease in the ventilatory equivalent of the mouse, hamster, and ground squirrel with progressive cooling, was accompanied by an increase in the percent of O_2 extracted at the lung (Fig. 2.3B). In the rat, lung O_2 extraction efficiency decreased with progressive cooling during hypoxia, but never to a significant extent (Fig. 2.3B). Generally, hypoxic individuals, had a higher lung O_2 extraction efficiency than normoxic individuals, however, the hypoxia-induced increase in lung O_2 extraction efficiency was only significant in the rats (P<0.05) and ground squirrels (P<0.001; Fig. 2.3B).

2.4 Discussion

The major question addressed in this study was whether adult heterotherms take advantage of their inherent ability to suppress metabolism, and exhibit greater reductions in their O_2 consumption rate and body temperature when challenged with cold and hypoxia than do adult homeotherms. In contrast to my predictions, during progressive cooling in hypoxia the homeothermic rat exhibited the largest reduction in O_2 consumption rate, associated with reductions in body temperature and ventilation, compared to their responses to progressive cooling in normoxia. The fall in O_2 consumption rate exceeded the fall in ventilation and so the ventilatory equivalent increased and the percentage of O_2 extracted from each breath fell. Heterotherms, when exposed to progressive cooling in hypoxia, also reduced their O_2 consumption rate relative to normoxic values but to a lesser extent than the homeothermic rat. Thus their O_2 consumption rates still increased during progressive cooling in hypoxia and were associated with a decrease or relatively constant level of ventilation, a decrease in the ventilatory

equivalent, and an increase in lung O_2 extraction efficiency compared to their normoxic response to progressive cooling. Thus, I conclude that during progressive cooling in hypoxia, heterotherms do not suppress O_2 consumption rate to a greater extent than do homeotherms, at least not during the euthermic season. Opposite to what I predicted, the ability to mount a thermogenic response in hypoxia was reduced most in the homeotherm, and least in the obligate heterotherm, with facultative heterotherms falling somewhere in between.

2.4.1 Series I- Responses to progressive cooling in normoxia

Maintaining a high body temperature in the cold is energetically expensive, especially for small mammals. As I predicted, all rodents in my study exhibited a significant increase in O_2 consumption rate as ambient temperature fell below the TNZ, with all species mounting a similar thermogenic response (similar slopes of the relationship between ambient temperature and O_2 consumption rate). All rodents in my study matched the increase in O_2 consumption rate as ambient temperature fell below their TNZ with a progressive increase in ventilation, a response typical of most mammals (for instance: weasels (Casey et al. 1979), deer mice (Chappell 1985), and ground squirrels (Barros et al. 2001). In all species, this was due to an increase in both breathing frequency and tidal volume. My findings align with those from rats (Gautier and Bonora 1992), chipmunks (Chappell 1992), and ground squirrels (Chappell 1992). An increase in breathing frequency at ambient temperatures below the TNZ may only be a characteristic of small mammals (less than 1 kg), as most larger mammals maintain a relatively constant breathing frequency and instead increase their tidal volume in hypoxia (Taylor and Sale 1969; Hulbert and Dawson 1974; Casey et al. 1979; Larcombe 2002). At ambient temperatures below the TNZ, the ventilatory equivalent and lung O_2 extraction efficiency were maintained in all species I investigated, consistent with the results of previous studies (Casey et al. 1979; Chappell 1985; Barros et al. 2001).

Despite these increases in O_2 consumption rate, only the rat maintained a constant body temperature. Whether the reductions in body temperature exhibited by heterothermic rodents when they were exposed to progressive cooling in normoxia reflect poor thermoregulatory ability and insufficient endogenous heat production, or a controlled, temperature dependent lowering of the thermoregulatory set-point, cannot be determined from my data. However, a reduction in

body temperature would curtail the energetic costs of thermogenesis, and is a beneficial adaptation to cold shared by both facultative and obligate heterotherms. Consistent with a temperature-induced reduction in thermoregulatory set-point, increases in ventilation and O_2 consumption rate during progressive cooling were matched, and lung O_2 extraction efficiency did not change in the heterothermic species despite the fall in body temperature.

2.4.2 Series II- Effects of hypoxia at each species' LCT (within the TNZ)

Smaller mammals have a higher resting mass-specific O_2 consumption rate, and tend to exhibit larger drops in their O_2 consumption rate in hypoxia than larger mammals (Hill 1959; Frappell et al. 1992). In my study, the mouse was smaller than all other species investigated (Table 2.1). Accordingly, it had the highest resting mass-specific O_2 consumption rate, and was the only species to decrease its O_2 consumption rate significantly (60%) in hypoxia within their TNZ. Since smaller mammals have higher thermogenic requirements than larger mammals (Kleiber 1975), an inhibition of thermogenesis in hypoxia would have a greater effect in reducing the O_2 consumption rates of smaller species than larger species. However, the hypoxia-induced decreases in O_2 consumption rates were not due to passive temperature effects procured by the reduction of body temperature in hypoxia, as in hypoxia the reductions in body temperature within the TNZ were minimal.

When exposed to hypoxia at thermoneutrality, most mammals increase ventilation, and do so by increasing breathing frequency and not tidal volume (Haldane et al. 1919; Frappell et al. 1992; Gautier and Bonora 1992; Barros et al. 2001). The relative magnitude of this HVR varies significantly among species (Frappell et al. 1992). Complementing these findings, I found that all rodents in my study increased ventilation in hypoxia, albeit to varying degrees. All rodents also increased ventilation relative to O_2 consumption rates when exposed to 7% O_2. The mouse exhibited the greatest increase in ventilation relative to O_2 consumption rates, increasing their ventilatory equivalent by 275% from normoxic levels, and slightly decreasing their lung O_2 extraction efficiency. Previous studies have found that an increase in ventilation in response to hypoxia is often, but not always, accompanied by a decrease in the amount of O_2 extracted by the lung from inspired air (Frappell et al. 1992; Barros et al. 2001) as a result of: 1) increased dead space ventilation; 2) diffusion limitation; or 3) a mismatch of ventilation and blood perfusion in

the lungs. Unlike the mouse, all other species exhibited both an increase in the ventilatory equivalent and an increase in the amount of O_2 they extracted at the lung. It is conceivable that the enhanced lung O_2 extraction efficiency of the rat, hamster, and ground squirrel, reflects the O_2 binding properties of the blood, as these species have a significantly lower P_{50} than the mouse at euthermic body temperatures (P_{50} rat- 33 torr; P_{50} mouse- 50 torr, P_{50} hamster- 28 torr; P_{50} ground squirrel- 18 torr; Schmidt-Nielsen and Larimer 1958; Maginniss and Milsom 1994). Blood with a higher affinity for O_2 will extract more O_2 from the lung at any given level of hypoxia. Thus, while all animals were exposed to the same level of environmental hypoxia, it may have resulted in different levels of hypoxemia. Other factors that could explain the higher lung O_2 extraction efficiency of these species in hypoxia include improved O_2 diffusion, or an increase in perfusion or capillary density.

2.4.3 Series III- Effects of hypoxia on the response to progressive cooling

The most notable and perhaps surprising result from my study was that in hypoxia, the rat progressively reduced its O_2 consumption rate and body temperature as ambient temperature fell below the TNZ, more so than any other species. Associated with this fall in O_2 consumption rate and body temperature was a fall in ventilation. The decreases in O_2 consumption rate, body temperature, and ventilation could all reflect an inability to extract sufficient O_2 from the inspired air. The ability to increase the rate of O_2 consumption as ambient temperature is reduced in hypoxia has been suggested to indicate that a hypoxia-induced reduction in O_2 consumption rate is not due to O_2 limitation, *per se*, but rather reflects a controlled reduction in O_2 consumption rate attributed to a lowering of the thermoregulatory set-point and the threshold for thermogenesis (Kuhnen et al. 1987; Dupré et al. 1988; Barros et al. 2001; Tattersall and Milsom 2003b; Tattersall and Milsom 2009). My results indicate that since the rats increased ventilation relative to their O_2 consumption rate (increased ventilatory equivalent), extracted less O_2 from every breath, and were unable to increase their O_2 consumption rate as ambient temperature was reduced, that the hypoxia-induced reductions in O_2 consumption rate and body temperature likely resulted from a limitation of O_2 supply. Given that rats are capable of tolerating 10% O_2 at varying ambient temperatures for weeks (Mortola and Seifert 2000; Cadena and Tattersall 2014),

it is conceivable that they may still exhibit a thermogenic response more typical of other small homeotherms in less severe hypoxia, but not at the levels used in the present study.

Interestingly, although the ground squirrels in my study exhibited reductions in their O_2 consumption rate in hypoxia relative to normoxia, they were better able than the other species to increase O_2 consumption rate as ambient temperatures were reduced. Furthermore, they did not exhibit a reduction in their body temperature compared to normoxic values at any ambient temperature investigated. These results also indicate that the hypoxia-induced decreases in O_2 consumption rates in this species were not due to an active reduction in the thermoregulatory set-point. This is in contrast to my predictions, and to the findings of Barros et al. (2001) on the golden-mantled ground squirrel. To my knowledge, the only other mammals that exhibit such a robust hypoxia-induced depression in O_2 consumption rates without a decrease in body temperature are newborn mammals (see Chapter 3) and the extremely hypoxia-tolerant naked mole rat (Pamenter et al. 2015). It is conceivable that temperature-independent reductions in O_2 consumption rates are a common phenomenon among hypoxia tolerant animals. A previous study from our lab has investigated the central regulation of body temperature and its role in the HMR of ground squirrels during the active euthermic season (Tattersall and Milsom 2009). To build on this work, experiments examining the central regulation of body temperature and its role in the HMR of rodents that range their degree of heterothermic expression, at multiple levels of hypoxia, and during the active and hibernating season warrant investigation.

Mice and hamsters, on the other hand, mounted a modest thermogenic response as ambient temperatures fell in hypoxia, and also exhibited O_2 consumption rates and body temperatures below those seen in normoxia. In the case of these two species, the reductions in body temperature were much greater than those seen in normoxia and would certainly contribute to declines in O_2 consumption rates. Whether these reductions in body temperature reflect a controlled, temperature-dependent lowering of the thermoregulatory set-point cannot be determined from my data, but are consistent with suggestions that as a line of protection against hypoxia, small mammals will reduce their body temperature (Gautier et al. 1987; Dupré et al. 1988; Wood 1991; Barros et al. 2001; Tattersall and Milsom 2003b; Branco et al. 2006; Tattersall and Milsom 2009). This is achieved if possible, through behavioural (i.e. huddling or postural changes) and physiological (i.e. peripheral vasodilation and heat dumping and inhibition of non-shivering thermogenesis) responses in order to reduce their metabolic need for O_2.

In hypoxia, all heterotherms either decreased (mice and hamsters), or maintained (ground squirrels) a constant level of ventilation with progressive cooling, despite increases in O_2 consumption rates. Thus, the heterotherms reduced their ventilatory equivalent and supported their increased O_2 demand with an increase in lung O_2 extraction efficiency. This increase in lung O_2 extraction efficiency was especially significant for the hamster and ground squirrel, which at their peak extracted more than 40% of the O_2 from each breath, again likely associated with changes in hemoglobin-O_2 affinity of the blood, and consistent with the results of Barros et al. (2001) in the golden-mantled ground squirrel.

In agreement with other studies (Gautier and Bonora 1992; Barros et al. 2001), I found that all rodents increased ventilation in response to hypoxia at temperatures within their TNZ. As ambient temperature fell, however, the HVR was abolished and ventilation dropped below those recorded in normoxia at low ambient temperatures. This appears to reflect a shift in the balance of increasing ventilation and O_2 supply to reducing O_2 consumption rate and O_2 demand, such that eventually ventilatory equivalents become lower in hypoxia than in normoxia. It is also conceivable that hypoxia-induced increases in ventilation decline in the cold because of a depressant effect on central control of respiration (Kiley et al. 1984; Maskrey 1990; Gautier 1996). Furthermore, reductions in body temperature are accompanied by an increase in O_2 affinity of the blood, and thus a lowering of the hypoxic ventilatory threshold (Boggs 1995). Indeed, in some rodents, the cold-induced shift in hemoglobin-O_2 affinity is so profound that levels of hypoxia as low as 1 to 3% O_2 at a body temperature of 4-7°C fail to elicit a ventilatory response (Milsom 1992). Thus, interpretation of this blunted HVR must take into account an increase in chemosensitive drive due to the stimulation of the peripheral chemoreceptors, a reduced metabolic drive linked to the fall in O_2 consumption rate and body temperature (in some species), and changes in hemoglobin-O_2 affinity (for a review see Frappell 1998).

2.4.4 Conclusions

How small mammals match O_2 supply and O_2 demand when they are exposed to the cold in hypoxia is intriguing, as they are faced with low O_2 availability at a time when staying warm is energetically taxing. I initially predicted that in normoxia, all species would respond to progressive cooling in a similar fashion given the fact that I conducted all experiments during the

active, euthermic season, and found that this was the case. I also predicted that within their TNZ, hypoxia would reduce O_2 consumption rates and body temperatures in all species. Surprisingly within the TNZ, inspiring 7% O_2 had little effect on O_2 consumption rates or body temperatures in species other than the mouse. Lastly, I hypothesized that hypoxia would reduce the ability of all species to mount a thermogenic response to decreasing ambient temperature, as indicated by a downward shift in the threshold temperature for thermogenesis (i.e. the LCT), a widening of the TNZ, a reduction in thermal conductance (i.e. a decrease in the slope of the relationship between O_2 consumption rate and ambient temperature), and a drop in body temperature with ambient temperature. I hypothesized that because facultative and obligate heterotherms are capable of reductions in their rate of O_2 consumption and body temperature when faced with energetic shortfalls (Ruf and Geiser 1995; Geiser 2004), they would be better able to reduce their O_2 consumption rate and body temperature in hypoxia than homeotherms. I found that hypoxia did reduce the thermogenic response of all species during progressive cooling, as indicated by an abolishment, or lowering, of the threshold temperature for thermogenesis (their LCT), a widening of the TNZ, and reduction in thermal conductance. However, contrary to my predictions, I found that in hypoxia the heterotherms still mounted a modest thermogenic response during progressive cooling. Thus, it appears that during the euthermic season, heterotherms do not rely solely on their remarkable ability to reduce their thermoregulatory set-point, O_2 consumption rate, and body temperature, but rather use an enhanced ability to extract O_2 from the inspired air as a part of their overall response for dealing with hypoxia in the cold.

Table 2.1. Species, number of individuals per treatment, sex, and body mass of adult homeothermic and heterothermic rodents exposed to progressive cooling in normoxia (21% O_2; Series I and II) and hypoxia (7% O_2; Series II and III). Body mass is presented as mean ± s.e.m.

Species	Degree of heterothermy	Treatment	Sample size		Body mass (g)
			female	male	
Rattus norvegicus rat	homeotherm	normoxia	5	0	450 ± 15
		hypoxia	5	0	582 ± 31
Mus musculus mouse	facultative heterotherm	normoxia	10	0	27 ± 2
		hypoxia	8	0	50 ± 3
Mesocricetus auratus hamster	facultative heterotherm	normoxia	4	5	209 ± 11
		hypoxia	3	5	170 ± 14
Ictidomys tridecemlineatus ground squirrel	obligate heterotherm	normoxia	10	1	245 ± 8
		hypoxia	10	0	303 ± 23

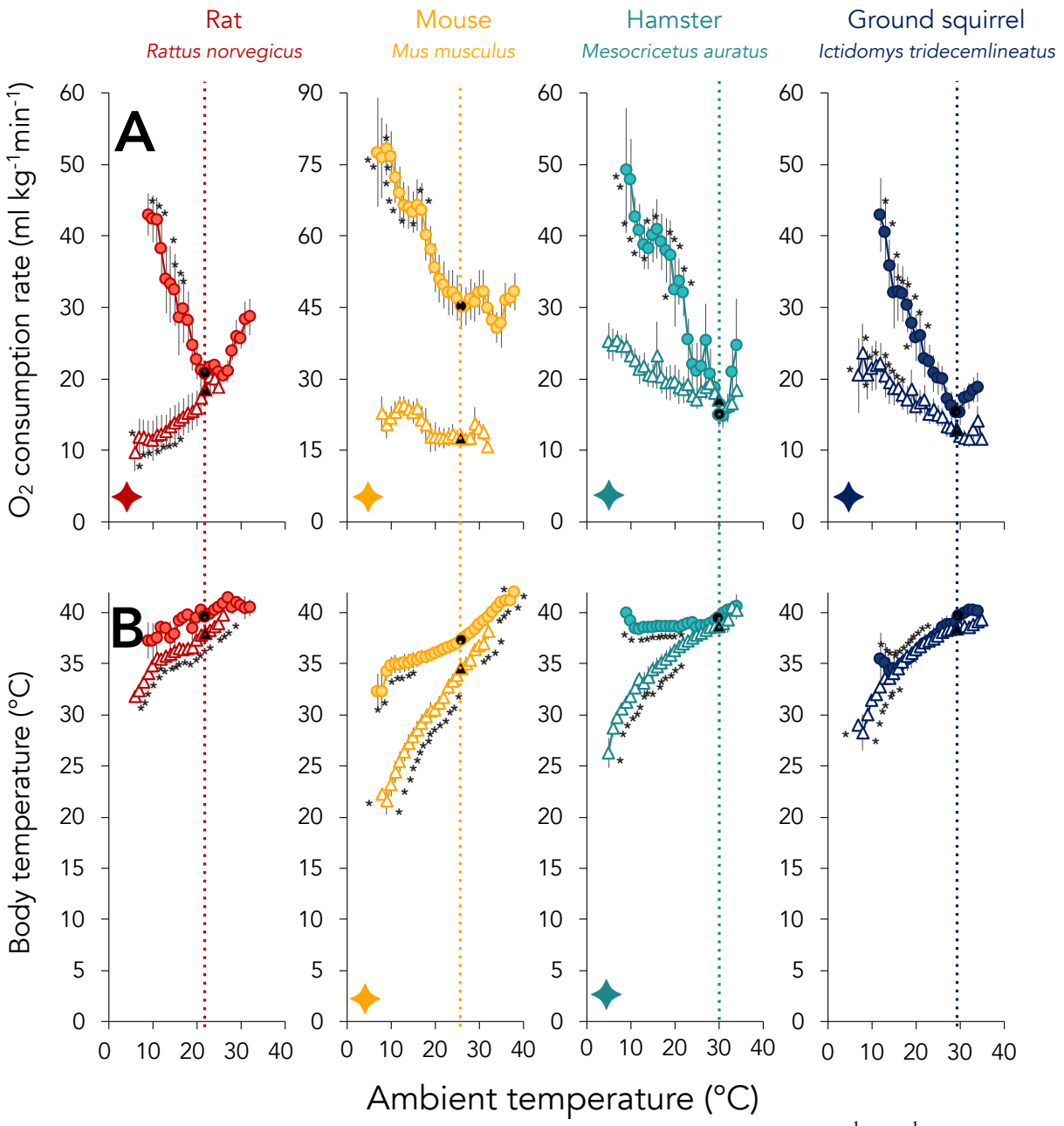

Figure 2.1. Effects of progressive cooling on **(A)** O_2 consumption rate (ml $kg^{-1}min^{-1}$), and **(B)** body temperature (°C) of normoxic (21% O_2; filled circles) and hypoxic (7% O_2; open triangles) adult homeothermic and heterothermic rodents. Symbols are as follows: dashed lines and black symbols mark the normoxic lower critical temperature (LCT) for each species. A significant difference from the LCT is indicated by an *. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m., and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=5, hypoxia: n=4. Facultative heterotherms: mice, normoxia: n=10, hypoxia: n=8; hamsters: n=9, hypoxia: n=8. Obligate heterotherms: ground squirrels, normoxia: n=11, hypoxia: n=10. Note that in panel **(A)** the y-axis scale is different for the mouse compared to the other species.

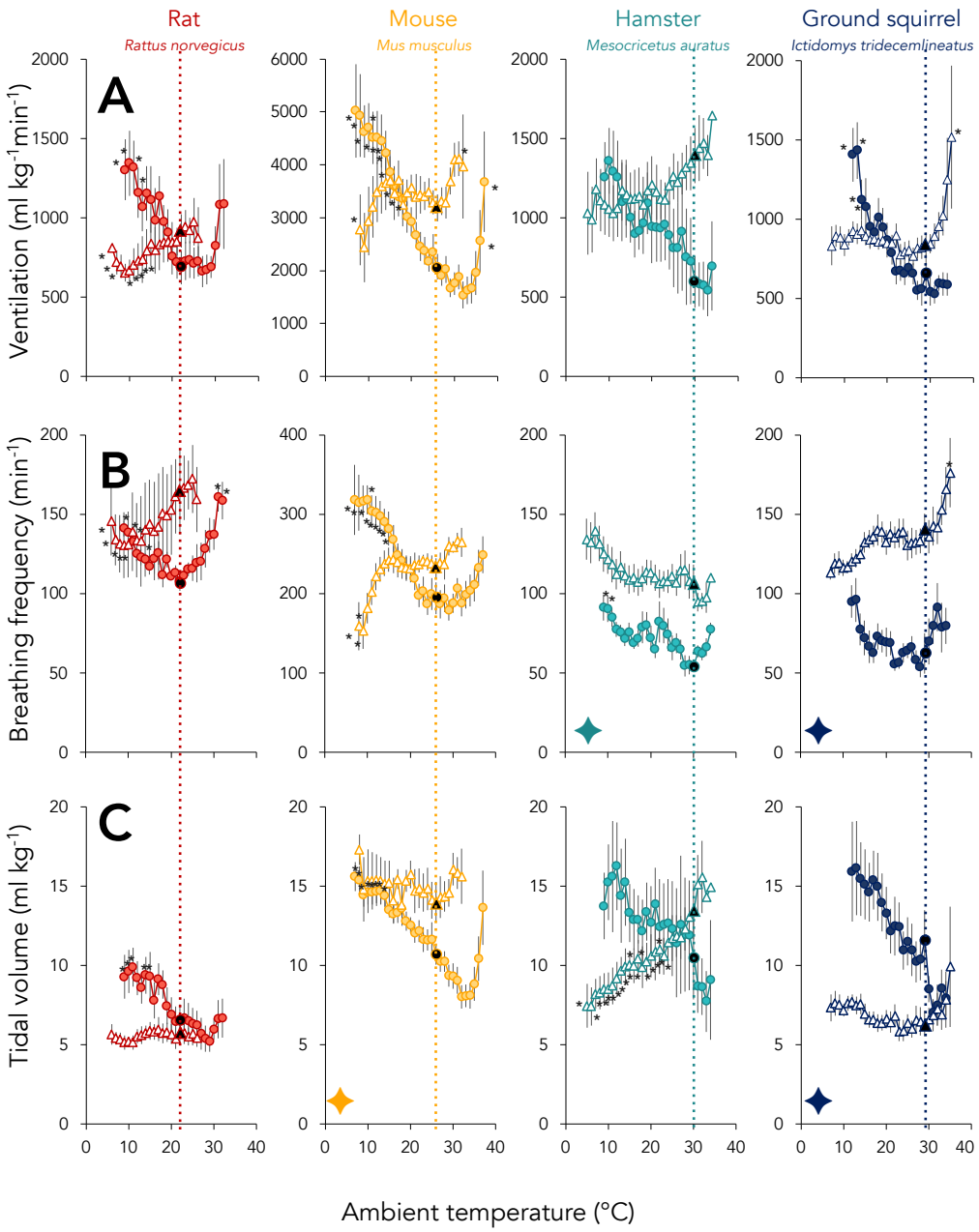

Figure 2.2. Effects of progressive cooling on **(A)** ventilation (ml $kg^{-1}min^{-1}$), **(B)** breathing frequency (min^{-1}), and **(C)** tidal volume (ml kg^{-1}) of normoxic (21% O_2; filled circles) and hypoxic (7% O_2; open triangles) adult homeothermic and heterothermic rodents. Symbols are as follows: dashed lines and black symbols mark the normoxic lower critical temperature (LCT) for each species. A significant difference from the LCT is indicated by an *. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m., and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=5, hypoxia: n=4. Facultative heterotherms: mice, normoxia: n=10, hypoxia: n=8; hamsters: n=9, hypoxia: n=8. Obligate heterotherms: ground squirrels, normoxia: n=11, hypoxia: n=10. Note that in panels **(A)** and **(B)** the y-axis scales are different for the mouse compared to the other species.

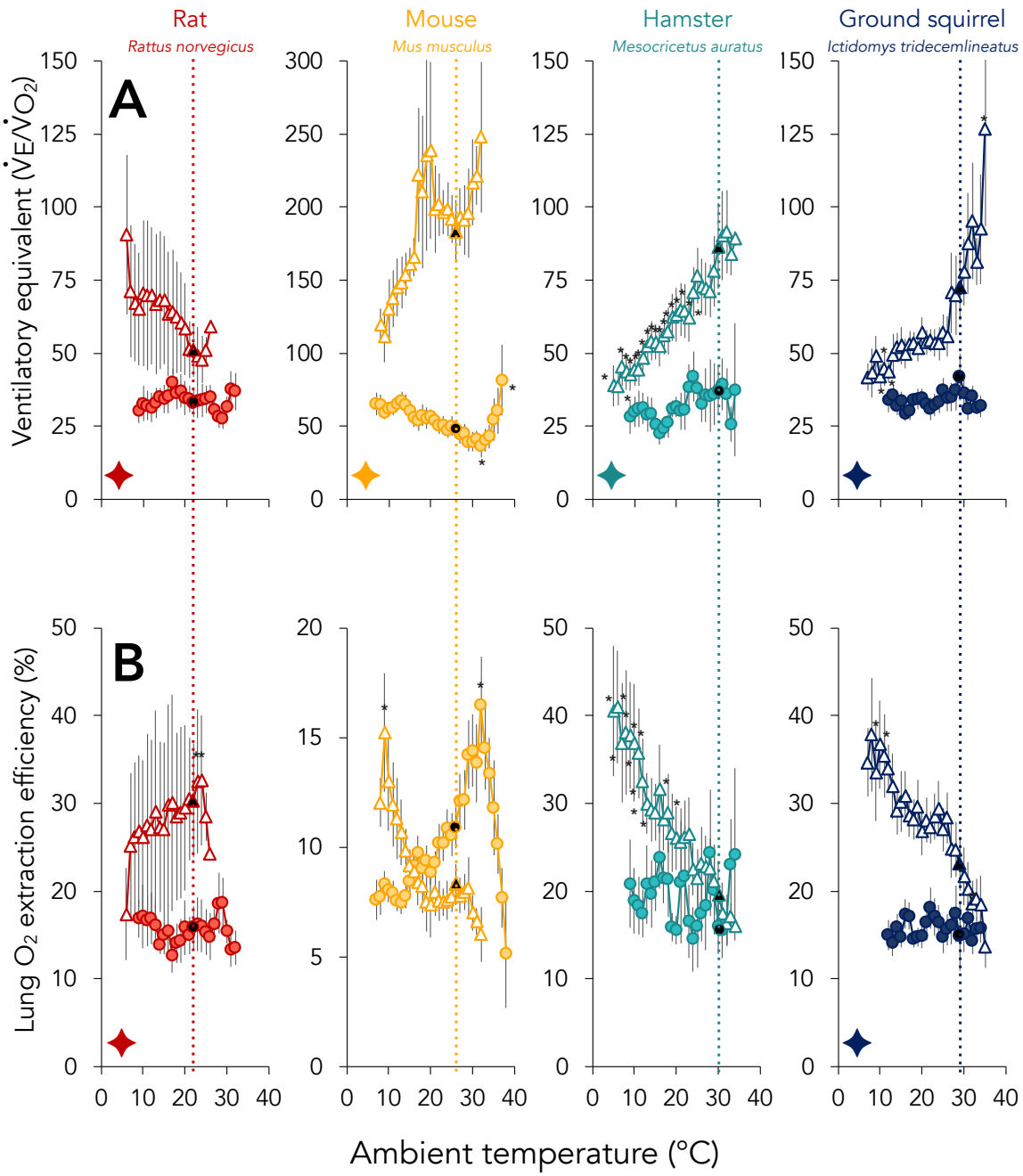

Figure 2.3. Effects of progressive cooling on **(A)** the ventilatory equivalent, and **(B)** lung O_2 extraction efficiency (%) of normoxic (21% O_2; filled circles) and hypoxic (7% O_2; open triangles) adult homeothermic and heterothermic rodents. Symbols are as follows: dashed lines and black symbols mark the normoxic lower critical temperature (LCT) for each species. A significant difference from the LCT is indicated by an *. ✦ indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m., and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=5, hypoxia: n=4. Facultative heterotherms: mice, normoxia: n=10, hypoxia: n=8; hamsters: n=9, hypoxia: n=8. Obligate heterotherms: ground squirrels, normoxia: n=11, hypoxia: n=10. Note that in panels **(A)** and **(B)** the y-axis scales are different for the mouse compared to the other species.

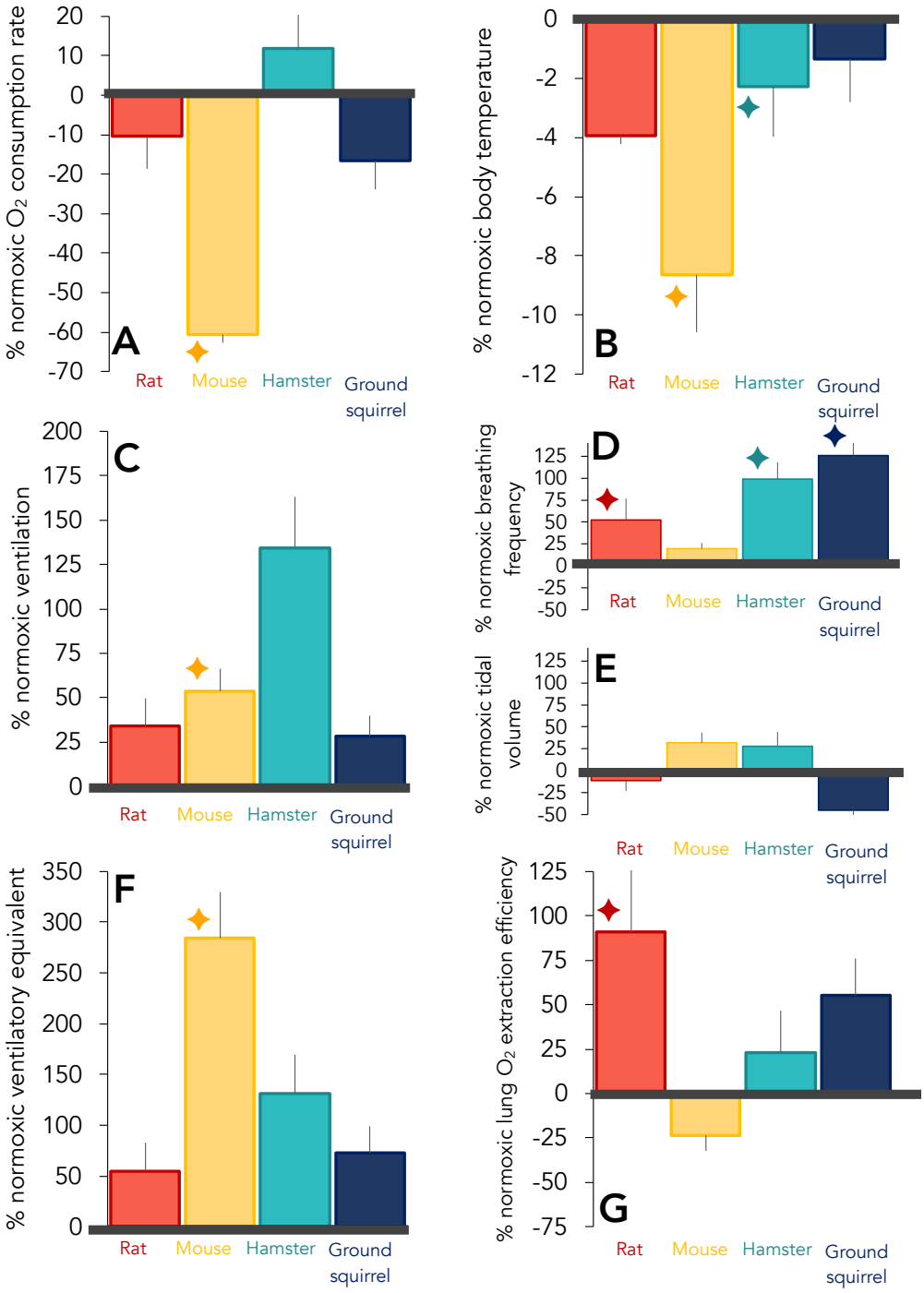

Figure 2.4. Hypoxic (7% O_2) **(A)** O_2 consumption rate, **(B)** body temperature, **(C)** ventilation, **(D)** breathing frequency, **(E)** tidal volume, **(F)** ventilatory equivalent, and **(G)** lung O_2 extraction efficiency represented as percent-change from normoxic values at the lower critical temperature (LCT) of adult homeothermic and heterothermic rodents. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m., and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=5, hypoxia: n=4. Facultative heterotherms: mice, normoxia: n=10, hypoxia: n=8; hamsters: n=9, hypoxia: n=8. Obligate heterotherms: ground squirrels, normoxia: n=11, hypoxia: n=10.

Chapter 3: The effects of progressive cooling on matching O_2 supply and O_2 demand in newborn rodents in normoxia and hypoxia

3.1 Introduction

Most adult mammals are unable to survive extreme reductions in body temperature (i.e. cold), or low levels of O_2 (for a review see Boutilier 2001). Species capable of hibernation (i.e. heterotherms), however, can survive these extreme environmental challenges significantly longer than most species that are incapable of hibernation (i.e. homeotherms) (Hiestand et al. 1950; Bullard et al. 1960; for a review see Drew et al. 2004). Interestingly, both newborn homeothermic and heterothermic rodents are more tolerant of cold and hypoxia than their adult counterparts (Avery and Johlin 1932; Kabat 1940; Fazekas et al. 1941; Glass et al. 1944; Adolph 1948a; Adolph 1948b; Hiestand et al. 1953; Adolph 1969; Singer 1999; Hill 2000; Fong 2010). In newborn rodents, the enhanced ability to withstand cold temperatures may be an adaptation to fluctuations in ambient temperature in their burrow when their mother leaves the nest to forage, attributed to the fact that newborns rely on parental care to stay warm. Their enhanced hypoxia tolerance, on the other hand, may stem from group-mediated depletion of O_2, a condition often experienced within their litters. Whatever the cause, cold and hypoxia tolerance in newborn mammals is well established (Avery and Johlin 1932; Kabat 1940; Fazekas et al. 1941; Glass et al. 1944; Adolph 1948a; Hiestand et al. 1953; Adolph 1969; Singer 1999; Hill 2000; Fong 2010); however, the basis of this tolerance is not fully understood.

Most adult rodents exposed to cold maintain a relatively constant body temperature (Ingram and Legge 1970; Chappell and Roverud 1990; for a review see Mortola and Frappell 2000). Unlike adults, however, most newborn rodents lack a dense pelage, liberal quantities of subcutaneous fat, and the ability to produce heat through shivering thermogenesis (Adolph 1957; Hissa and Lagerspetz 1964; Hissa 1968; Alexander 1975; Maxwell and Morton 1975; McClure and Porter 1983; Hill 1983; Spiers and Adair 1986; Knight 1987; Newkirk et al. 1995). Although newborn rodents combine behavioural and physiological thermoregulatory responses in an attempt to maintain a constant body temperature in the cold, due to their poor insulation, large surface area to volume ratio, and poor thermogenic capacity, they are unable to do so (Moore and Underwood 1963; Hissa 1968; Newkirk et al. 1995). Thus, their body temperatures and O_2

consumption rates fall linearly with ambient temperature (Taylor 1960; Mortola and Dotta 1992).

When exposed to hypoxia, most adult rodents increase ventilation in order to compensate for the reduction in inspired O_2, and sustain O_2 supply and normal O_2 consumption rates (Kuhnen et al. 1987; Dupré et al. 1988; Frappell et al. 1992; Chapter 2). The literature suggests that in most heterothermic rodents, however, the increase in ventilation is modest, and instead that heterotherms rely on a reduction in their rate of O_2 consumption, and hence O_2 demand (Kuhnen et al. 1987; Dupré et al. 1988; Barros et al. 2001; Tattersall and Milsom 2003b; Tattersall and Milsom 2009). My results in Chapter 2 are only partly consistent with this, suggesting that the combination of changes in O_2 consumption rates and ventilation can be more variable than previously believed, and that the hypoxic response is highly dependent on ambient temperature, time in hypoxia, and severity of the hypoxic exposure.

Newborn rodents on the other hand, appear to rely solely on a reduction in O_2 demand to survive prolonged and severe hypoxic challenges, and ventilation is often reduced below levels seen in normoxia (Cross et al. 1958; Mortola et al. 1989). While clearly there are differences in the responses to cold and hypoxia in newborn rodents compared to their adult counterparts, it remains unknown whether newborn homeothermic and heterothermic rodents exhibit the same responses when challenged with cold and hypoxia, either alone or in combination. There are differences in how adult homeotherms and heterotherms match O_2 supply and O_2 demand when exposed to cold and hypoxia (Saiki et al. 1994; Barros et al. 2001; Chapter 2) but whether these differences are already present in newborn animals is unknown. I hypothesized that they are not, and that newborn rodents that range in their degree of heterothermic expression are indistinguishable in how they match O_2 supply and O_2 demand during thermal and hypoxic challenges. To test this hypothesis I investigated the metabolic, thermoregulatory, and ventilatory responses of newborn rats (*Rattus norvegicus*), mice (*Mus musculus*), hamsters (*Mesocricetus auratus*), and ground squirrels (*Ictidomys tridecemlineatus*), all less than 5 days of age (Table 3.1). Because body size and state of development at the time of birth have been shown to have an effect on the relative magnitude of the hypoxic response (Mortola et al. 1989), I conducted experiments solely on altricial rodents matched for body size and developmental stage (Table 3.1). I selected these species because they range in their expression of heterothermy as adults. The rat is a true homeotherm. It is not known to use torpor and maintains a high body temperature and O_2 consumption rate independent of season and environmental conditions (Yoda

et al. 2000). Mice and hamsters are facultative, non-seasonal heterotherms (Lyman 1948; Hudson and Scott 1979), they use torpor any time of the year when confronted with energetic stressors, such as low ambient temperature (Ellison and Skinner 1992; Heldmaier et al. 1999), or limitations in food or energy reserves (Lyman 1948; Hudson and Scott 1979). The ground squirrel is an obligate heterotherm, and uses torpor seasonally, only during the winter (Wang and Lee 2011).

The objectives of this study were to investigate the thermoregulatory, metabolic, and ventilatory responses of newborn rodents that range in their degree of heterothermic expression to determine how newborn rodents match O_2 supply and O_2 demand when exposed to: (1) falling ambient temperature in normoxia; (2) hypoxia at the ambient temperature that they are often exposed to in their litter (their preferred ambient temperature; Nagy 1993); and (3) hypoxia at temperatures below their preferred ambient temperature.

3.2 Methods

3.2.1 Experimental animals and housing

Pregnant rats, mice, and hamsters were purchased from Charles River Laboratories (Portage, MI), while adult ground squirrels were live-trapped in June 2013 and 2014 in Carman, MB, Canada (49°30′ N, 98°01′ W) and transferred to the animal care housing facility at the University of British Columbia in Vancouver, BC, Canada. Ground squirrels were trapped with the approval of Manitoba Conservation and Water Stewardship, under wildlife scientific permit WB15027. All animals were provided with food and water *ad libitum*. Adult rats, mice, and hamsters were fed Purina Rodent Chow (no. 5001, Purina LabDiet, St. Louis, MO), while ground squirrels were given 1:1 IAMS dog food (Mars Incorporated, Mount Olive, NJ) and Purina Rodent Chow supplemented with sunflower seeds, fruit, and vegetables. Pregnant rodents were housed in an environmental chamber, held under a 07:00 to 19:00 light cycle, an ambient temperature of 21 to 23°C, and 45-55% relative humidity. Newborns were born at the animal care holding facility, and housed with their mothers until the day of the experiment. Each species was split into two groups: a normoxic group (21% O_2) and a hypoxic group (7% O_2) (Table 3.1). Experimental trials began in the morning and continued until early evening, with each

experimental trial lasting ~ 5 hours (280 ± 18 minutes for normoxia; 298 ± 18 minutes for hypoxia). All experimental procedures in this study conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the UBC Committee on Animal Care (under protocol A13-0091).

3.2.2 Experimental protocol

On the day of the experiment a newborn was separated from its mother, instrumented with a thermal probe, and placed into a 20 to 30 ml head-out chamber. The newborn's muzzle protruded through a small hole in a pliable piece of latex that provided a tight seal around the newborn's muzzle and separated the chamber into two parts: a head compartment, and a body compartment. The head compartment was flushed with normoxic air and set inside a temperature-controlled environmental chamber set to 33°C, which is about the ambient temperature within the litter of most rodent species (i.e. their preferred ambient temperature) (Nagy 1993). Ambient temperature was continuously recorded throughout the experiment using a temperature datalogger (iButton Maxim Integrated, Chandler, CA, USA) placed inside the chamber. Animals were given at least one hour to acclimate to the experimental setup. Across the 4 species, a total of 58 newborns were used in these experiments (28 normoxia (Series I and II), and 30 hypoxia (Series II and III).

3.2.2.1 Series I- Responses to progressive cooling in normoxia

To determine how newborn rodents match O_2 supply and O_2 demand during progressive cooling in normoxia (Series I), following acclimation and baseline recordings of body temperature, O_2 consumption rate, and ventilation at 33°C, ambient temperature was gradually increased up to 40°C and then reduced down to 10°C at an average rate of 0.21 ± 0.05°C min^{-1}. This temperature range was chosen to simulate situations that newborn rodents are exposed to naturally in the wild, but more importantly to identify the TNZ of the newborn rodents. All of these variables were continuously measured as ambient temperature was manipulated.

3.2.2.2 Series II- Effects of hypoxia at the preferred ambient temperature (TNZ)

To determine how newborn rodents match O_2 supply and O_2 demand in normoxia (Series I) and hypoxia (Series III) at their preferred ambient temperature (TNZ), body temperature, O_2 consumption rate, and ventilation were compared under each condition (normoxia and hypoxia) at 33°C. These data were calculated once all data for Series I and III for each species were collected.

3.2.2.3 Series III- Effects of hypoxia on the response to progressive cooling

To determine how newborn rodents match O_2 supply and O_2 demand during progressive cooling in hypoxia (Series III), following acclimation in normoxic air (1 hour) at 33°C, each animal was exposed to hypoxic gas (7% O_2) until body temperature, O_2 consumption rate, and ventilation reached steady state (165 ± 60 minutes). I selected this level of inspired O_2 as it is: (1) low enough to desaturate the arterial blood of adults of the species investigated in this study (Schmidt-Nielsen and Larimer 1958; Maginniss and Milsom 1994); (2) within the range of environmental O_2 levels that some of these species would face in the wild (Studier and Proctor 1971; Maclean 1981; Kuhnen 1986); and (3) identical to that used in Chapter 2 on adults of the same species. Because the same level of inspired O_2 results in different degrees of blood and tissue hypoxia (hypoxemia) among species, the results of the present study should be examined in the context of the experimental conditions in which they have been obtained. Different levels and durations of environmental hypoxia may modify species relationships reported here.

An identical protocol was followed as that in Series I. Once hypoxic (7% O_2) steady-state values at 33°C were recorded, ambient temperature was gradually increased up to 40°C, and then reduced down to 10°C, at an average rate of 0.25 ± 0.02°C min^{-1}. All variables were continuously recorded as ambient temperature was manipulated. Normoxic (Series I) and hypoxic (Series III) experiments were performed on different animals. Each experimental trial lasted ~ 5 hours (280 ± 18 mins for normoxia (Series I); 298 ± 18 mins for hypoxia (Series III)).

3.2.3 Body temperature measurements

Prior to being placed into the animal chamber, each individual was instrumented with a sterile, 0.64 mm diameter copper-constantan thermocouple wire (Physitemp IT-18, Clifton, NJ,

USA). The thermal probe was calibrated with a National Bureau of Standards certified thermometer. Prior to thermal probe insertion the area around the anus was disinfected with an antiseptic (Betadine®, Purdue Pharma, Stamford, CT, USA), and 95% ethanol. Following disinfection, a topical anaesthetic (2% Xylocaine®, AstraZeneca Canada Inc., Mississauga, ON, Canada) was administered to the area around the anus using a cotton swab. The tip of the thermal probe was lubricated and inserted about 5 to 10 mm into the animal's rectum. The thermal probe was secured to the animal's skin using tissue adhesive (3M™ Vetbond™, 3M, London, ON, Canada). The procedure took less than 10 minutes. Following each experimental trial, the insertion site was disinfected, topical anaesthetic administered, and the thermal probe removed.

3.2.4 O_2 consumption rate measurements

Oxygen consumption rate was determined using open-flow respirometry. Individuals were placed in the animal chamber with incurrent airflow pushed through the head chamber at a flow rate of 5.0 to 26.5 ml min^{-1} using calibrated flowmeters (PRSFM4302-1; Praxair Technology Inc., Danbury, CT, USA). The incurrent airflow was adjusted for each individual to ensure that neither the O_2 concentration, nor CO_2 concentration of the air leaving the head chamber were altered more than 1% by the animal's metabolism. Hypoxia was achieved by mixing compressed dry air and nitrogen in appropriate ratios using calibrated flowmeters. The incurrent and excurrent airflow, as well as the composition of the gas mixture were continuously monitored using a Field Metabolic System (Sable Systems, Las Vegas, NV, USA) with a built in flowmeter and O_2 and CO_2 gas analyzers. The gas analyzers were calibrated for O_2 and CO_2 before and after each experiment, with dry commercial air (Praxair Technology Inc., Danbury, CT, USA) and a premixed gas mixture (1.55% CO_2 balanced with N_2; Praxair Technology Inc., Danbury, CT, USA), respectively. Excurrent flow was passed through a desiccant media (DM-060-24; Perma Pure LLC, Toms River NJ, USA) before entering the cells of the O_2 and CO_2 analyzers. Incurrent gas concentrations and gas flow were checked every 15 minutes throughout the experiment. Oxygen consumption rate was calculated using equation 10.6 in Lighton (2008):

$$\dot{V}O_2 = \dot{V}i\,[(FiO_2 - FeO_2) - FeO_2\,(FeCO_2 - FiCO_2)]/(1 - FeO_2) \qquad (1)$$

Where $\dot{V}O_2$ is O_2 consumption rate (ml min^{-1}), $\dot{V}i$ is incurrent flow rate (ml min^{-1}), FiO_2 and $FiCO_2$ are fractional concentrations of incurrent O_2 and CO_2 of dry gas, respectively, and FeO_2 and $FeCO_2$ are fractional concentrations of excurrent O_2 and CO_2 of dry gas, respectively. Barometric pressure and chamber temperature were used to convert O_2 consumption rate measurements to STPD.

3.2.5 Ventilatory measurements

Ventilatory measurements were acquired using head-out plethysmography as described by Mortola and Frappell (2013). The head compartment of the animal chamber was connected to a pneumotachograph and a differential pressure transducer (DP103-18; Validyne, Northridge, CA, USA) was used to measure ventilation-induced pressure oscillations. To determine tidal volume from pressure oscillations, the system was calibrated at the beginning of each experimental trial by injecting and withdrawing known volumes of air (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 ml) at a rate similar to the animal's breathing frequency. Tidal volume was calculated by integrating expiratory flow. Breathing frequency was calculated directly from the ventilation-induced pressure oscillations, while ventilation was calculated from the product of tidal volume and breathing frequency. All ventilatory measurements are reported at BTPS.

3.2.6 Calculation of the ventilatory equivalent and lung O_2 extraction efficiency

The ventilatory equivalent (the quotient of ventilation and O_2 consumption rate), and lung O_2 extraction efficiency were calculated from ventilatory and O_2 consumption rate measurements. The percent of O_2 extracted from each breath was calculated by dividing O_2 consumption rate by ventilation multiplied by the fractional concentration of O_2 in inspired air (O_2 delivery to the lungs) and multiplying by 100.

3.2.7 Data collection and analysis

All signals (body temperature, incurrent and excurrent O_2 concentration, and the ventilation-induced pressure signal) were amplified, filtered, and recorded on a PowerLab 8/35

data acquisition system (ADInstruments Pty Ltd., Colorado Springs, CO, USA). From the recorded signals, and calculated dependent variables, I determined average body temperature, O_2 consumption rate, breathing frequency, tidal volume, ventilation, the ventilatory equivalent, and lung O_2 extraction efficiency, for the entire period the animal was exposed to each ambient temperature.

Statistical analyses were performed using R (R Core Team 2017, Vienna, AT, EUR). For Series I and III, I used linear mixed effects models (lme4 package; Bates et al. 2015) to account for any effects that body mass and sex may have had on the dependent variables, and to account for repeated sampling of the same individual with changes in ambient temperature, with individual treated as a random effect. For Series II, I ran a general linear model to account for any effects that body mass and sex may have had on the dependent variables. When visual inspection of residuals, and q-q plots revealed deviations from the assumptions of general linear, and linear mixed effects models (normality, homogeneity of variances, linearity, and independence), I log transformed the dependent variable. For each series, ambient temperature, level of inspired O_2, species, body mass, and sex were fixed effects in my initial models. I tested all 2- and 3-way interactions of ambient temperature, level of inspired O_2, and species. When body mass or sex did not have a significant effect on the dependent variables, they were removed from the model. I did not remove any other terms from my models given the importance of all independent variables and interactions to my research objectives. I used lmerTest in R to obtain F-statistics and *P*-values for each model (Kuznetsova 2016). Following the methods of Fangue et al. (2009), when interaction terms were significant the data were separated and analyzed independently using a one-way ANOVA, followed by a Tukey-Holm post-hoc analysis to determine differences among ambient temperatures, O_2 level, and species, and to correct for multiple pairwise comparisons. All results are presented as mean ± s.e.m., with statistical significance set as $P<0.05$.

3.3 Results

3.3.1 Series I- Responses to progressive cooling in normoxia

3.3.1.1 Newborn rodents reduce body temperature and O_2 consumption rate in the cold in normoxia

In normoxia, the body temperatures of newborn rodents decreased linearly, and significantly, as ambient temperature was reduced ($F_{1,28}$=3820.0, P<0.0001; Fig. 3.1A). Despite this, all species, except for ground squirrels, initially increased their body temperature-ambient temperature (Tb-Ta) differential (Fig. 3.1B). At lower ambient temperatures the Tb-Ta differential narrowed again (Fig. 3.1B). Although these trends are noteworthy there was no significant effect of ambient temperature (P=0.68), species (P=0.12), or an interaction between ambient temperature and species (P=0.95) on the Tb-Ta differential of newborn rodents as ambient temperature fell in normoxia (Fig. 3.1B).

Oxygen consumption rate also decreased in all newborn rodents as ambient temperature was reduced (Fig. 3.1C). The manner in which it fell, however, varied among species, as exhibited by a significant interaction between ambient temperature and species ($F_{3,25}$=4.5, P<0.05; Fig.3.1C). Oxygen consumption rate did not fall significantly until 16°C in rats, 25°C in mice, 23°C in hamsters, and 18°C in ground squirrels, compared to their O_2 consumption rate at 33°C (Fig. 3.1C). Below these temperatures, O_2 consumption rate fell dramatically (Fig. 3.1C). Rats were the only species to initially increase their O_2 consumption rate in response to a reduction in ambient temperature from 33°C in normoxia (Fig. 3.1C).

3.3.1.2 Newborn rodents exhibit ventilatory depression in the cold

In normoxia, ventilation varied significantly among species as ambient temperature was reduced from 33°C ($F_{3,25}$=14.8, P<0.0001), but in all species ventilation generally followed the changes in O_2 consumption rate (Fig. 3.2A). Ventilation did not fall significantly until 11°C in rats, 25°C in mice, and 23°C in hamsters and ground squirrels (Fig. 3.2A). Below these temperatures ventilation fell dramatically (Fig. 3.2A). Rats were the only species to initially increase ventilation as ambient temperature fell from 33°C in normoxia (Fig. 3.2A). The cold-induced reduction in ventilation, which is the product of breathing frequency and tidal volume, was solely due to decreases in breathing frequency ($F_{3,28}$=6.3, P<0.01; Fig. 3.2B). Tidal volume increased progressively and significantly as ambient temperature was reduced from 33°C in all

species but the ground squirrel ($F_{3,26}$=14.5, P<0.0001; Fig. 3.2C). The ground squirrel maintained a relatively constant tidal volume (Fig. 3.2C)

3.3.1.3 Newborn rodents differ in how they match O_2 supply and O_2 demand in the cold

There was a significant interaction between ambient temperature and species on the ventilatory equivalent and lung O_2 extraction efficiency of newborn rodents in normoxia (ventilatory equivalent: $F_{3,26}$=16.5, P<0.0001; Fig. 3.3A; lung O_2 extraction efficiency: $F_{3,25}$=17.5, P<0.0001; Fig. 3.3B). In normoxia, with a reduction in ambient temperature from 33°C down to 20°C, ventilation in rats and hamsters mirrored O_2 consumption rates, and thus their ventilatory equivalent remained constant (Fig. 3.3A). Below 20°C, however, rats and hamsters decreased ventilation less than their O_2 consumption rates, resulting in an increase in the ventilatory equivalent of 35-55% compared to values at 33°C (Fig. 3.3A). This increase in ventilatory equivalent only became significant at 13°C in rats, and never reached significance in hamsters (Fig. 3.3A). Unlike rats and hamsters, below 20°C, mice and ground squirrels decreased ventilation more than their O_2 consumption rates, resulting in a decrease in their ventilatory equivalent of 40-50%, however, compared to the ventilatory equivalent at 33°C the decrease was not statistically significant in either species (Fig. 3.3A). This slight decrease in the ventilatory equivalent was accompanied by an increase in lung O_2 extraction efficiency of 70-100%, that reached significance at 16°C in mice, and 21°C in ground squirrels compared to their lung O_2 extraction efficiency at 33°C (Fig. 3.3B). The percent of O_2 extracted from each breath remained constant in rats and hamsters (Fig. 3.3B).

3.3.2 Series II- Effects of hypoxia at the preferred ambient temperature (TNZ)

3.3.2.1 At their preferred ambient temperature all newborn rodents exhibit a hypoxia-induced reduction in their O_2 consumption rate, only in part due to a lowering of body temperature

Because the newborn's normoxic body temperature was only a few degrees above ambient temperature there was not much scope for body temperature to fall in hypoxia.

However, all species did reduce body temperature by 4-6% when exposed to hypoxia at their preferred ambient temperature of 33°C ($F_{1,31}$=29.2, P<0.0001; Fig. 3.4A). This fall was significant in all species except the ground squirrel (P=0.13; Fig. 3.4A). There was no significant interaction between O_2 level and species on the fall in body temperature of newborn rodents (P=0.92; Fig. 3.4A).

Relative to normoxic individuals, all newborn rodents exposed to 7% O_2 at 33°C significantly reduced their O_2 consumption rate ($F_{1,31}$=37.7, P<0.0001; Fig. 3.4B). Although there was no significant interaction between O_2 level and species (P= 0.16; Fig. 3.4B), the relative magnitude of the hypoxic decrease in O_2 consumption rates was least in rats (30%), and greatest in the ground squirrels (60%) (Fig. 3.4B).

3.3.2.2 The HVR varies among newborn rodents at their preferred ambient temperature

Ventilation was affected by the interaction between O_2 level and species ($F_{3,31}$=8.2, P<0.001; Fig. 3.4C). When exposed to hypoxia at 33°C, rats significantly increased ventilation by 140% (P<0.0001; Fig. 3.4C). Mice (P<0.01), and hamsters (P<0.001) exhibited a modest ventilatory response to hypoxia, increasing ventilation by 75-80% (Fig. 3.4C). Ground squirrels, on the other hand, had no significant ventilatory response to hypoxia (P= 0.25; Fig. 3.4C). In the rat, the HVR was due to significant increases in both breathing frequency ($F_{1,31}$=14.8, P<0.001; Fig. 3.4D) and tidal volume ($F_{1,31}$=6.7, P<0.05; Fig. 3.4E). Although ground squirrels did not exhibit a significant HVR at 33°C, they did change their breathing pattern in hypoxia, increasing their breathing frequency by 80% (P=0.14), but reducing their tidal volume by 40% (P=0.37) (Fig. 3.4C&E). However, these changes in breathing pattern were not statistically significant.

3.3.2.3 Hypoxia increases the ventilatory equivalent but does not alter lung O_2 extraction efficiency in newborn rodents at their preferred ambient temperature

All newborn rodents significantly increased ventilation relative to their O_2 consumption rates when exposed to 7% O_2 at 33°C, with a significant interaction between O_2 level and species on their ventilatory equivalent ($F_{3,31}$=7.4, P<0.001; Fig. 3.4F). Rats increased their ventilatory equivalent the most, by 310%, while mice, hamsters, and ground squirrels increased their

ventilatory equivalent by 200-220% (Fig. 3.4F). In hypoxia at 33°C, there was a tendency for lung O_2 extraction efficiency to fall more in rats (by 25%), than in the other species (by 5-15%), but there was no significant interaction between O_2 level and species on lung O_2 extraction efficiency (P=0.95), nor significant difference between normoxic and hypoxic lung O_2 extraction efficiency for any of the species investigated (P=0.09; Fig. 3.4G).

3.3.3 Series III- Effects of hypoxia on the response to progressive cooling

3.3.3.1 Newborn rodents exhibit greater falls O_2 consumption rates when challenged with cold and hypoxia, than with cold alone

As in normoxia, exposure to hypoxia decreased body temperature of all newborns as ambient temperature was reduced from 33°C, however, the relative magnitude of this decrease was significantly different among species, and was affected by a significant interaction between ambient temperature, O_2 level, and species ($F_{3,58}$=3.6, P<0.05; Fig. 3.1A). Rats (P=0.15) and hamsters (P= 0.84) did not exhibit a significant difference in body temperature between normoxia and hypoxia as ambient temperature fell (Fig. 3.1A). Mice (P<0.01) and ground squirrels (P<0.01), however, significantly decreased body temperature below normoxic levels at all ambient temperatures investigated (Fig. 3.1A). There was also a significant interaction between ambient temperature, O_2 level, and species on the Tb-Ta differential ($F_{3,58}$=3.6, P<0.05; Fig. 3.1B). As in normoxia, hypoxic rats and hamsters initially increased their Tb-Ta differential as ambient temperature dropped, with no significant difference between normoxia and hypoxia (Fig. 3.1B). Mice (P<0.01) and ground squirrels (P<0.01), on the other hand, decreased their Tb-Ta differential significantly between normoxia and hypoxia at all ambient temperatures investigated (Fig. 3.1B). In fact, the differential between body temperature and ambient temperature in the ground squirrels was completely abolished in hypoxia (Fig. 3.1B).

There was no significant 3-way interaction between ambient temperature, O_2 level, and species on O_2 consumption rate (P=0.49; Fig. 3.1C). However, there were differences in the effects of ambient temperature on O_2 consumption rates among species, as reflected by a significant interaction between ambient temperature and species ($F_{3,58}$=11.9, P<0.0001; Fig. 3.1C). In hypoxia, all species progressively reduced their O_2 consumption rate as ambient

temperature fell below 33°C (Fig. 3.1C). Compared to their O_2 consumption rate at 33°C, O_2 consumption rates fell significantly at 25°C in rats, 31°C in mice, 20°C in hamsters, and 30°C in ground squirrels (Fig. 3.1C). Compared to all other species, ground squirrels exhibited the greatest hypoxia-induced reduction in O_2 consumption rate at all ambient temperatures investigated, ultimately reducing their O_2 consumption rate by 75% compared to normoxic values (Fig. 3.1C). In the rat, mouse, and hamster, as ambient temperature was reduced, the relative difference between normoxic and hypoxic O_2 consumption rates also progressively decreased (Fig. 3.1C).

3.3.3.2 All newborn rodents exhibit an HVR in the warm and the cold, except for the ground squirrel

Ventilation was significantly affected by the interaction between ambient temperature, O_2 level, and species ($F_{3,61}$=3.1, P<0.05; Fig. 3.2A). As in normoxia, in hypoxia all newborns decreased ventilation as ambient temperature fell below 33°C (Fig. 3.2A). This decrease in ventilation became significant at 17°C in rats, 27°C in mice, 25°C in hamsters, and 30°C in ground squirrels, compared to ventilation levels at 33°C (Fig. 3.2A). As in normoxia, the reduction in ventilation in hypoxia was due to a decrease in breathing frequency ($F_{1,63}$=29.0, P<0.0001; Fig. 3.2B), as tidal volume significantly increased with decreasing ambient temperature in all species but the ground squirrel ($F_{3,59}$=2.9, P<0.05; Fig. 3.2C).

When exposed to 7% O_2, however, all species, except for the ground squirrels, significantly increased ventilation relative to normoxic values at all ambient temperatures investigated. In the ground squirrels, ventilation was unaffected by hypoxia at any ambient temperature (P=0.10; Fig. 3.2A). In those species that exhibited an HVR, the increase in ventilation above normoxic levels was primarily due to an increase in breathing frequency (P<0.01; Fig. 3.2B). Tidal volume, however, was significantly elevated relative to normoxic values at all ambient temperatures in rats (P<0.01; Fig. 3.2C). In mice it was only elevated compared to normoxic values below 18°C (Fig. 3.2C). Tidal volume was never elevated above normoxic values in hamsters (Fig. 3.2C). Although ground squirrels did not exhibit an HVR at any ambient temperature, they did exhibit a change in breathing pattern as ambient temperature fell in hypoxia (Fig. 3.2C). Compared to normoxic values, ground squirrels increased breathing

frequency in hypoxia (P<0.05); however, this increase was less than that seen in the other three species (Fig. 3.2B), and their tidal volume in hypoxia was reduced below normoxic values (Fig. 3.2C).

3.3.3.3 Newborn rodents differ in how they match O_2 supply and O_2 demand in response to hypoxia in the cold

There was no significant interactive effect between ambient temperature, O_2 level, and species on the ventilatory equivalent of newborn rodents (P=0.09; Fig. 3.3A). However, there was a significant interaction between ambient temperature and O_2 level ($F_{1,54}$=26.2, P<0.0001) as well as O_2 level and species ($F_{3,55}$=4.8, P<0.01) (Fig. 3.3A). Unlike in normoxia where all species generally maintained their ventilatory equivalent down to an ambient temperature of 20°C, in hypoxia as ambient temperature fell below 33°C the ventilatory equivalent initially increased in all species (Fig. 3.3A). It then progressively fell as ambient temperature was reduced (Fig. 3.3A). The fall in ventilatory equivalent reached significance at 18°C in mice, and 25°C in ground squirrels, in contrast to the rats and hamsters where the fall in ventilatory equivalent never reached significance compared to their ventilatory equivalent at 33°C (Fig. 3.3A).

Due to the increases in ventilation and/or decreases in O_2 consumption rates in hypoxia, the ventilatory equivalent was significantly elevated above normoxic levels at all ambient temperatures in all species (Fig. 3.3A). The relative increase in the ventilatory equivalent in hypoxia compared to normoxia was maintained as temperature was initially reduced but progressively decreased at the lowest levels of ambient temperature in rats (at 16°C), mice and hamsters (at 23°C) (Fig. 3.3A). Ground squirrels, however, maintained the relative increase in their ventilatory equivalent as ambient temperature fell (Fig. 3.3A).

Lung O_2 extraction efficiency was significantly affected by the interaction between ambient temperature and species on lung O_2 extraction efficiency ($F_{3,47}$=41.5, P<0.0001; Fig. 3.3B). Just as in normoxia, rats and hamsters maintained a constant level of O_2 extraction at the lung as ambient temperature fell in hypoxia (P=0.51) while the percent O_2 extracted from each breath increased as ambient temperature fell in mice and ground squirrels (Fig. 3.3B). Generally, newborns did not exhibit a significant difference lung O_2 extraction efficiency between normoxic and hypoxic individuals (Fig. 3.3B).

3.4 Discussion

The major question that I addressed in this study was whether newborn rodents that range in their degree of heterothermic expression differ in the way they match O_2 supply and O_2 demand in response to thermal and hypoxic challenges. Collectively, my data indicate that the newborn's response to cold and hypoxia is highly dependent on their degree of heterothermic expression: homeotherms, facultative heterotherms, and obligate heterotherms all exhibit a different balance of changes in O_2 supply and O_2 demand when challenged with cold and hypoxia, either alone, or in combination. Relative to normoxic individuals, all newborns when challenged with cold and hypoxia: i) reduced their body temperature, with rats maintaining the greatest body to ambient temperature differential, and ground squirrels the smallest; ii) exhibited greater metabolic depression, with rats depressing O_2 consumption the least, and ground squirrels the most; iii) increased ventilation, except for ground squirrels, in which the HVR was absent; and iv) increased ventilation relative to O_2 consumption, thus their ventilatory requirement significantly increased; this increase was greatest in rats, and smallest in ground squirrels. The facultative heterotherms exhibited responses in between these two. I conclude that even at birth the ground squirrel, an obligate heterotherm, employs a potentially more cost effective response than the homeothermic rat, with the facultative heterotherms exhibiting responses in between these two extreme phenotypes.

3.4.1 Series I- Responses to progressive cooling in normoxia

At birth, some mammals exhibit a TNZ, a range of ambient temperatures over which body temperature can be maintained by passive mechanisms, and respond to a reduction in ambient temperature outside of this zone by increasing heat production, and their O_2 consumption rate (Thompson and Moore 1968; Alexander 1975; Mortola 2005). Most altricial rodents, however, display little or no thermogenic response to cold at birth (Moore and Underwood 1963; Hissa 1968; Newkirk et al. 1995). All of the species in my study were altricial, born with relatively little insulation, and with a limited ability to control heat loss. While no species mounted a metabolic response (i.e. an increase in O_2 consumption rate) sufficient to maintain a constant body temperature, all species except the ground squirrels, attempted a

thermogenic response, as indicated by an increase in their Tb-Ta differential. This increase in the Tb-Ta differential coincided with a smaller fall in O_2 consumption rate with ambient temperature; both indicative of increased thermogenesis. Rats were the only species that exhibited an initial increase in their O_2 consumption rate at ambient temperatures below their narrow TNZ of 30-33°C, and they exhibited a greater Tb-Ta differential than all other species. As ambient temperature continued to fall, however, even rats did not maintain an elevated O_2 consumption rate, and ultimately O_2 consumption rate plummeted. Conversely, ground squirrels had the lowest Tb-Ta differential and exhibited the steepest immediate fall in O_2 consumption rate. Given that thermogenesis is energetically costly, and the thermogenic capacity of these newborns appears limited, by maintaining a low Tb-Ta differential, and not attempting to mount a thermogenic response in the cold, the ground squirrel's response could be considered more cost effective.

It should be noted that the newborns in the present study were unable to use behavioural means to thermoregulate, as each newborn was studied in isolation. Huddling to reduce heat loss, and maintain a constant body temperature and O_2 consumption rate represents an important behavioural component of the newborn's defense against cold (Alberts 1978; Saiki and Mortola 1996); a strategy that is less energetically costly than recruiting physiological mechanisms, and may be the primary mechanism these species rely on when faced with cold conditions in the wild. My findings on rats are in agreement with those of previous studies (Taylor 1960; Mortola and Dotta 1992); however, to my knowledge there are no studies of the thermal and metabolic responses to a progressive reduction in ambient temperature of species of newborn rodents other than the homeothermic rat.

Since the newborn mammals in my study did not mount a significant thermogenic response, O_2 demand dropped with ambient temperature, and ventilation followed. My findings are in line with those of previous studies (Saiki and Mortola 1996; Tattersall and Milsom 2003a; for a review see Mortola 2005). In all species, the cold-induced reduction in ventilation was solely due to a decrease in breathing frequency, as the tidal volume of each breath increased in rats, mice, and hamsters, and remained relatively constant in ground squirrels. Regulating ventilation in the cold through changes in breathing frequency, rather than tidal volume has also been observed in other studies (Tattersall and Milsom 2003a; MacFarlane and Frappell 2004; Mortola 2005). Reducing ventilation solely by reducing breathing frequency prevents the

increase in dead space ventilation and reduction in effective ventilation that would ensue if tidal volume were to decrease also.

In all species, the reduction in O_2 supply initially paralleled decreases in O_2 demand (measured as O_2 consumption rate). However, below an ambient temperature of 20°C, rats and hamsters decreased ventilation less with the fall in O_2 consumption rate, and thus their ventilatory equivalent increased, and lung O_2 extraction efficiency decreased. To my knowledge, this is a response unique among mammals, but typical of ectotherms in response to cold. In many ectotherms, when body temperature decreases the ventilatory equivalent increases to eliminate CO_2 (Reeves 1972; Reeves 1977; Nattie 1990). The ensuing hypocapnic alkalosis matches the temperature induced changes in neutral pH, and ensures that despite changes in body temperature pH-dependent properties of proteins remain constant, a process known as alphastat regulation (Reeves 1972; Reeves 1977; Nattie 1990). This response, however, comes at a cost as increases in ventilation increase O_2 consumption rates, as well as heat loss through the respiratory system. Conversely, below an ambient temperature of 20°C, mice and ground squirrels decrease ventilation more than O_2 consumption rates and increase lung O_2 extraction efficiency. This response is more metabolically efficient, and reduces the rate of heat dissipation across the respiratory tract (Mortola 2005). Consistent with this, mice and ground squirrels in my study generally maintained their Tb-Ta differential with a reduction in ambient temperature, while rats and hamsters did not. Why some species differ in how they match O_2 supply and O_2 demand is not clear.

3.4.2 Series II- Effects of hypoxia at the preferred ambient temperature (TNZ)

My study confirms that newborns reduce O_2 demand in response to limited O_2 availability (Cross et al. 1958; Mourek 1959; Adolph 1969; for a review see Mortola 1999; Singer 1999; Mortola 2004; Fong 2010). However, I found that the relative magnitude of the HMR (the hypoxia- induced suppression of O_2 consumption rate) varied among species, ground squirrels exhibited the greatest suppression of O_2 demand, rats exhibited the least, and mice and hamsters fell somewhere in between. Reducing O_2 demand in hypoxia is an advantageous response as it decreases the O_2 requirement of tissues and greatly extends the time energy reserves can sustain O_2 consumption rates in a low O_2 environment. Here I report a clear relationship between the degree of O_2 consumption rate suppression and the degree of

heterothermy. There are, however, disadvantages of O_2 consumption rate suppression that only become apparent when hypoxic conditions persist. In the developing rodent, prolonged O_2 consumption rate suppression inhibits functions, such as tissue and organ growth, tissue differentiation, and cell repair (Frappell and Mortola 1994). Ultimately, the inhibition of these functions leads to permanent modifications in structure and function, and may hinder the newborn's survival over the long term (Frappell and Mortola 1994).

While there was little scope for body temperature to fall in hypoxia, as the Tb-Ta differential was minimal, all newborn rodents decreased body temperature in hypoxia by roughly 5% (~ 2°C). In agreement with the trends I report here, a previous study by Mortola and colleagues (1989) found that body temperature decreased slightly, or remained unchanged in newborn mammals acutely exposed to hypoxia. It has been suggested that a reduction in O_2 demand in hypoxia is a phenomenon largely based on the inhibition of behavioural, shivering, and non-shivering thermogenesis, as a result of a resetting of the thermoregulatory set-point to a lower level of body temperature (Hill 1959; Mortola and Gautier 1995; Mortola 1999). In the present study, however, the large reduction in O_2 consumption rate observed in all newborns could not result solely from passive temperature effects due to the small reduction in body temperature (Q_{10} effect). In Figure 3.5 I calculated what the relationship would be between ambient temperature and O_2 consumption rate due solely to the fall in body temperature in hypoxia assuming a Q_{10} of 3. The fall in the rate of O_2 consumption would be far less than what was observed (Fig. 3.5). Whether this body-temperature independent fall in O_2 consumption rate is due to O_2 limitation or active suppression of O_2 consumption rate remains to be determined. Whatever the case, the ground squirrel had a significantly lower body temperature in hypoxia than any of the other species investigated. This may be an important response underlying enhanced hypoxia tolerance in this species, as lower body temperatures have been found to extend survival time in hypoxia (Wood and Stabenau 1998), reduce O_2 demands at rest (Frappell et al. 1992), and increase the affinity of hemoglobin for O_2 (Musacchia and Volkert 1971; Maginniss and Milsom 1994).

In hypoxia, I found that rats increased ventilation the most, mice and hamsters exhibited a modest ventilatory response, and ground squirrels did not increase ventilation. It has long been recognized that in hypoxia extreme suppression of O_2 consumption rate is a phenomenon that reduces tissue deoxygenation, contributes to a blunted HVR, and reduces the costs associated

with thermogenesis and the cost of increasing ventilation to support it (Cross et al. 1958; Adolph and Hoy 1960; Mortola et al. 1989; Mortola 1991). Furthermore, mammals capable of hibernation generally have a hemoglobin with a greater affinity for O_2 than their homeothermic counterparts (Maginniss and Milsom 1994). Thus, it is conceivable that the blunted HVR in the ground squirrel, the only obligate heterotherm in this study, reflects the greater hypoxia-induced fall in O_2 consumption rate, and the greater hemoglobin-O_2 affinity of this species. Given that among the species investigated, the ground squirrel has the highest hemoglobin-O_2 affinity, and the lowest P_{50} (partial pressure of O_2 at which the blood is 50% saturated; Maginniss and Milsom 1994) it would be the least hypoxemic at 7% O_2. Thus, ground squirrels may be less affected by environmental hypoxia than the other species.

Of the species that increased ventilation in hypoxia, the increase was primarily the result of an increase in breathing frequency. Mortola et al. (1989) also found that the hypoxia-mediated increase in ventilation of newborn mammals is primarily due to an increase in breathing frequency, as tidal volume varies among species, and either slightly increases, decreases, or remains constant in hypoxia. The same is true in the present study. These findings highlight species differences among newborns in hypoxic respiratory sensitivity (the relative magnitude of the ventilatory response) and breathing pattern responses. The consequences of these differences, however, are modest, as seen by the examination of changes in the ventilatory equivalent and lung O_2 extraction efficiency, as discussed next.

Upon exposure to hypoxia, all newborns reduced their rate of O_2 consumption and increased ventilation, this combination resulted in a large increase in the ventilatory equivalent. Because each volume of gas inspired contains less O_2, unless a larger percentage of the O_2 in each breath is extracted, the amount of gas that must be delivered to the lungs for each volume of O_2 consumed, must increase to compensate. The relative magnitude of the changes in each of these two variables varied among species (Mortola et al. 1989). In the present study, lung O_2 extraction efficiency fell by roughly 25% in rats, but remained relatively constant in the other three species. As a result, rats increased ventilation relative to their O_2 consumption rate by over 300%, while all other species increased their ventilatory equivalents by roughly 200%. The reduced emphasis of rats on extracting O_2 from each breath during hypoxia could reflect species differences in lung morphology, lung perfusion, or the carrying capacity of the blood. This remains to be explored.

3.4.3 Series III- Effects of hypoxia on the responses to progressive cooling

While rats and hamsters still mounted a modest thermogenic response when ambient temperature was reduced in hypoxia, mice and ground squirrels did not. Regardless, body temperature decreased with the fall in ambient temperature in hypoxia in all newborns and in all species the rates of O_2 consumption in hypoxia were lower relative to those in normoxia. In rats, mice, and hamsters the hypoxia-induced reduction in O_2 consumption rates (the difference between normoxic and hypoxic O_2 consumption rates expressed as a percent change) was greater at higher ambient temperatures than at lower ambient temperatures. Ground squirrels, on the other hand, maintained the relative difference between their normoxic and hypoxic O_2 consumption rates as ambient temperature fell, and exhibited the greatest hypoxia-induced depression in O_2 consumption rate at each ambient temperature. My findings indicate that although a reduction in the thermoregulatory set-point may be an important mechanism in reducing O_2 consumption rates in newborn rats (Hill 1959; Mortola and Gautier 1995; Mortola 1999), body temperature did not fall more than 1-2°C and thus the fall in body temperature *per se* could not be the primary mechanism responsible for the significant reduction in O_2 demand of newborn rodents. Regardless, the reduction in the rate of O_2 consumption provides substantial energy savings. This energy savings would be beneficial in increasing survival when environmental conditions are unfavourable, and allocating residual energy for development when environmental conditions improve.

Just as in normoxia, in hypoxia all newborns matched the decrease in O_2 demand, with a decrease in ventilation as ambient temperature fell. However, at each ambient temperature, ventilation in hypoxia remained elevated relative to normoxic values, except in the ground squirrels, which did not exhibit an HVR. As in normoxia, all newborns decreased breathing frequency in hypoxia as ambient temperature fell. However, all newborn rodents, but ground squirrels, also increased tidal volume. This slower, deeper breathing pattern should be more effective for gas exchange but could be more metabolically costly (for a review see Mortola and Frappell 2000).

To attenuate reductions in O_2 supply, in hypoxia rats and hamsters initially increased their ventilatory equivalent as ambient temperature fell, progressively decreasing their ventilatory equivalent at lower ambient temperatures, while maintaining lung O_2 extraction efficiency.

Conversely, mice and ground squirrels progressively decreased their ventilatory equivalent as ambient temperature fell in hypoxia, increasing lung O_2 extraction efficiency from 20% to as much as 60-80% of the O_2 in the inspired air. These findings are in accordance with those of Barros et al. (2001) on adult ground squirrels. Among newborns, ground squirrels are unique as they are the only species that do not exhibit an HVR at any ambient temperature investigated. Instead of increasing ventilation to compensate for a drop in O_2 supply in hypoxia, ground squirrels solely relied on their enhanced ability to extract O_2 at the lung. The increase in lung O_2 extraction efficiency with the reduction in ambient temperature may be due to a combination of factors, such as longer resident time of gas in the lungs (lower breathing frequency), relative increases in heart rate, stroke volume, hematocrit, or hemoglobin-O_2 affinity of the blood.

3.4.4 Conclusions

It has been hypothesized that heterotherms retain traits common to all newborn mammals (Harris et al. 2004). These traits include exceptional tolerance to reductions in body temperature and inspired O_2. However, even as newborns, heterotherms are more tolerant of cold and O_2 limitation than homeotherms (Hiestand et al. 1953; Fong 2010). My study is the first to report that newborn rodents differ in how they match O_2 supply and O_2 demand when exposed to thermal and hypoxic challenges, with their responses reflecting the degree of heterothermic expression, as well as level of tolerance. When newborn rodents are exposed to thermal and hypoxic challenges, the homeothermic rat exhibited the greatest thermogenic response, depressed their metabolic demand for O_2 the least, and balanced O_2 supply and O_2 demand by increasing ventilation. On the other hand, the ground squirrel, an obligate heterotherm, did not mount a thermogenic response, exhibited greater reductions in their metabolic demand for O_2, and increased O_2 uptake, not by increasing ventilation, but by extracting up to 80% of the O_2 from each inspired breath. The responses of the facultative heterotherms, the mouse and hamster, fell somewhere between those of the rat and ground squirrel. Thus, even as newborns, homeotherms and heterotherms diverge in how they match O_2 supply and O_2 demand under cold and/or hypoxic conditions. My findings indicate that not all newborn rodents are wired the same, and that elements of the adult phenotype are already present at birth.

Table 3.1. Species, number of individuals per treatment, sex, age, and body mass of newborn homeothermic and heterothermic rodents exposed to progressive cooling in normoxia (21% O_2; Series I and II) and hypoxia (7% O_2; Series II and III). Age and body mass are presented as mean ± s.e.m.

Species	Degree of heterothermy	Treatment	Sample size		Age (days)	Body mass (g)
			female	male		
Rattus norvegicus rat	homeotherm	normoxia	4	3	2.3 ± 0.4	9.6 ± 0.8
		hypoxia	6	5	2.6 ± 0.4	8.9 ± 0.5
Mus musculus mouse	facultative heterotherm	normoxia	6	3	2.6 ± 0.4	2.7 ± 0.2
		hypoxia	3	3	1.7 ± 0.2	2.6 ± 0.1
Mesocricetus auratus hamster	facultative heterotherm	normoxia	4	4	2.5 ± 0.4	4.3 ± 0.4
		hypoxia	4	5	4.4 ± 0.3	5.6 ± 0.4
Ictidomys tridecemlineatus ground squirrel	obligate heterotherm	normoxia	2	2	2.6 ± 0.3	5.3 ± 0.2
		hypoxia	2	2	3.2 ± 0.4	6.1 ± 0.3

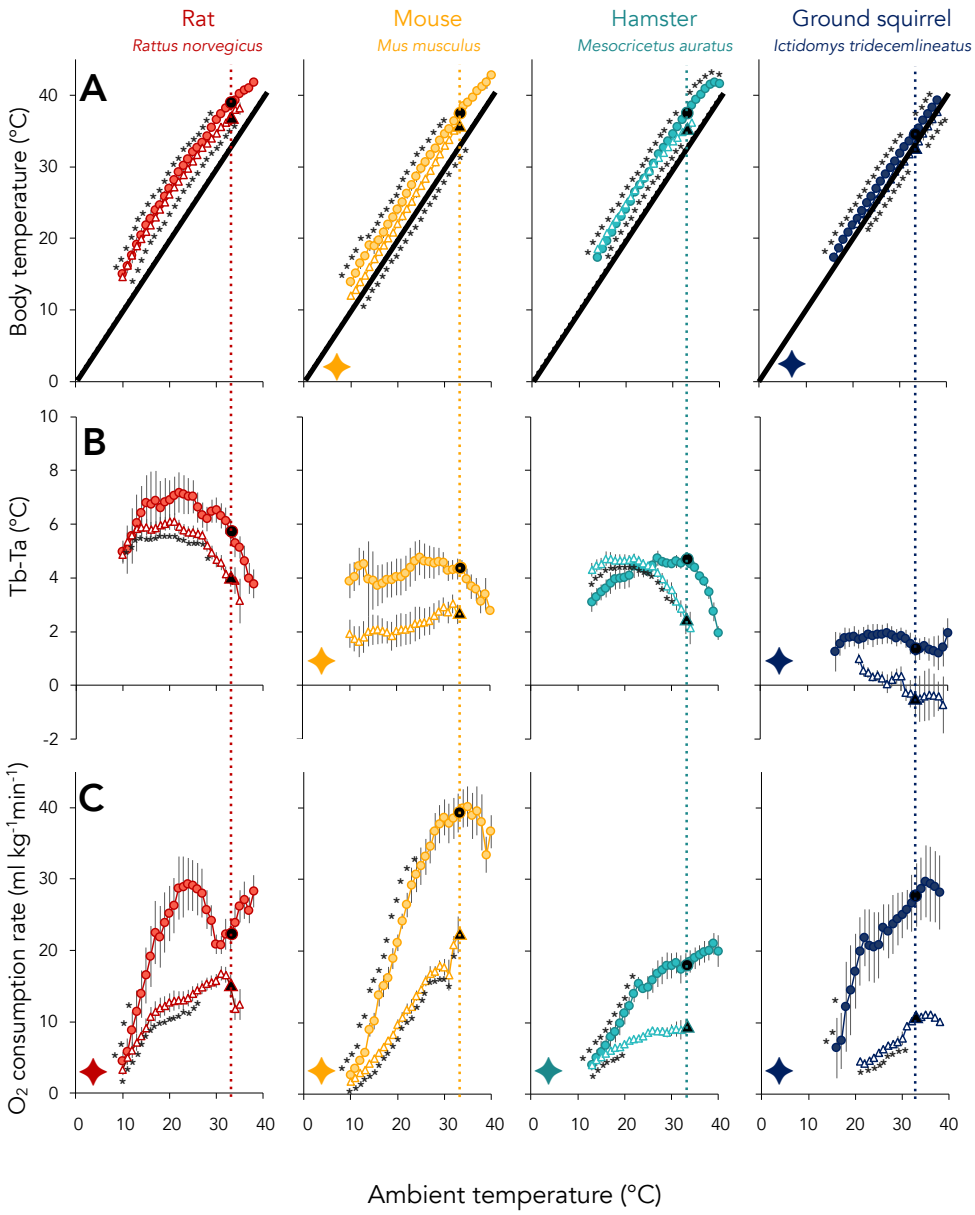

Figure 3.1. Effects of progressive cooling on **(A)** body temperature (°C), **(B)** body temperature-ambient temperature (Tb-Ta) differential (°C), and **(C)** O_2 consumption rate (ml $kg^{-1}min^{-1}$) of normoxic (21% O_2; filled circles) and hypoxic (7% O_2; open triangles) newborn homeothermic and heterothermic rodents. Symbols are as follows: solid black line in panel **(A)** represents the line of unity (body temperature=ambient temperature). Dashed lines and black symbols mark the preferred ambient temperature (33°C) of newborn rodents. An asterisk (*) indicates a significant difference from the preferred ambient temperature of newborn rodents. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=7: hypoxia: n=11. Facultative heterotherms: mice, normoxia: n=9: hypoxia: n=6; hamsters: n=8: hypoxia: n=9. Obligate heterotherms: ground squirrels, normoxia: n=4: hypoxia: n=4.

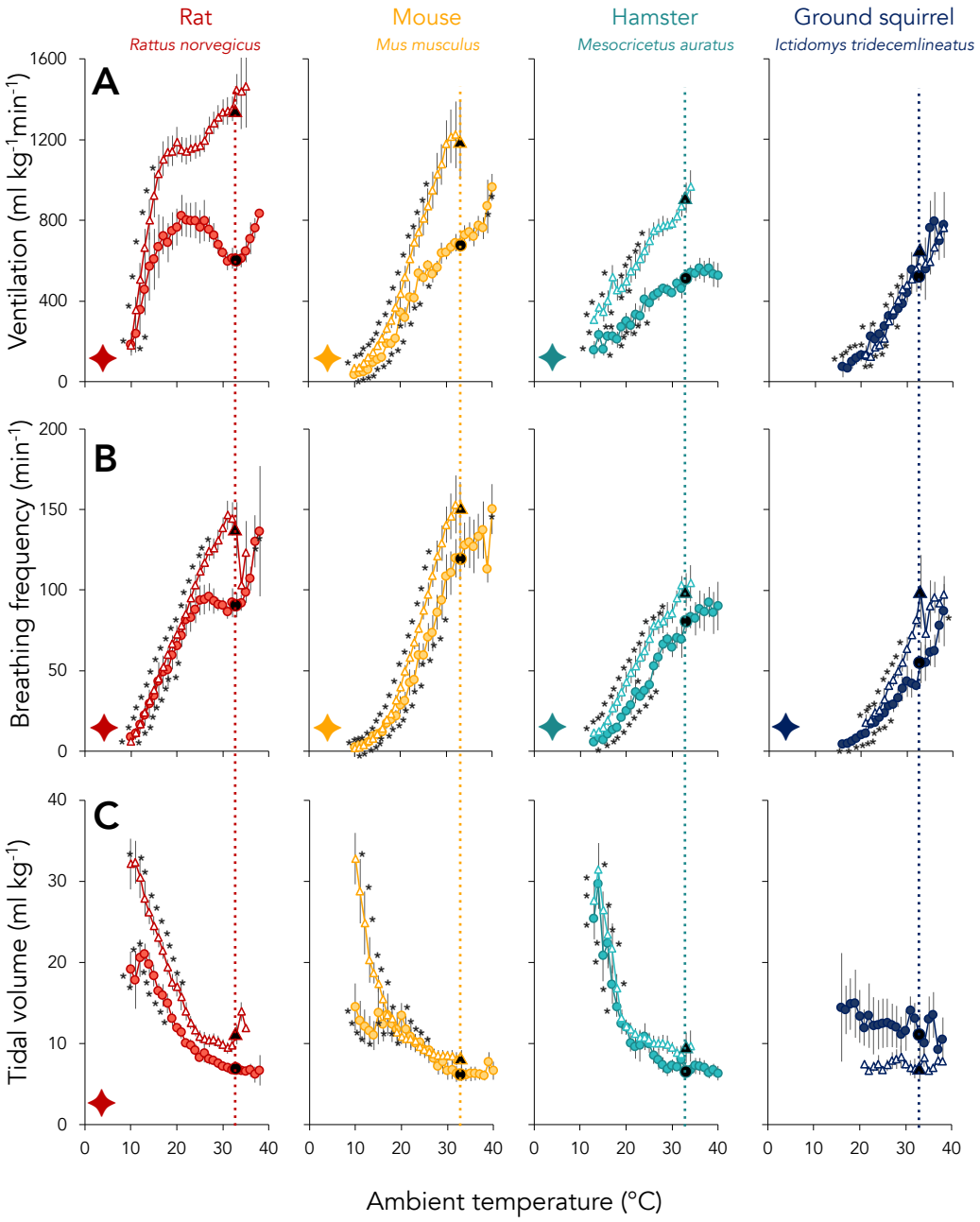

Figure 3.2. Effects of progressive cooling on **(A)** ventilation (ml $kg^{-1}min^{-1}$), **(B)** breathing frequency (min^{-1}), and **(C)** tidal volume (ml kg^{-1}) of normoxic (21% O_2; filled circles) and hypoxic (7% O_2; open triangles) newborn homeothermic and heterothermic rodents. Symbols are as follows: dashed lines and black symbols mark the preferred ambient temperature (33°C) of newborn rodents. An asterisk (*) indicates a significant difference from the preferred ambient temperature of newborn rodents. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=7: hypoxia: n=11. Facultative heterotherms: mice, normoxia: n=9: hypoxia: n=6; hamsters: n=8: hypoxia: n=9. Obligate heterotherms: ground squirrels, normoxia: n=4: hypoxia: n=4.

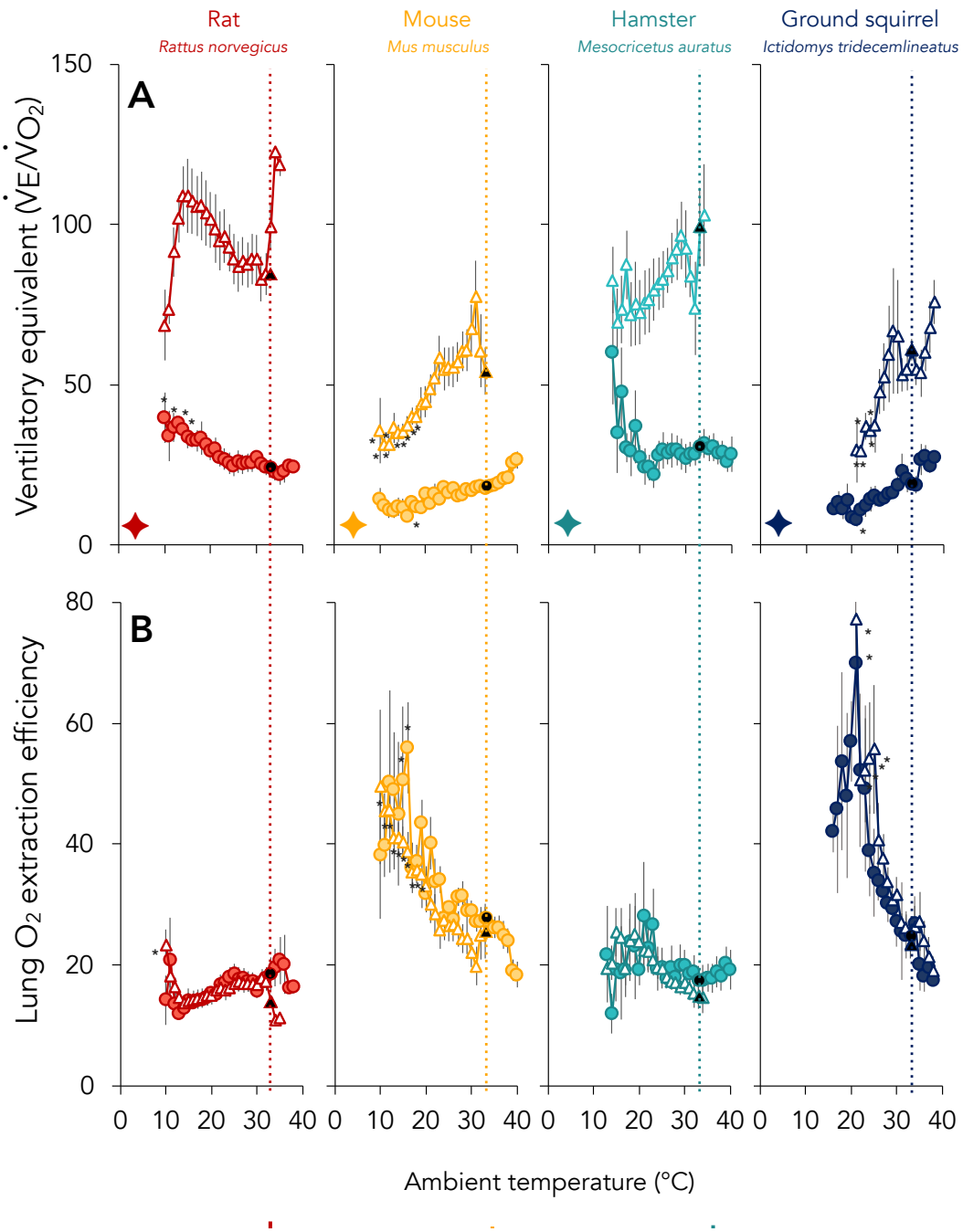

Figure 3.3. Effects of progressive cooling on **(A)** the ventilatory equivalent, and **(B)** lung O_2 extraction efficiency (%) of normoxic (21% O_2; filled circles) and hypoxic (7% O_2; open triangles) newborn homeothermic and heterothermic rodents. Symbols are as follows: dashed lines and black symbols mark the preferred ambient temperature (33°C) of newborn rodents. An asterisk (*) indicates a significant difference from the preferred ambient temperature of newborn rodents. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=7: hypoxia: n=11. Facultative heterotherms: mice, normoxia: n=9: hypoxia: n=6; hamsters: n=8: hypoxia: n=9. Obligate heterotherms: ground squirrels, normoxia: n=4: hypoxia: n=4.

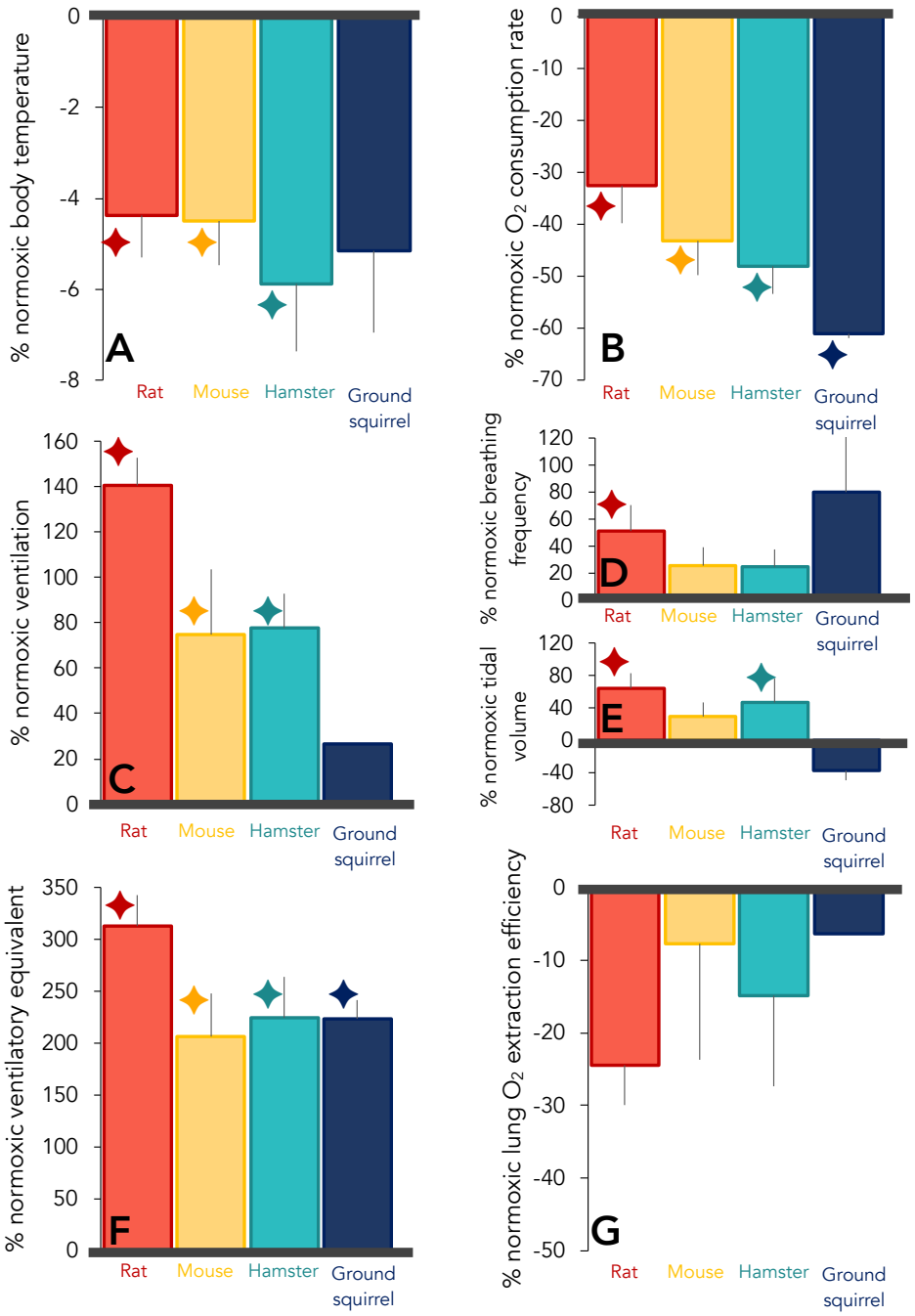

Figure 3.4. Hypoxic (7% O_2) **(A)** body temperature, **(B)** O_2 consumption rate, **(C)** ventilation, **(D)** breathing frequency, **(E)** tidal volume, **(F)** ventilatory equivalent, and **(G)** lung O_2 extraction efficiency represented as percent-change from normoxic values at the preferred ambient temperature of newborn homeothermic and heterothermic rodents. ✦indicates a significant difference between normoxic and hypoxic individuals of the same species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=7: hypoxia: n=11. Facultative heterotherms: mice, normoxia: n=9: hypoxia: n=6; hamsters: n=8: hypoxia: n=9. Obligate heterotherms: ground squirrels, normoxia: n=4: hypoxia: n=4.

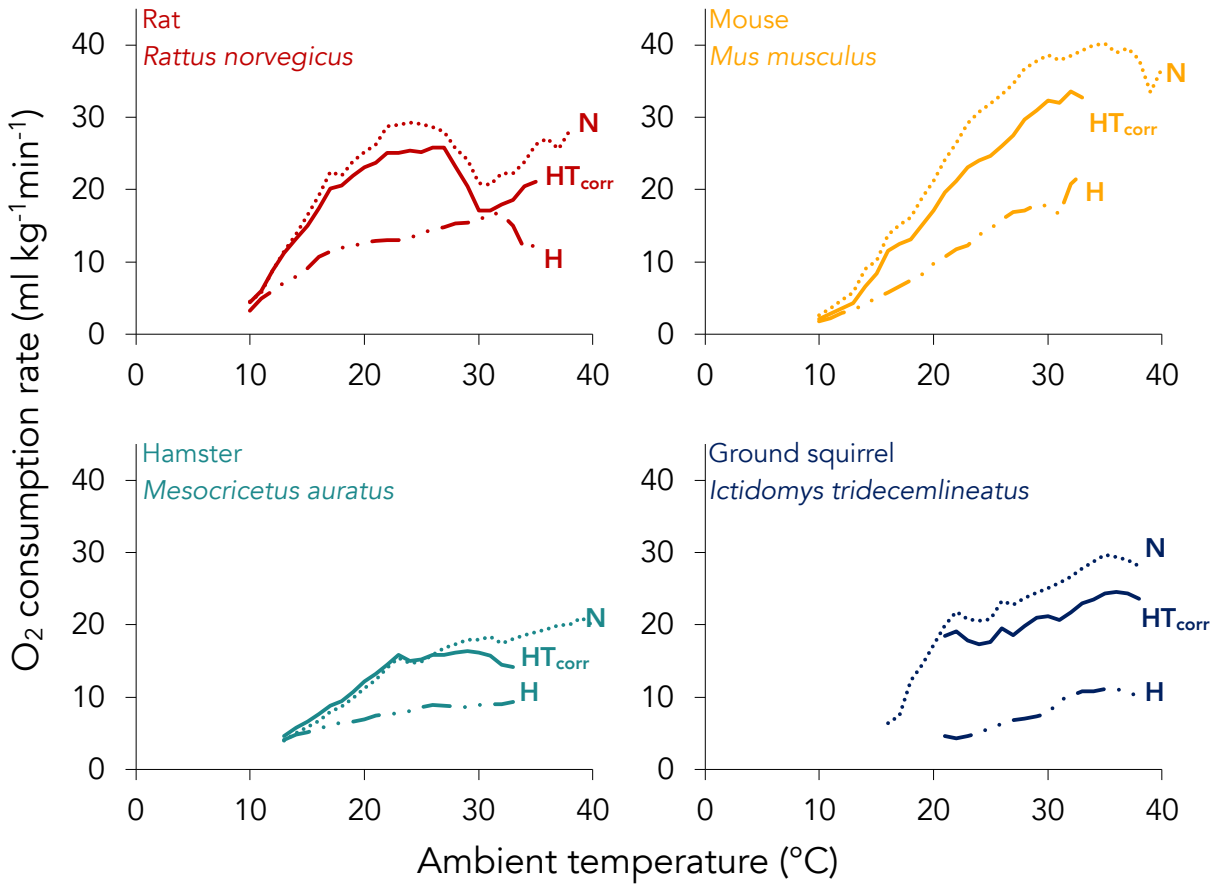

Figure 3.5. Effects of progressive cooling on normoxic (21% O_2; dotted lines; N) and hypoxic (7% O_2; dashed lines; H) O_2 consumption rates (ml $kg^{-1}min^{-1}$) of newborn homeothermic and heterothermic rodents. Solid lines represent hypothetical hypoxic O_2 consumption rates for the drop in body temperature in hypoxia from normoxia if a Q_{10} of 3 is assumed (HT_{corr}). Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats, normoxia: n=7: hypoxia: n=11. Facultative heterotherms: mice, normoxia: n=9: hypoxia: n=6; hamsters: n=8: hypoxia: n=9. Obligate heterotherms: ground squirrels, normoxia: n=4: hypoxia: n=4.

Chapter 4: The effects of progressive hypoxia on matching O_2 supply and O_2 demand in rodents within their thermoneutral zone

4.1 Introduction

Many animals have adapted to living in hypoxia and have evolved various responses for matching O_2 supply and O_2 demand in low O_2 environments (for a review see Dzal et al. 2015). Some animals remain active in hypoxia and mitigate the reduction in O_2 availability by increasing ventilation, the HVR (e.g. Scott 2011). Other animals conform to reduced O_2 availability, and reduce O_2 demand by suppressing their O_2 consumption rate, the HMR (e.g. Frappell et al. 1992; Barros et al. 2001; Mortola 2004). What little data there are indicate that both homeothermic and heterothermic newborn rodents respond to hypoxia primarily by reducing O_2 demand (Cross et al. 1958; Mortola et al. 1989; Mortola 1991; Mortola and Dotta 1992; Mortola 2004). However, even as newborns, heterotherms exhibit a greater HMR than homeotherms (Chapter 3).

As homeothermic rodents develop into adults they maintain energy balance in hypoxia by changing from a reliance on decreasing O_2 demand as a newborn to a reliance on increasing O_2 supply (via an increase in ventilation) as an adult (Mortola 1991; Frappell et al. 1992; but see Chapter 2). This developmental transition coincides with a reduction in hypoxia tolerance (Avery and Johlin 1932; Kabat 1940; Glass et al. 1944; Adolph 1969; for a review see Singer 1999 and references within). Adult heterotherms, on the other hand, appear to be more similar to newborn rodents, both relying on their remarkable ability to reduce O_2 demand in response to hypoxia (McArthur and Milsom 1991; Mortola 1991; Frappell et al. 1992; Frappell and Mortola 1994; Barros et al. 2001; Tattersall and Milsom 2009; Chapter 2). However, adult heterotherms also increase ventilation in response to hypoxia (McArthur and Milsom 1991; Mortola 1991; Frappell et al. 1992; Frappell and Mortola 1994; Barros et al. 2001; Tattersall and Milsom 2009; Chapter 2). Interestingly, adult heterotherms do not exhibit a decrease in hypoxia tolerance with development, or at least not to the same extent as adult homeotherms (Avery and Johlin 1932; Kabat 1940; Glass et al. 1944; Adolph 1969; for a review see Singer 1999 and references within). Thus, it is conceivable that the ability to reduce O_2 demand in response to lowered O_2 availability reflects a response retained from neonatal life and may explain the enhanced hypoxia

tolerance of newborns and adult heterotherms. However, data on adult heterotherms are scarce, as are comparable data on newborn homeotherms and heterotherms.

In view of this, it was my intent to determine how rodents that range in heterothermic expression match O_2 supply and O_2 demand during progressive hypoxia as newborns and as adults to elucidate: (1) whether adult homeothermic and heterothermic rodents differ in how they match O_2 supply and O_2 demand in hypoxia; and if so (2) whether their responses reflect different developmental trajectories. If heterotherms retain physiological traits common to all newborns, I hypothesized that they would not change how they match O_2 supply and O_2 demand in hypoxia during postnatal development. Experiments were conducted on four species of newborn and adult rodents: rats (*Rattus norvegicus*), mice (*Mus musculus*), hamsters (*Mesocricetus auratus*), and ground squirrels (*Ictidomys tridecemlineatus*) (Table 4.1). All experiments on adults were conducted during the non-hibernating season when they were euthermic and active. Because body size, and state of development at the time of birth have an effect on the relative magnitude of the hypoxic response I conducted my experiments solely on small, altricial mammals, belonging to the order Rodentia. More importantly, I selected these species as they range in their expression of heterothermy as adults. The rat is a true homeotherm that is not known to use torpor and maintains a high body temperature and O_2 consumption rate independent of season and environmental conditions (Yoda et al. 2000). Mice and hamsters are facultative, non-seasonal heterotherms that use torpor at any time of the year when confronted with energetic stressors such as low ambient temperature (Ellison and Skinner 1992; Heldmaier et al. 1999), or limitations in food or energy reserves (Lyman 1948; Hudson and Scott 1979). The ground squirrel is an obligate heterotherm, using torpor seasonally. When exposed to cold during the non-hibernating, active season, obligate heterotherms generally increase heat production to maintain body temperature, similar to homeothermic species (Wang and Lee 2011). I predicted that in response to progressive hypoxia homeothermic rodents would go from decreasing O_2 demand (i.e. suppressing their O_2 consumption rate) as newborns, to increasing O_2 supply (i.e. increasing ventilation) as adults, and that facultative and obligate heterotherms would be similar as newborns and as adults, responding to hypoxia primarily with a decrease in O_2 demand. Because heterotherms are capable of greater reductions in body temperature and O_2 consumption rate than homeotherms, I predicted that newborn and adult heterotherms would exhibit a blunted HVR and an enhanced HMR compared to adult homeotherms. Coincident with

this, I hypothesized that hypoxia tolerance would be associated with a lower O_2 response threshold, and that the level of inspired O_2 at which a significant HVR was mounted, and the relative magnitude of this HVR, would be lowest in newborn mammals < obligate heterotherms < facultative heterotherms < homeotherms.

4.2 Methods

4.2.1 Experimental animals and housing

Pregnant and non-reproductive rats, mice, and hamsters were purchased from Charles River Laboratories (Portage, MI), while ground squirrels were live-trapped in June 2013 and 2014 in Carman, MB, Canada (49°30′ N, 98°01′ W) and transferred to the animal care housing facility at the University of British Columbia in Vancouver, BC, Canada. Ground squirrels were trapped with the approval of Manitoba Conservation and Water Stewardship, under wildlife scientific permit WB15027. All animals were provided with food and water *ad libitum*. Adult rats, mice, and hamsters were fed Purina Rodent Chow (no. 5001, Purina LabDiet, St. Louis, MO), while ground squirrels were given 1:1 IAMS dog food (Mars Incorporated, Mount Olive, NJ) and Purina Rodent Chow supplemented with sunflower seeds, fruit, and vegetables. Pregnant and non-reproductive adults were housed in an environmental chamber, held under a 07:00 to 19:00 photoperiod cycle, an ambient temperature of 21 to 23°C, and 45-55% relative humidity. Rodents were born at the animal care holding facility and housed with their mothers until the day of the experiment. Animals were not fasted prior to experiments. All experimental procedures in this study conformed to the guidelines of the Canadian Council on Animal Care and were approved by the UBC Committee on Animal Care (under protocol A13-0091).

4.2.2 Experimental protocol

On the day of the experiment an individual newborn or adult rodent was instrumented with a thermal probe, and placed into a 20 to 30 ml, or 200 to 1000 ml chamber depending on the size of the animal. The animal chamber was flushed with normoxic air (21% O_2) and set inside a temperature-controlled environmental chamber set to 32.49 ± 0.09°C for newborn rodents, and

26.24 ± 0.18°C for adult rodents. The selected ambient temperature for newborns was similar to the ambient temperature within their litter and within the TNZ of newborn rats (refer to Chapter 3 in this thesis). The selected ambient temperature for adults was an ambient temperature within the TNZ, or an ambient temperature at which O_2 consumption rate and body temperature did not statistically differ from that within the TNZ of all species investigated (refer to Chapter 2 in this thesis). Thus, throughout this thesis I refer to these ambient temperatures as within the newborns' and adults' TNZ. Ambient temperature was continuously recorded throughout the experiment using a temperature datalogger (iButton Maxim Integrated, Chandler, CA, USA) placed inside the animal chamber. Animals were given at least one hour to acclimate to the experimental setup, followed by baseline recordings of O_2 consumption rate, body temperature, and ventilation in normoxia. To determine how rodents match O_2 supply and O_2 demand in hypoxia, metabolic, thermoregulatory, and ventilatory measurements were collected at different levels of inspired O_2. The fractional O_2 composition of gas introduced into the chamber was reduced in a progressive, stepwise manner, with each rodent being exposed to 21%, 12%, 9%, and 7% O_2. Rodents were held in each condition until O_2 consumption rate, body temperature, and ventilation reached a steady state (~ 45 minutes), after which the O_2 was reduced to the next level. The most severe level of hypoxia (7% O_2) was followed by a normoxic recovery period. Each experimental trial lasted ~ 4 hours (230 ± 8 minutes for newborns; 269 ± 12 minutes for adults). Across the 4 species, a total of 46 newborns and 26 adults were used in these experiments (Table 4.1).

4.2.3 Body temperature measurements

Prior to being placed into the animal chamber, each individual was instrumented with a sterile, 0.64 mm diameter copper-constantan thermocouple wire (Physitemp IT-18, Clifton, NJ, USA). The thermal probe was calibrated with a National Bureau of Standards certified thermometer, and was used to determine the body temperature of rodents with changes in inspired O_2.

In newborns, prior to thermal probe insertion the area around the anus was disinfected with an antiseptic (Betadine®, Purdue Pharma, Stamford, CT, USA), and 95% ethanol. Following disinfection, a topical anaesthetic (2% Xylocaine®, AstraZeneca Canada Inc., Mississauga, ON, Canada) was administered to the area around the anus using a cotton swab.

The tip of the thermal probe was lubricated and inserted about 5 to 10 mm into the animal's rectum. The thermal probe was secured to the animal's skin using tissue adhesive (3M™ Vetbond™, 3M, London, ON, Canada). The procedure took less than 10 minutes. Following each experimental trial, the insertion site was disinfected, the topical anaesthetic administered, and the thermal probe removed.

In adults, each animal was anaesthetized by gaseous inhalation of isoflurane (1-5% isoflurane: 21% O_2 mixture; flow rate = 1-5 L min^{-1}). Once the animal was anaesthetized, the fur over the abdomen and between the shoulder blades was cropped, treated with a depilatory cream, and the skin underneath was cleaned with distilled water, and disinfected with an antiseptic surgical scrub (Betadine®), followed by 95% ethanol. Two small subcutaneous incisions (< 0.5 cm) were made, one in the cleaned abdominal area, and another in between the animal's shoulder blades. A 16.5 G needle was inserted into the peritoneal cavity through the incision site in the cleaned abdominal area of the animal. The thermal probe was fed through the 16.5 G needle, and once ~1 cm of the thermal probe was within the peritoneal cavity, the 16.5 G needle was removed and the thermal probe was sutured to the skin using prolene sutures. The thermal probe was fed through to the shoulder blades via a 30 cm long trocar needle. The trocar needle ran subcutaneously from the incision site in the abdominal area, through the back of the animal, and exited at the cleaned incision site between the shoulder blades. Once the thermal probe was fed through to the shoulder blades, the trocar needle was removed, and both incision sites were sutured shut. Placement of the thermal probe in this location minimized the likelihood of the animal tugging, chewing, or removing the thermal probe during the experiment. The procedure took less than 20 minutes to complete. Surgical analgesic (Metacam (Boehringer Ingelheim Vetmedica Inc., Duluth, Georgia, USA: 1-5 mg kg^{-1}) was administered subcutaneously, pre-operatively. All animals were fully recovered from anesthesia (~2 hours) before initiating experiments.

4.2.4 O_2 consumption rate measurements

Oxygen consumption rate was determined using open-flow respirometry. The advantage of an open-flow system is that the animal chamber is constantly flushed with air so that O_2 depletion and CO_2 accumulation are avoided, and O_2 consumption rate and ventilation can be

continuously and simultaneously monitored. Newborn and adult rodents were placed in their individual chambers, with incurrent airflow pushed through each individual chamber at a flow rate of 8 to 34 ml min^{-1} for newborns, and 150 to 1100 ml min^{-1} for adults, using calibrated flowmeters (PRSFM4302-1; Praxair Technology Inc., Danbury, CT, USA). The incurrent airflow was adjusted for each individual to ensure that neither the O_2 concentration nor CO_2 concentration of the air leaving the chamber were altered more than 1% by the animal's metabolism. Different levels of hypoxia were achieved by mixing compressed dry air and nitrogen in appropriate ratios using the calibrated flowmeters. The incurrent and excurrent airflow, as well as the composition of the gas mixture were continuously monitored using a Field Metabolic System (Sable Systems, Las Vegas, NV, USA) with a built in flowmeter and O_2 and CO_2 gas analyzers. The gas analyzers were calibrated for O_2 and CO_2 before and after each experiment, with dry commercial air (Praxair Technology Inc., Danbury, CT, USA) and a premixed gas mixture (1.55% CO_2 balanced with N_2; Praxair Technology Inc., Danbury, CT, USA), respectively. A subsample, or entire excurrent flow was passed through a desiccant media (DM-060-24; Perma Pure LLC, Toms River NJ, USA) before entering the cells of the O_2 analyzer and CO_2 analyzer. Incurrent gas concentrations and gas flow were checked before and after exposure to each O_2 level. Oxygen consumption rate was calculated using equation 10.6 in Lighton (2008):

$$\dot{V}O_2 = \dot{V}i\,[(FiO_2 - FeO_2) - FeO_2\,(FeCO_2 - FiCO_2)]/(1 - FeO_2) \qquad (1)$$

where $\dot{V}O_2$ is O_2 consumption rate (ml min^{-1}), $\dot{V}i$ is incurrent flow rate (ml min^{-1}), FiO_2 and $FiCO_2$ are fractional concentrations of incurrent O_2 and CO_2 of dry gas, respectively, and FeO_2 and $FeCO_2$ are fractional concentrations of excurrent O_2 and CO_2 of dry gas, respectively. Barometric pressure and chamber temperature were used to convert O_2 consumption rate measurements to STPD.

4.2.5 Ventilatory measurements

In newborns, ventilatory measurements were acquired using head-out plethysmography as described by Mortola and Frappell (2013). Newborns were separated from their mothers on

the day of the experiment, and placed into a head-out chamber. The animal's muzzle protruded through a small hole in a pliable piece of latex, which provided a tight seal around the animal's muzzle, and separated the chamber into two parts: a head compartment, and a body compartment. The head compartment of the chamber was connected to a pneumotachograph and a differential pressure transducer (DP103-18; Validyne, Northridge, CA, USA) was used to measure ventilation-induced pressure oscillations. To determine tidal volume from pressure oscillations, the system was calibrated at the beginning of each experimental trial by injecting and withdrawing known volumes of air (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 ml) at a rate similar to the animal's breathing frequency. Tidal volume was calculated by integrating expiratory flow.

In adults, ventilatory measurements were acquired using barometric whole-body plethysmography, in an open-flow system (e.g. Chapin 1954; Drorbaugh and Fenn 1955; Jacky 1978; Jacky 1980; Mortola and Frappell 2013). This method relies on the difference in ambient temperature and humidity between the chamber and experimental animal, and is therefore an unsuitable technique for measuring ventilation in animals that do not have a Tb-Ta differential (i.e. newborn rodents). The experimental set-up consisted of two identical Plexiglass chambers, one being the designated animal chamber, and the other the designated reference chamber. Individuals were placed, unrestrained, within the animal chamber. Ventilation-induced pressure oscillations were detected with a differential pressure transducer (DP103-18; Validyne, Northridge, CA, USA) connected between the animal and reference chamber. As the animal breathed within its chamber it resulted in pressure oscillations induced by the warming and humidification of inspired air, these pressure oscillations were compared to the pressure of the reference chamber. To determine tidal volume, the system was calibrated with the animal present in the chamber, as described by McArthur and Milsom (1991). To determine tidal volume from pressure oscillations, the system was calibrated prior to each experiment by injecting and withdrawing known volumes of air (0.2 to 2.0 ml) into the animal chamber at a rate similar to the animal's breathing frequency to produce pressure oscillations at least 10 times as great as that of the animal's breathing. Tidal volume was calculated by integrating expiratory ventilatory flow, and using the equation of Drorbaugh and Fenn (1955) modified for flow-through plethysmography by Jacky (1978). In both newborn and adult rodents, breathing frequency was calculated directly from the ventilation-induced pressure oscillations, while ventilation was

calculated from the product of tidal volume and breathing frequency. All ventilatory measurements are reported at BTPS.

4.2.6 Calculation of O_2 delivery, the ventilatory equivalent and lung O_2 extraction efficiency

The ventilatory equivalent (the quotient of ventilation and O_2 consumption rate), and the percent of O_2 extracted by the lung from each breath were calculated from ventilatory and O_2 consumption rate measurements. Oxygen delivery to the lungs was calculated as ventilation multiplied by the fractional concentration of O_2 in inspired air. To determine the percent of O_2 extracted from each breath O_2 consumption rate was divided by ventilation multiplied by the fractional concentration of O_2 in inspired air (O_2 delivery to the lungs) and multiplied by 100.

4.2.7 Data collection and analysis

All signals (body temperature, incurrent and excurrent O_2 concentration, and the ventilation-induced pressure signal) were amplified, filtered, and recorded onto a PowerLab 8/35 data acquisition system (ADInstruments Pty Ltd., Colorado Springs, CO, USA). At each inspired O_2 level I determined average values for each dependent variable for the last 10 minutes of steady state recordings. The dependent variables analysed were: O_2 consumption rate, body temperature, breathing frequency, tidal volume, ventilation, O_2 delivery, the ventilatory equivalent, and lung O_2 extraction efficiency for the entire period the animal was exposed to each ambient temperature.

Statistical analyses were performed using R (R Core Team 2017, Vienna, AT, EUR). I used linear mixed effects models (lme4 package in R; Bates et al. 2015) to account for repeated sampling of the same individual with changes in inspired O_2, with individual treated as a random effect. When visual inspection of residuals, and q-q plots revealed deviations from the assumptions of linear mixed effects models (normality, homogeneity of variances, linearity, and independence), I log transformed the dependent variable. Oxygen level, age, species, body mass, and sex were fixed effects in my initial models. I tested all 2- and 3-way interactions of O_2 level, age, and species. When body mass or sex did not have a significant effect on the dependent variables they were removed from the model. I did not remove any other terms from my models

given the importance of all independent variables and interactions to my research objectives. I used lmerTest in R to obtain F-statistics and *P*-values for each model (Kuznetsova 2016). Following the methods of Fangue et al. (2009), when interaction terms were significant the data were separated and analyzed independently using a one-way ANOVA, followed by a Tukey-Holm post-hoc analysis to determine differences in dependent variables between O_2, age, and species of rodents, correcting for multiple pairwise comparisons. All results are presented as mean ± s.e.m., with statistical significance set as $P<0.05$.

4.3 Results

4.3.1 Newborn and adult rodents exhibit a progressive reduction in O_2 consumption rate as inspired O_2 levels fall.

All rodents progressively reduced O_2 consumption rate as inspired levels of O_2 were reduced. However, there was a significant 3-way interaction between O_2 level, age, and species on O_2 consumption rates ($F_{12,217}=3.4$, $P<0.001$; Fig. 4.1A). All adult rodents had a higher normoxic mass-specific O_2 consumption rate than their newborn counterparts (Fig. 4.1.A). During exposure to progressive hypoxia, all newborn rodents exhibited a significant reduction in their O_2 consumption rate, reaching values 45-55% lower than normoxic values when breathing 7% O_2 (Fig. 4.1A). Adult rodents did not reduce their O_2 consumption rates from normoxic values as much as newborns, and exhibited species differences in the relative magnitude of their HMR. At 7% O_2 adults of all species, but the ground squirrel, exhibited a modest HMR, reducing their O_2 consumption rate by 30-40% relative to normoxic levels (Fig. 4.1A). Adult ground squirrels, on the other hand, reduced their rate of O_2 consumption by only 15% (Fig. 4.1A).

In all groups, this hypoxia-induced reduction in the rate of O_2 consumption was only in part due to a hypoxia-induced reduction in body temperature (Fig. 4.1B). There was a significant 3-way interaction between O_2 level, age, and species on body temperature ($F_{12,218}=3.8$, $P<0.0001$; Fig. 4.1B). Newborn rats and mice significantly reduced their body temperature during progressive hypoxia, whereas newborn hamsters and ground squirrels maintained their body temperature at each level of hypoxia (Fig. 4.1B). All adults, except for the ground squirrel, exhibited a greater hypoxia-induced reduction in body temperature than their newborn

counterparts (Fig. 4.1B). Note, that the Tb-Ta differential for newborns was extremely small. Just as in newborn ground squirrels, adult ground squirrels maintained their body temperature as inspired levels of O_2 were reduced (Fig. 4.1B). In both newborns and adults, the drop in body temperature was greatest in the rat, and least in the ground squirrel, with no species dropping their body temperature by more than 4°C (Fig. 4.1B).

4.3.2 Newborn and adult rodents progressively increase ventilation with reductions in inspired O_2, however, the relative magnitude of the HVR varies with age and species

All rodents progressively increased ventilation as inspired air was reduced down to 7% O_2. However, the O_2 response threshold, and the relative magnitude of this HVR varied with O_2 level, age, and species ($F_{12,218}$=2.1, P<0.05; Fig. 4.2A). At 7% O_2 all species, except for the ground squirrel, exhibited a modest 80-150% increase in ventilation from normoxic rates, with no difference in the relative magnitude of these responses between newborns and adults of any species (Fig. 4.2A). Unlike the other species, ground squirrels exhibited a 55% increase in ventilation as newborns, but as adults significantly increased their ventilation by more than 350% (Fig. 4.2A). In newborn and adult rats, the changes in ventilation were primarily due to increases in tidal volume, as breathing frequency only increased by 12-40%, whereas in newborn and adult mice, hamsters, and ground squirrels, changes in ventilation were primarily due to changes in breathing frequency, as tidal volume only increased by 15-40%, and in the case of the adult hamster tidal volume even decreased (Fig. 4.2B&C). These trends gave rise to a significant 3-way interaction between O_2 level, species, and age on breathing frequency ($F_{12,223}$=4.6, P<0.0001; Fig. 4.2B) and tidal volume ($F_{12,213}$=2.2, P<0.05; Fig. 4.2C).

Newborn rodents exhibited species differences in their ventilatory O_2 response threshold, increasing ventilation when inspired O_2 levels fell to 9% O_2 in hamsters, and 7% O_2 in rats, mice and ground squirrels (Fig. 4.2A). Postnatal development also seemed to affect the ventilatory O_2 response threshold of species differently. Adult rats and mice had a higher ventilatory response threshold as an adult than as a newborn (Fig. 4.2A). The ventilatory O_2 response thresholds of hamsters and ground squirrels did not differ between newborns and adults (Fig. 4.2A).

4.3.3 Hypoxia increases the ventilatory equivalent of newborn and adult rodents, however,

the relative magnitude of this increase varies with species and age

All rodents significantly increased ventilation as inspired O_2 was reduced (Fig.4.3A). However, adult ground squirrels were the only group that mounted a ventilatory response large enough to maintain O_2 delivery to the lungs when environmental O_2 levels plummeted ($F_{4, 219}$=3.4, P<0.05; Fig. 4.3A&B). There was no significant 3-way interaction between O_2 level, species, and age on O_2 delivery (P= 0.18; Fig. 4.3B).

All rodents progressively increased ventilation relative to their O_2 consumption rate when inspired O_2 was reduced down to 7% O_2, with a significant interaction between O_2 level, species, and age on their ventilatory equivalent ($F_{12,223}$=4.0, P<0.0001; Fig.4.4A). Rats and ground squirrels exhibited age-related differences in their ventilatory equivalent (Fig. 4.4A). Compared to normoxic values, rats exposed to 7% O_2 increased their ventilatory equivalent by more than 500% as newborns, and only 65% as adults, whereas the opposite was true in ground squirrels (Fig. 4.4A). Compared to normoxic values, ground squirrels exposed to 7% O_2 increased their ventilatory equivalent by 175% as newborns, and more than 400% as adults (Fig. 4.4A). Mice and hamsters maintained a relative hypoxia-induced increase in their ventilatory equivalent as newborns and adults (Fig. 4.4A). When exposed to 7% O_2, both species as newborns and adults increased their ventilatory equivalent by ~250% compared to normoxic values (Fig. 4.4A). Lung O_2 extraction efficiency was significantly affected by the interaction between O_2 level, species, and age ($F_{12,220}$=2.0, P<0.05; Fig. 4.4B). However, at 7% O_2 newborn and adult rats were the only groups to exhibit a significant decrease in the amount of O_2 extracted at the lung, with all other groups generally maintaining their lung O_2 extraction efficiency (Fig. 4.4B).

4.4 Discussion

If heterotherms retain physiological traits common to all newborns, I hypothesized that they would not change how they match O_2 supply and O_2 demand in hypoxia during postnatal development. I predicted that the newborns of all species would respond to progressive hypoxia by reducing their rate of O_2 consumption, and this was the case. Relative to normoxic values, all newborn rodents exhibited significant reductions in their O_2 consumption rate of 45-55% when breathing 7% O_2. I predicted that in response to progressive hypoxia homeothermic rodents

would go from primarily decreasing O_2 demand (i.e. suppressing O_2 consumption rate) as newborns, to primarily increasing O_2 supply (i.e. increasing ventilation) as adults, and that facultative and obligate heterotherms would respond to hypoxia like the newborns, primarily decreasing their metabolic demand for O_2. Contrary to my predictions, I found that during progressive hypoxia, both newborns and adults of all species reduced O_2 demand and increased O_2 supply, although homeotherms and heterotherms did so to varying degrees.

As predicted, newborns relied on a decrease in their metabolic demand for O_2 more than their adult counterparts. Contrary to my predictions, however, newborns did not solely rely on a reduction in O_2 demand in hypoxia, nor did they exhibit a blunted HVR compared to that of their adult counterparts. As a result, and also contrary to my predictions, the only rodent to exhibit an enhanced HVR was the adult obligate heterotherm, the ground squirrel, and not the adult homeothermic rat. Lastly, while hypoxia tolerance (based on literature values) did correlate with a lower O_2 response threshold, with newborns and adult heterotherms exhibiting lower O_2 thresholds than adult homeotherms, it did not correlate with a lower hypoxic sensitivity.

I conclude that the basis of enhanced hypoxia tolerance of newborn and adult heterothermic rodents is not due to differences in the way in which they match O_2 supply and O_2 demand at the whole-animal level, nor is it associated with a lower hypoxic sensitivity, at least not at ambient temperatures within their TNZ.

4.4.1 Effects of progressive hypoxia on O_2 demand

At rest, most newborn mammals have a higher mass-specific O_2 consumption rates than their adult counterparts (Kleiber 1975). It is possible that newborns require these high O_2 consumption rates for tissue growth, but also the maintenance of body temperature, as their large body surface to body mass ratio encourages heat loss. However, in species that weigh less than 1 kg as adults, the opposite is more common (Mortola 1991; Mortola 2004). All species in the present study weigh less than 1 kg as adults (Table 4.1), and as newborns exhibited a lower mass-specific O_2 consumption rate than their adult counterparts. The reason behind this phenomenon remains unknown.

In hypoxia, most small mammals exhibit a rapid and reversible decrease in their rate of O_2 consumption, and thus O_2 demand (Frappell et al. 1992). In the present study, this reduction

in the rate of O_2 consumption (the HMR) was found to be greater in newborn rodents (45 to 55%) than in adults (15 to 40%) of both homeotherms and heterotherms. Furthermore, the relative magnitude of the HMR was similar in newborns of all species, homeotherms and heterotherms, consistent with the results of earlier studies (Mortola et al. 1989; Frappell et al., 1992). In Chapter 3 I also found that all newborn rodents exposed to 7% O_2 at 33°C significantly reduced their rate of O_2 consumption relative to normoxic individuals, but in that study the relative magnitude of the hypoxic decrease in O_2 consumption rates was least in rats (30%), and greatest in the ground squirrels (60%), albeit these differences were not significant.

Previously, the relative magnitude of the HMR was shown to be greater in adult heterotherms than homeotherms (Frappell et al. 1992; Barros et al. 2001). For example, when exposed to 10% O_2 adult homeothermic rats reduced their O_2 consumption rate by 20% from normoxic values (Frappell et al. 1992) whereas, adult heterothermic mice and hamsters reduced their O_2 consumption rates by 30 and 50%, respectively (Frappell et al. 1992). Golden-mantled ground squirrels (*Spermophilus lateralis*) have also been found to reduce their O_2 consumption by 40% when exposed to 7% O_2 (Barros et al. 2001). To my surprise, I found no difference in the relative magnitude of the HMR between adult rats, mice, and hamsters exposed to 7% O_2 (all reduced their rate of O_2 consumption by 30 to 40%). In fact, in contrast to my predictions, adult ground squirrels (obligate heterotherms) reduced their O_2 consumption rate the least (15%). I obtained similar results in Chapter 2 when adults were exposed to 7% O_2 within their TNZ, although in Chapter 2 the falls in O_2 consumption rates were small not only in adult ground squirrels but adult rats and hamsters also exhibited a reduction in their rate of O_2 consumption of less than 15%, compared to normoxic values. Furthermore, adult mice exhibited greater falls in their O_2 consumption rates in Chapter 2 than in the present study (60% vs. 40% reduction in their rate of O_2 consumption compared to normoxic values). The reason for the differences between these two chapters is not clear but may reflect different time domains for the HMR (Bishop et al, 2001; Mortola and Seifert, 2000), as animals in Chapter 2 had been exposed to 7% O_2 for three hours, while those in the present study were exposed to progressive hypoxia and were only held at 7% O_2 for ~ 45 minutes.

The different results between the present study and previous studies on rats, mice and hamsters (Frappell et al., 1992) may stem from differences in the ambient temperature at which the experiments were run. Unlike the present study, Frappell et al., (1992) ran experiments at

22°C, an ambient temperature well below the TNZ of mice and hamsters, but within the TNZ of rats (see Chapter 2). Ambient temperature plays a pervasive role in determining the relative magnitude of the HMR; it has been shown to be more pronounced at ambient temperatures below the TNZ (Hill 1959; Dupré et al. 1988; Saiki et al. 1994; Barros et al. 2001). This is explored more fully in Chapter 5 of this thesis. Regardless of the explanation, my findings support the prediction that the enhanced hypoxia tolerance of newborn rodents relative to their adult counterparts may stem from an enhanced HMR, however, contrary to my predictions, the enhanced hypoxia tolerance of adult heterothermic rodents, at least within their TNZ, cannot.

4.4.2 Effects of progressive hypoxia on body temperature

Hypoxia exposure in mammals has been shown to result in a controlled reduction in the thermoregulatory set-point (Barros et al. 2001; Tattersall and Milsom 2009), and this hypoxia-induced reduction in body temperature reduces the rate of O_2 consumption. As noted above, in the present study the reductions in the rate of O_2 consumption (the HMR) were greater in newborn rodents (45 to 55%) than in adults (15 to 40%) of both homeotherms and heterotherms although no group dropped body temperature by more than 4°C at the most severe level of hypoxia (7% O_2). It is important to note that the ambient temperature for the newborns was only 4-5°C below their normoxic body temperature.

My findings are intriguing because they indicate that the reduction in O_2 consumption rate observed in all animals during hypoxia was not primarily due to a reduction in body temperature, but can only be explained by active physiological inhibition. Unfortunately, the mechanisms determining the reduction in O_2 consumption rate within the TNZ in these rodents are still not known. Potential mechanisms contributing to the hypoxia-induced reduction in O_2 consumption rate within the TNZ include: (i) inhibition of protein synthesis and protein degradation; (ii) ion channel arrest; (iii) increase in inhibitory neurotransmission; (iv) and suppression of substrate oxidation. Nevertheless, my data indicate that within the TNZ, reductions in body temperature do not play an important role in the strategies newborn and adult heterotherms use to match O_2 supply and O_2 demand.

4.4.3 Effects of progressive hypoxia on O_2 supply

Because newborns and adult heterotherms are capable of greater reductions in their O_2 consumption rate during energetic shortfalls than adult homeotherms, I predicted that newborns of both groups of animals would rely more on a reduction in O_2 demand during progressive hypoxia than on an increase in O_2 supply, and would exhibit a blunted HVR compared to adult homeotherms. Furthermore, if heterotherms retain physiological traits common to all newborn mammals, I predicted that in response to progressive hypoxia homeothermic rodents would go from primarily decreasing O_2 demand (i.e. suppressing O_2 consumption rate) as newborns, to primarily increasing O_2 supply (i.e. increasing ventilation) as adults, but that facultative and obligate heterotherms would still rely more on a decrease in O_2 demand than on an increase in ventilation. This is not what I found.

Contrary to my predictions, newborns did not solely rely on a reduction in O_2 consumption rate to match O_2 supply and O_2 demand in hypoxia, nor did they exhibit a blunted HVR compared to that of their adult counterparts. Among newborns, I found that rats exhibited the greatest HVR, whereas ground squirrels exhibited the smallest HVR. This is consistent with the hypothesis that heterotherms exhibit a blunted HVR, even at birth and consistent with data from Chapter 3. However, contrary to my predictions, as they developed into adults, the rat, mouse, and hamster continued to exhibit a similar HVR. Although the ground squirrel, an obligate heterotherm, exhibited the smallest HVR as a newborn, it exhibited an enhanced HVR as an adult; and not the homeothermic rat as I originally predicted. While these findings are in contrast to my predictions, similar data exist in the literature. While some studies have found that adult homeothermic rats have an enhanced HVR, compared to that of the heterothermic hamster (Mortola 1991; Frappell et al. 1992), others have found that adult homeothermic rats and heterothermic hamsters exhibit similar ventilatory responses to progressive hypoxia (Walker et al. 1985). Furthermore, adult golden-mantled and Columbian ground squirrels also exhibit a strong ventilatory response when exposed to progressive hypoxia during the euthermic season (McArthur and Milsom 1991). The relative magnitude of their reported HVR is similar to that reported here for the 13-lined ground squirrel, and even greater than that of other similar-sized adult homeothermic rodents (Cragg and Drysdale 1983; Holloway and Heath 1984).

As previously reported for most mammals, the hypoxia-mediated increase in ventilation in this study was achieved mainly through an increase in breathing frequency, as changes in tidal volume were variable (Dempsey and Forster 1982; Walker et al. 1985; Mortola et al. 1989;

McArthur and Milsom 1991). Generally, increases in breathing frequency prevail in moderate hypoxia, while an increase in tidal volume manifests itself at more severe levels of hypoxia. Collectively, my findings indicate that enhanced hypoxia tolerance of newborn and adult heterotherms is also not a result of an enhanced ability to increase ventilation in hypoxia, although it may be an important response employed by euthermic adult ground squirrels.

4.4.4 Matching O_2 supply and O_2 demand during progressive hypoxia

Most mammals increase ventilation with respect to their metabolic requirements when challenged with hypoxia (Mortola et al. 1989; Mortola 1991; Frappell et al. 1992; but see Pamenter et al. 2015), this can result from either an increase in ventilation or a reduction in O_2 consumption rate. Contrary to my predictions, homeothermic and heterothermic rodents did not differ in their reliance on the HMR or HVR to match O_2 supply and O_2 demand as newborns, nor as adults. During progressive hypoxia all rodents both reduced their metabolic demand for O_2 by reducing O_2 consumption rate, and increased O_2 supply by increasing ventilation. I had predicted that all newborns would primarily reduce O_2 consumption rate, and that this would also be the case for adult heterotherms. On the other hand, I predicted that adult homeotherms would primarily increase ventilation. To my surprise, it was the adult ground squirrels that exhibited an enhanced ability to maintain O_2 supply by increasing ventilation and exhibited a higher ventilatory equivalent than all other animals in my study.

Hyperventilation, as indicated by an increase in the ventilatory equivalent, is associated with hypocapnia, and a respiratory alkalosis, which tends to inhibit breathing. Although the mechanism behind the ground squirrel's enhanced HVR in hypoxia is unclear, it is conceivable that the adult ground squirrel is less sensitive to changes in CO_2/pH and better able to sustain increases in ventilation during hypoxia than other animals. Collectively, my data indicate that the increase in the ventilatory equivalent within the TNZ was due to a 100-400% increase in ventilation, and a 40-60% drop in O_2 consumption rates in all animals, and not solely due to a reduction in O_2 consumption rate in newborn and adult heterothermic rodents as I originally predicted.

4.4.5 Hypoxia tolerance correlates with a lower O_2 response threshold but not a lower

sensitivity to hypoxia

The preceding data chapters (Chapters 2 and 3) examined the effects of a single level of severe environmental hypoxia (7% O_2) on the thermogenic response of small mammals. However, the same level of inspired O_2 does not mean a similar degree of hypoxemia in all species. Thus, in order to examine the hypoxic sensitivity and thresholds of newborn and adult homeothermic and heterothermic rodents, in the present study I exposed animals to a progressive reduction in environmental O_2. The low resting arterial O_2 levels typical of newborns and heterotherms (Musacchia and Volkert 1971; Lahiri 1975; Mortola 2001) suggest that their O_2 response threshold should be lower than that of adult homeotherms, i.e. it should require a lower level of O_2 to initiate a ventilatory response. Indeed, newborns had a lower O_2 response threshold than their adult counterparts. Furthermore, adult heterotherms exhibited a lower O_2 response threshold than adult homeothermic rats.

My results for the newborn and adult heterothermic rodents are not surprising given that their blood typically has a greater O_2-carrying capacity than that of homeothermic rodents (Deveci et al. 2001). Their hemoglobin concentration, hematocrit levels, and erythrocyte counts are all elevated (Musacchia and Volkert 1971; Maginniss and Milsom 1994; Deveci et al. 2001). Levels of organo-phosphates in their blood are reduced, causing a left-shifted O_2 equilibrium curve that further enhances their O_2-carrying capacity (Musacchia and Volkert 1971; Maginniss and Milsom 1994; Deveci et al. 2001). More importantly, heterothermic species have a high hemoglobin-O_2 affinity and a correspondingly low P_{50} (partial pressure of O_2 at which the blood is 50% saturated) with P_{50} values as low as 18 torr; half that of most mammals (measured at pHa 7.49 and 37°C; Maginniss and Milsom 1994). These hematologic adaptations suggest that it would take a lower level of inspired O_2 to desaturate the blood of heterotherms than that of homeotherms.

Although O_2 response sensitivities have been found to be slightly reduced in newborn rodents and adult heterotherms (Mortola et al. 1989; Mortola 1991; Frappell et al. 1992; Barros et al. 2001), other studies have found that O_2 response sensitivities of heterothermic species do not differ from values reported for homeothermic mammals (Mortola et al. 1989; McArthur and Milsom 1991; Frappell et al. 1992; Osborne and Milsom 1993). Contrary to my predictions, hypoxic sensitivity was not lower in newborns, and did not change with development in rats,

mice and hamsters. To my surprise, hypoxic sensitivity drastically increased during postnatal development in the ground squirrel, opposite to what I predicted (as discussed above). My findings indicate that while hypoxia tolerance does correlate with a lower O_2 response threshold, it does not correlate with a lower ventilatory sensitivity to hypoxia.

4.4.6 Conclusions

I report that the enhanced hypoxia tolerance of newborn and adult heterothermic rodents is not due to an enhanced ability to decrease O_2 demand, nor to an increased ventilatory response to hypoxia compared to homeothermic adult rodents. I found that all rodents, both as newborns and adults, reduced O_2 demand and increased ventilation while generally maintaining the amount of O_2 extracted at the lungs. Since this was true of both homeotherms and heterotherms my data do not support the "hibernator as a neonate" hypothesis. However, I only explored the first of many steps in the O_2 transport pathway that also includes diffusion of O_2 into the blood, circulation of O_2 throughout the body, and diffusion from the blood to the mitochondria in the tissues. Important adaptations that increase O_2 flux along this pathway could exist at any one of these steps, and could possibly explain the enhanced hypoxia tolerance in these animals.

Table 4.1. Species, number of individuals per treatment, sex, age, and body mass of newborn and adult homeothermic and heterothermic rodents exposed to progressive hypoxia (21, 12, 9, and 7% O_2) within their thermoneutral zone (TNZ; newborns: 33°C; adults: 26°C). Age and body mass are presented as mean ± s.e.m.

Species	Degree of heterothermy	Age group	Sample size		Age (days)	Body mass (g)
			female	male		
Rattus norvegicus rat	homeotherm	newborn	2	6	1.6 ± 0.5	9.1 ± 0.9
		adult	3	3	> 70	368 ± 46
Mus musculus mouse	facultative heterotherm	newborn	8	7	3.7 ± 0.4	3.1 ± 0.1
		adult	4	3	> 70	46 ± 6
Mesocricetus auratus hamster	facultative heterotherm	newborn	6	6	3.2 ± 0.4	5.0 ± 0.3
		adult	4	2	> 70	163 ± 5
Ictidomys tridecemlineatus ground squirrel	obligate heterotherm	newborn	8	3	3.7 ± 0.3	7.1 ± 0.5
		adult	5	2	> 70	258 ± 22

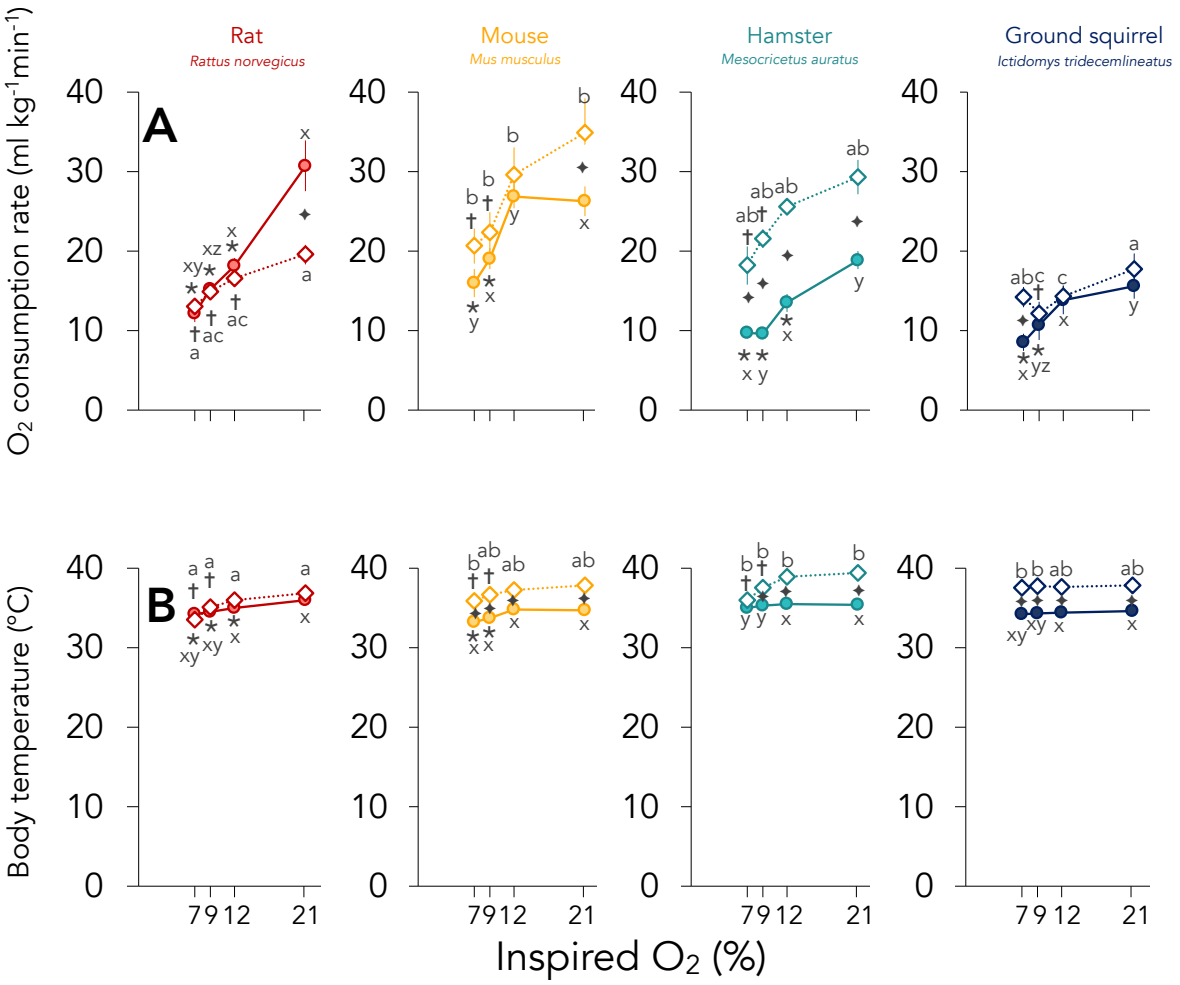

Figure 4.1. Effects of progressive hypoxia on **(A)** O_2 consumption rate (ml $kg^{-1}min^{-1}$), and **(B)** body temperature (°C) of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents at thermoneutrality (an ambient temperature of 33°C for newborns and 26°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦indicates a significant difference between newborns and adults of each species at each level of O_2. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=8, adults- n=6. Facultative heterotherms: mice (*Mus musculus*): newborns- n=15, adults- n=7; hamsters (*Mesocricetus auratus*): newborns- n=12, adults- n=6. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=11, adults-n=7.

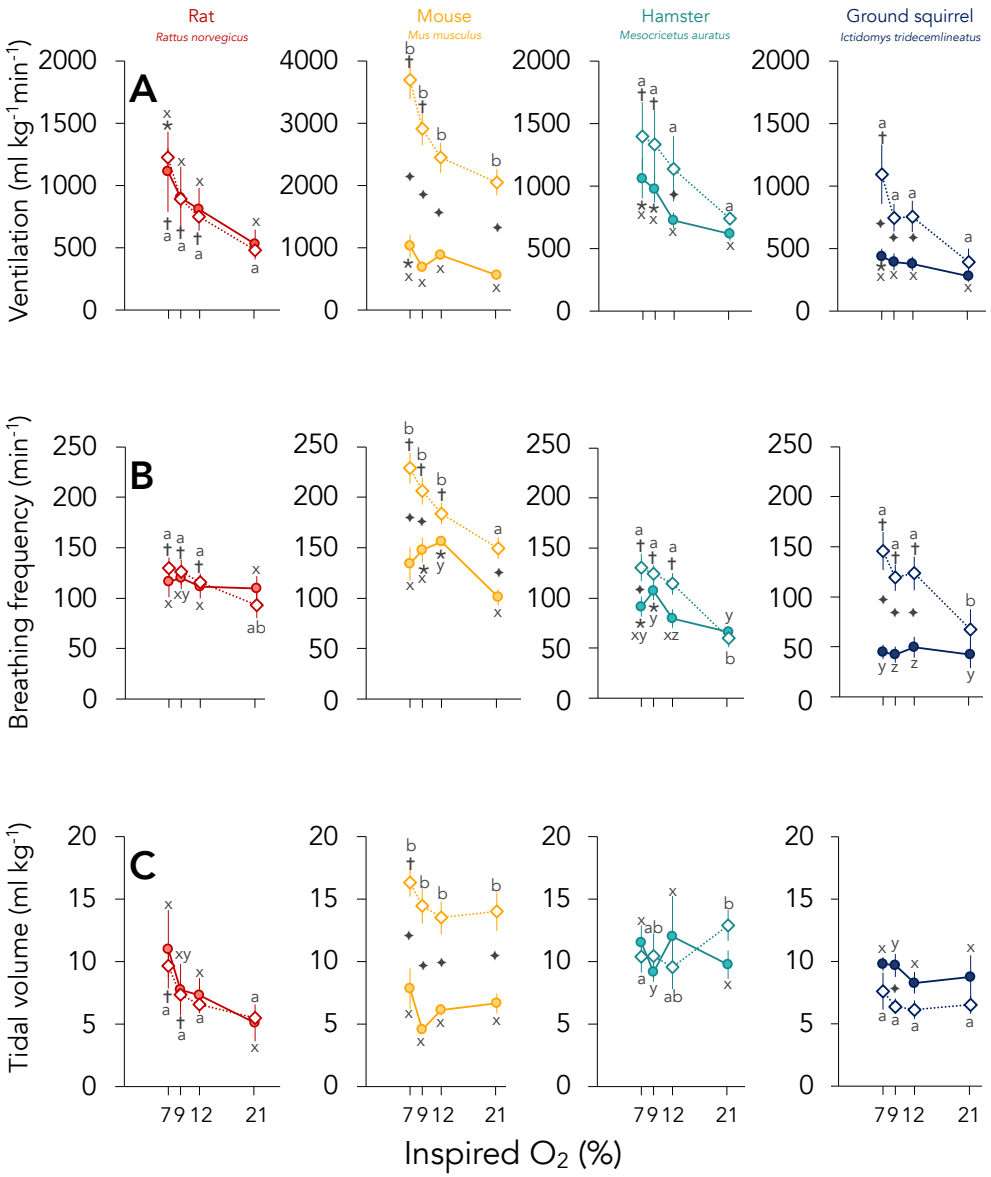

Figure 4.2. Effects of progressive hypoxia on **(A)** ventilation (ml $kg^{-1}min^{-1}$), **(B)** breathing frequency (min^{-1}), and **(C)** tidal volume (ml kg^{-1}) of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents at thermoneutrality (an ambient temperature of 33°C for newborns and 26°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦indicates a significant difference between newborns and adults of each species at each level of O_2. Note the y-axis in panel **(A)** for mouse ventilation is different from that of the other species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=8, adults- n=6. Facultative heterotherms: mice (*Mus musculus*): newborns- n=15, adults- n=7; hamsters (*Mesocricetus auratus*): newborns- n=12, adults- n=6. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=11, adults- n=7.

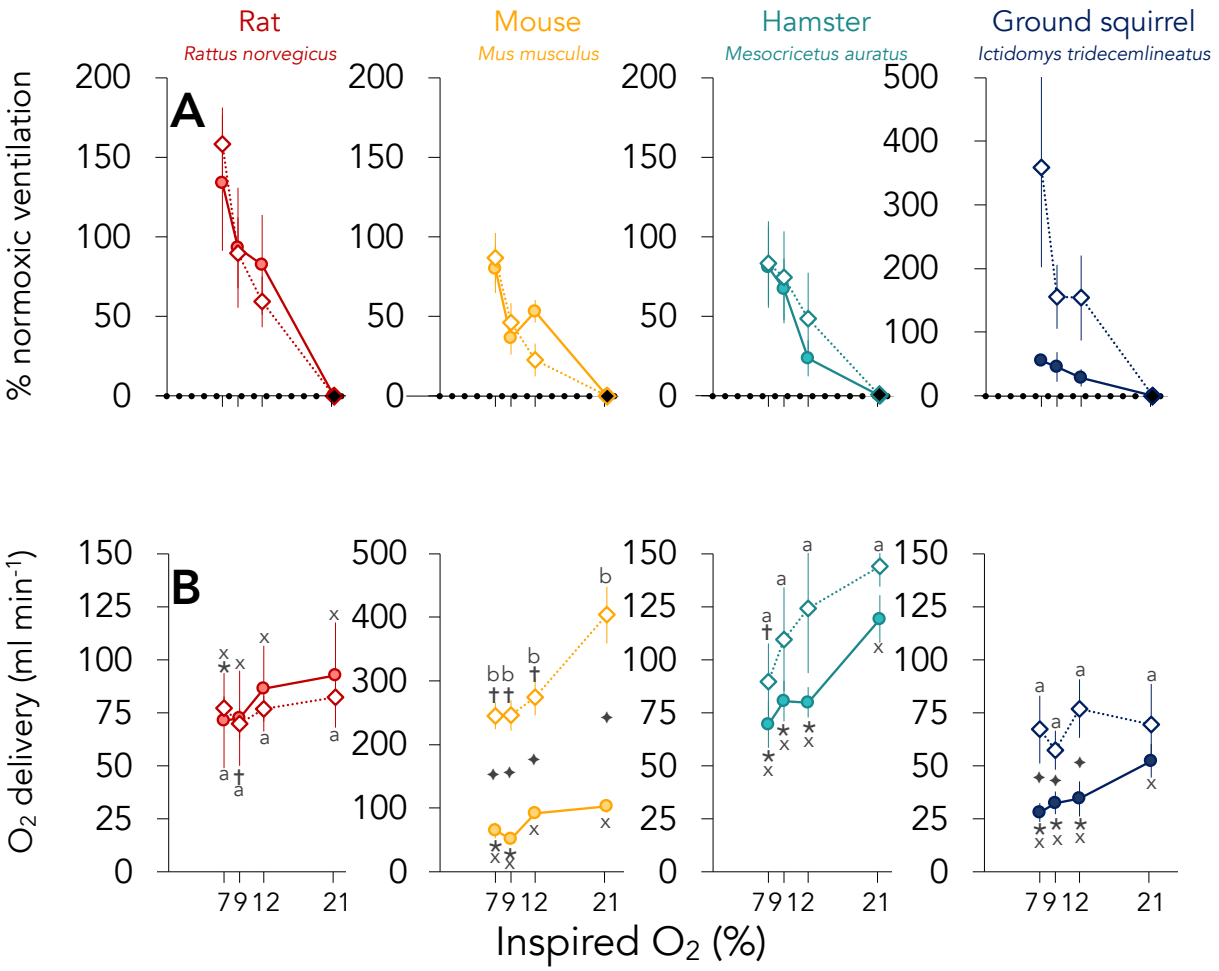

Figure 4.3. Effects of progressive hypoxia on **(A)** percent normoxic ventilation, and **(B)** O_2 delivery (ml min^{-1}), of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents at thermoneutrality (an ambient temperature of 33°C for newborns and 26°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦indicates a significant difference between newborns and adults of each species at each level of O_2. Note the y-axis in panel **(B)** for mouse O_2 delivery is different from that of the other species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=8, adults- n=6. Facultative heterotherms: mice (*Mus musculus*): newborns- n=15, adults- n=7; hamsters (*Mesocricetus auratus*): newborns- n=12, adults- n=6. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=11, adults- n=7.

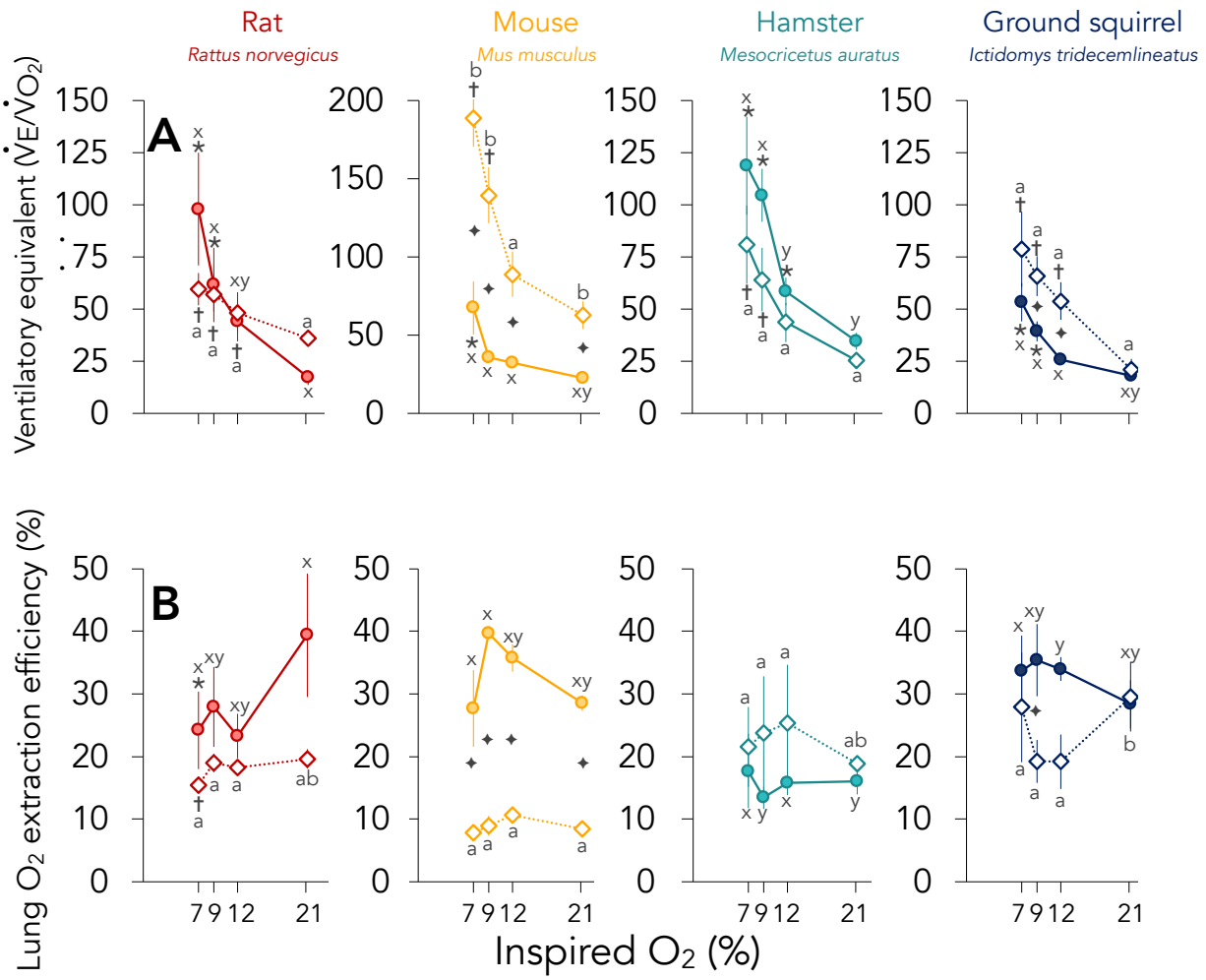

Figure 4.4. Effects of progressive hypoxia on **(A)** the ventilatory equivalent, and **(B)** lung O_2 extraction efficiency (%) of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents at thermoneutrality (an ambient temperature of 33°C for newborns and 26°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦indicates a significant difference between newborns and adults of each species at each level of O_2. Note the y-axis in panel **(A)** for mouse lung O_2 ventilatory equivalent is different from that of the other species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=8, adults- n=6. Facultative heterotherms: mice (*Mus musculus*): newborns- n=15, adults- n=7; hamsters (*Mesocricetus auratus*): newborns- n=12, adults- n=6. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=11, adults- n=7.

Chapter 5: The effects of progressive hypoxia on matching O_2 supply and O_2 demand in rodents in the cold

5.1 Introduction

When mammals are exposed to ambient temperatures below their TNZ they increase thermogenesis, and thus O_2 consumption rate, in order to maintain a high body temperature (Chappell 1992; Gautier and Bonora 1992; Barros et al. 2001). In most small mammals, hypoxia induces a reduction in the rate of O_2 consumption (the HMR), which is often greater in the cold than within the TNZ (Barros et al. 2001; Mortola and Maskrey 2011). It has been hypothesized that the relative magnitude of the HMR is not dependent on ambient temperature, *per se*, but rather on the normoxic mass-specific O_2 consumption rate of the animal (Hill 1959; Frappell et al. 1992). Thus, the greater the level of thermogenesis in normoxia, the larger the suppression of O_2 consumption rate and body temperature in hypoxia (Hill 1959; Frappell et al. 1992). This hypothesis is supported by an extensive line of evidence that suggests that the enhanced HMR in the cold is primarily due to a lowering of the thermoregulatory set-point and inhibition of thermogenesis (Hill 1959; Blatteis and Lutherer 1973; Dupré et al. 1988; Mortola and Feher 1998; Barros et al. 2001; Steiner and Branco 2002; Tattersall and Milsom 2003b; Tattersall and Milsom 2009; but see Chapters 2 and 3).

In most small mammals, hypoxia also results in an increase in ventilation, the HVR (Mortola et al. 1989; Frappell et al. 1992). Although data are sparse, the HVR appears to be reduced in the cold (Saiki et al 1994; Barros et al. 2001). It has been hypothesized that this temperature-dependent depression of the HVR is due to the enhanced HMR in the cold (Saiki et al. 1994; Barros et al. 2001). Thus, if the HMR is pronounced, ventilation can be depressed to levels lower than those in normoxia (Barros et al. 2001). Whether this is also true of newborn rodents is unknown, as the effects of cold on the HMR and HVR of newborn rodents have never been investigated simultaneously, although they have been measured in isolation (Mortola and Dotta 1992; Merazzi and Mortola 1999; Cameron et al. 2000).

Generally, newborns are more hypoxia tolerant than their adult counterparts (Kabat 1940; Glass et al. 1944), and hibernating heterotherms are more hypoxia tolerant than euthermic heterotherms and homeotherms (Frerichs and Hallenbeck 1998). For example, in an *in vitro*

study where hippocampal slices of the euthermic ground squirrel (a heterotherm) and rat (a homeotherm) were held at a normal body temperature (37°C) they exhibited similar levels of hypoxia tolerance (Frerichs and Hallenbeck 1998). However, when the temperature of the hippocampal slices was reduced to 20°C, hypoxia tolerance increased in the euthermic ground squirrel but not in the rat (Frerichs and Hallenbeck 1998). This enhanced hypoxia tolerance at low temperatures is intriguing as it may reflect an adaptation specific to heterotherms, induced by cold alone. However, even a mild reduction in body temperature of 3-5°C exerts a powerful protective effect against damage from hypoxia in both homeotherms and heterotherms (Wood and Gonzales 1996; Wagner et al. 1999; for a review see Erecinska et al. 2003).

While it is well established that cold exposure and moderate decreases in body temperature are protective to animals subjected to hypoxia (Miller and Miller 1966; Wood 1991; Wood and Gonzalez 1996; Frerichs and Hallenbeck 1998), few studies have quantified the temperature induced changes in hypoxia tolerance in mammals. Even the most basic system-level responses, including metabolic, thermoregulatory, and ventilatory responses to hypoxia in the cold remain to be characterized in newborn and adult homeotherms and heterotherms. Thus, the aims of this study were to examine these responses in newborn and adult rodents exposed to progressive decreases in inspired O_2 at an ambient temperature below their TNZ, or below their preferred ambient temperature. I hypothesized that exposure to hypoxia and cold would result in an enhanced HMR and a blunted HVR than exposure to hypoxia at a warmer ambient temperature within the animal's TNZ, or at its preferred ambient temperature (Nagy 1993). Experiments were conducted on four species of newborn and adult rodents: rats (*Rattus norvegicus*), mice (*Mus musculus*), hamsters (*Mesocricetus auratus*), and ground squirrels (*Ictidomys tridecemlineatus*) (Table 5.1). All experiments on adults were conducted during the non-hibernating season when all rodents were euthermic and active. Because body size, and state of development at the time of birth have an effect on the relative magnitude of the hypoxic response I conducted experiments solely on small, altricial mammals, belonging to the order Rodentia. More importantly, I selected these species as they range in their expression of heterothermy as adults. The rat is a true homeotherm. It is not known to use torpor and maintains a high body temperature and O_2 consumption rate independent of season and environmental conditions (Yoda et al. 2000). Mice and hamsters are facultative, non-seasonal heterotherms that use torpor at any time of the year when confronted with energetic stressors such as low ambient

temperature (Ellison and Skinner 1992; Heldmaier et al. 1999), or limitations in food or energy reserves (Lyman 1948; Hudson and Scott 1979). The ground squirrel is an obligate heterotherm, using torpor seasonally. When exposed to cold during the non-hibernating, active season obligate heterotherms generally increase heat production to maintain body temperature, similar to homeothermic species (Wang and Lee 2011). I predicted that, because heterotherms are capable of more extreme reductions in O_2 consumption rate and body temperature than homeotherms, the effects of cold on the HMR and the HVR would be greater in heterotherms (facultative and obligate). Owing to their poor thermogenic capacity, and thus lower body temperature in the cold, I predicted that the effects of cold on the HMR and the HVR would be even greater in newborns.

5.2 Methods

5.2.1 Experimental animals and housing

Pregnant and non-reproductive rats, mice, and hamsters were purchased from Charles River Laboratories (Portage, MI), while ground squirrels were live-trapped in June 2013 and 2014 in Carman, MB, Canada (49°30′ N, 98°01′ W) and transferred to the animal care housing facility at the University of British Columbia in Vancouver, BC, Canada. Ground squirrels were trapped with the approval of Manitoba Conservation and Water Stewardship, under the wildlife scientific permit WB15027. All animals were provided with food and water *ad libitum*. Adult rats, mice, and hamsters were fed Purina Rodent Chow (no. 5001, Purina LabDiet, St. Louis, MO), while ground squirrels were given 1:1 IAMS dog food (Mars Incorporated, Mount Olive, NJ) and Purina Rodent Chow supplemented with sunflower seeds, fruit, and vegetables. Pregnant and non-reproductive adults were housed in an environmental chamber, held under a 07:00 to 19:00 photoperiod cycle, at an ambient temperature of 21 to 23°C, and 45-55% relative humidity. Rodents were born at the animal care holding facility, and housed with their mothers until the day of the experiment. Animals were not fasted prior to experiments. All experimental procedures in this study conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the UBC Committee on Animal Care (under protocol A13-0091).

5.2.2 Experimental protocol

On the day of the experiment an individual newborn or adult rodent was instrumented with a thermal probe, and placed into a 20 to 30 ml, or 200 to 1000 ml chamber depending on the size of the animal. The animal chambers were flushed with normoxic air (21% O_2) and set inside a temperature-controlled environmental chamber set to 20.94 ± 0.06°C for newborn rodents, and 15.16 ± 0.29°C for adult rodents. The selected ambient temperature for newborns was about 13°C below the ambient temperature within their litter, as well as the TNZ of newborn rats (see Chapter 3). The selected ambient temperature for adults was also about 13°C below the TNZ of all species investigated (see Chapter 2). Ambient temperature was continuously recorded throughout the experiment using a temperature datalogger (iButton Maxim Integrated, Chandler, CA, USA) placed inside the animal chamber. Animals were given at least one hour to acclimate to the experimental setup, followed by baseline recordings of O_2 consumption rate, body temperature, and ventilation in normoxia. To determine how rodents match O_2 supply and O_2 demand in hypoxia, O_2 consumption rate, body temperature, and ventilatory measurements were collected at different levels of inspired O_2. The fractional O_2 composition of inspired gas through the animal chamber was reduced in a progressive, stepwise manner, with each rodent being exposed to 21%, 12%, 9%, and 7% O_2. Rodents were held in each condition until O_2 consumption rate, body temperature, and ventilation reached a steady state (~ 45 minutes), after which O_2 was reduced to the next level. The most severe level of hypoxia (7% O_2) was followed by a normoxic recovery period. Each experimental trial lasted ~ 4.5 hours (228 ± 8 minutes for newborns; 304 ± 14 minutes for adults). Across the 4 species, a total of 25 newborns and 27 adults were used in these experiments (Table 5.1).

5.2.3 Body temperature measurements

Prior to being placed into the animal chamber, each individual was instrumented with a sterile, 0.64 mm diameter copper-constantan thermocouple wire (Physitemp IT-18, Clifton, NJ, USA). The thermal probe was calibrated with a National Bureau of Standards certified thermometer.

In newborns, prior to thermal probe insertion the area around the anus was disinfected

with an antiseptic (Betadine®, Purdue Pharma, Stamford, CT, USA), and 95% ethanol. Following disinfection, a topical anaesthetic (2% Xylocaine®, AstraZeneca Canada Inc., Mississauga, ON, Canada) was administered to the area around the anus using a cotton swab. The tip of the thermal probe was lubricated and inserted about 5 to 10 mm into the animal's rectum. The thermal probe was secured to the animal's skin using tissue adhesive (3M™ Vetbond™, 3M, London, ON, Canada). The procedure took less than 10 minutes. Following each experimental trial, the insertion site was disinfected, the topical anaesthetic administered, and the thermal probe removed.

In adults, each animal was anaesthetized by gaseous inhalation of isoflurane (1-5% isoflurane: 21% O_2 mixture; flow rate = 1-5L min^{-1}). Once the animal was anaesthetized, the fur over the abdomen and between the shoulder blades was cropped, treated with a depilatory cream, and the skin underneath was cleaned with distilled water, and disinfected with an antiseptic surgical scrub (Betadine®), followed by 95% ethanol. Two small subcutaneous incisions (< 0.5 cm) were made, one in the cleaned abdominal area, and another in between the animal's shoulder blades. A 16.5 G needle was inserted into the peritoneal cavity through the incision site in the cleaned abdominal area of the animal. The thermal probe was fed through the 16.5 G needle, and once ~1 cm of the thermal probe was within the peritoneal cavity, the 16.5 G needle was removed and the thermal probe was sutured to the skin using prolene sutures. The thermal probe was fed through to the the shoulder blades via a 30 cm long trocar needle. The trocar needle ran subcutaneously from the incision site in the abdominal area, through the back of the animal, and exited at the cleaned incision site between the shoulder blades. Once the thermal probe was fed through to the shoulder blades, the trocar needle was removed, and both incision sites were sutured shut. Placement of the thermal probe in this location minimized the likelihood of the animal tugging, chewing, or removing the thermal probe during the experiment. The procedure took less than 20 minutes to complete. Surgical analgesic (Metacam (Boehringer Ingelheim Vetmedica Inc., Duluth, Georgia, USA: 1-5 mg kg^{-1}) was administered subcutaneously, pre-operatively. All animals were fully recovered from anesthesia (~2 hours) before initiating experiments.

5.2.4 O_2 consumption rate measurements

Oxygen consumption rate was determined using open-flow respirometry. Newborn and adult rodents were placed in their individual chambers, with incurrent airflow pushed through the animal chamber at a flow rate of 5 to 34 ml min^{-1}, and 161 to 1010 ml min^{-1}, respectively, using calibrated flowmeters (PRSFM4302-1; Praxair Technology Inc., Danbury, CT, USA). The incurrent airflow was adjusted for each individual to ensure that neither the O_2 concentration, nor CO_2 concentration of the air leaving the animal chamber were altered more than 1% by the animal's metabolism. Different levels of hypoxia were achieved by mixing compressed dry air and nitrogen in appropriate ratios using the calibrated flowmeters. The incurrent and excurrent airflow, as well as the composition of the gas mixture were continuously monitored using a Field Metabolic System (Sable Systems, Las Vegas, NV, USA) with a built in flowmeter and O_2 and CO_2 gas analyzers. The gas analyzers were calibrated for O_2 and CO_2 before and after each experiment with dry commercial air (Praxair Technology Inc., Danbury, CT, USA) and a premixed gas mixture (1.55% CO_2 balanced with N_2; Praxair Technology Inc., Danbury, CT, USA), respectively. A subsample, or entire excurrent flow, was passed through a desiccant media (DM-060-24; Perma Pure LLC, Toms River NJ, USA) before entering the cells of the O_2 and CO_2 analyzers. Incurrent gas concentrations and gas flow were checked before and after exposure to each O_2 level. Oxygen consumption rate was calculated using equation 10.6 in Lighton (2008):

$$\dot{V}O_2 = \dot{V}i\,[(FiO_2 - FeO_2) - FeO_2\,(FeCO_2 - FiCO_2)]/(1 - FeO_2) \qquad (1)$$

Where $\dot{V}O_2$ is O_2 consumption rate (ml min^{-1}), $\dot{V}i$ is incurrent flow rate (ml min^{-1}), FiO_2 and $FiCO_2$ are fractional concentrations of incurrent O_2 and CO_2 of dry gas, respectively, and FeO_2 and $FeCO_2$ are fractional concentrations of excurrent O_2 and CO_2 of dry gas, respectively. Barometric pressure and chamber temperature were used to convert O_2 consumption rate to STPD.

5.2.5 Ventilatory measurements

In newborns, ventilatory measurements were acquired using head-out plethysmography as described by Mortola and Frappell (2013). Newborns were separated from their mothers on

the day of the experiment, and placed into a plastic head-out chamber. The animal's muzzle protruded through a small hole in a pliable piece of latex, which provided a tight seal around the animal's muzzle, and separated the chamber into two parts: a head compartment, and a body compartment. The head compartment of the animal chamber was connected to a pneumotachograph and a differential pressure transducer (DP103-18; Validyne, Northridge, CA, USA) was used to measure ventilation-induced pressure oscillations. To determine tidal volume from pressure oscillations, the system was calibrated at the beginning of each experimental trial by injecting and withdrawing known volumes of air (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 ml) at a rate similar to the animal's breathing frequency. Tidal volume was calculated by integrating expiratory flow.

In adults, ventilatory measurements were acquired using barometric whole-body plethysmography, in an open-flow system (e.g. Chapin 1954; Drorbaugh and Fenn 1955; Jacky 1978; Jacky 1980; Mortola and Frappell 2013). This method relies on the difference in ambient temperature and humidity between the chamber and experimental animal, and is therefore an unsuitable technique for measuring ventilation in animals that do not have a body Tb-Ta differential (i.e. newborn rodents). The experimental set-up consisted of two identical Plexiglass chambers, one being the designated animal chamber, and the other the designated reference chamber. Individuals were placed, unrestrained, within the animal chamber. Ventilation-induced pressure oscillations were detected with a differential pressure transducer (DP103-18; Validyne, Northridge, CA, USA) connected between the animal and reference chamber. As the animal breathed within its chamber, it resulted in pressure oscillations induced by the warming and humidification of inspired air. These pressure oscillations were compared to the pressure of the reference chamber. To determine tidal volume, the system was calibrated with the animal present in the chamber, as described by McArthur and Milsom (1991). To determine tidal volume from pressure oscillations, the system was calibrated prior to each experiment by injecting and withdrawing known volumes of air (0.1 to 5.0 ml) into the animal chamber at a rate similar to the animal's breathing frequency to produce pressure oscillations at least 10 times as great as that of the animal's breathing. Tidal volume was calculated by integrating expiratory flow, and using the equation of Drorbaugh and Fenn (1955) modified for flow-through plethysmography by Jacky (1978). In both newborn and adult rodents, breathing frequency was calculated directly from the ventilation-induced pressure oscillations, while ventilation was calculated from the

product of tidal volume and breathing frequency. All ventilatory measurements are reported at BTPS.

5.2.6 Calculation of O_2 delivery, the ventilatory equivalent and lung O_2 extraction efficiency

The ventilatory equivalent (the quotient of ventilation and O_2 consumption rate), and the percent of O_2 extracted by the lung from each breath were calculated from ventilatory and O_2 consumption rate measurements. Oxygen delivery to the lungs was calculated as ventilation multiplied by the fractional concentration of O_2 in inspired air. To determine the percent of O_2 extracted from each breath O_2 consumption rate was divided by ventilation multiplied by the fractional concentration of O_2 in inspired air (O_2 delivery to the lungs) and multiplied by 100.

5.2.7 Data collection and analysis

All signals (body temperature, incurrent and excurrent O_2 concentration, and the ventilation-induced pressure signal) were amplified, filtered, and recorded onto a PowerLab 8/35 data acquisition system (ADInstruments Pty Ltd., Colorado Springs, CO, USA). At each inspired O_2 level I determined average values for each dependent variable for the last 10 minutes of steady state. The dependent variables analysed were: O_2 consumption rate, body temperature, breathing frequency, tidal volume, ventilation, O_2 delivery, the ventilatory equivalent, and lung O_2 extraction efficiency.

Statistical analyses were performed using R (R Core Team 2017, Vienna, AT, EUR). I used a repeated measures 3-way ANOVA to account for repeated sampling of the same individual with changes in inspired O_2, with individual treated as a random effect. When visual inspection of residuals and q-q plots revealed deviations from the assumptions of the 3-way ANOVA (normality, homogeneity of variances, linearity, and independence), I log transformed the dependent variable. Oxygen level, age, species, body mass, and sex were fixed effects in my initial models. I tested all 2- and 3-way interactions of O_2 level, age, and species. When body mass, or sex did not have a significant effect on the dependent variables they were removed from the model. I did not remove any other terms from my models given the importance of all

independent variables and interactions to my research objectives. Following the methods of Fangue et al. (2009), when interaction terms were significant the data were separated and analyzed independently using a one-way ANOVA, followed by a Tukey-Holm post-hoc analysis to determine differences in dependent variables between O_2, age, and species of rodents, correcting for multiple pairwise comparisons. All results are presented as mean ± s.e.m., with statistical significance set as $P<0.05$.

5.3 Results

5.3.1 The hypoxia-mediated decrease in O_2 consumption rate and body temperature varies with age and species

All newborn rodents reduced their O_2 consumption rate by 40-70% as inspired O_2 levels fell to 7% O_2 in the cold, except for mice (Fig. 5.1A). Unlike other newborns, mice maintained their O_2 consumption rate as inspired O_2 levels fell, exhibiting only a small non-significant reduction in O_2 consumption rate at 7% O_2 (Fig. 5.1A). At 7% O_2 all adult rodents also reduced their O_2 consumption rate by 30-50% relative to normoxic levels (Fig. 5.1A). As newborns, rats and ground squirrels exhibited a greater relative reduction in O_2 consumption rate (i.e. percent change) than as adults (Fig. 5.1A). The relative magnitude of the HMR (i.e. percent decrease) remained constant in mice and hamsters as they developed into adults (Fig. 5.1A). These trends resulted in a significant 3-way interaction between O_2 level, age, and species on O_2 consumption rates ($F_{9,129}=9.3$, $P<0.0001$; Fig. 5.1A).

There was a significant 3-way interaction between O_2 level, age, and species on body temperature ($F_{9,114}=13.3$, $P<0.0001$; Fig. 5.1B). In normoxia the body temperature of all newborns was significantly reduced in the cold, tracking ambient temperature (Fig. 5.1B; Fig. 5.5B). Newborn rats and hamsters also significantly reduced their body temperature during progressive hypoxia, albeit neither species dropped their body temperature by more than 2°C (Fig. 5.1B). Newborn mice and ground squirrels, on the other hand, maintained their body temperature at each level of hypoxia (Fig. 5.1B). Note that in all cases the normoxic body temperatures of the newborns were within 3-5°C of ambient temperature, leaving little room for body temperature to decrease in hypoxia (Fig. 5.1.B). Unlike the newborns, all adults maintained

their body temperature in normoxia in the cold (Fig. 5.1B; Fig. 5.7B). However, all adults also exhibited a hypoxia-induced reduction in body temperature (Fig. 5.1B). The drop in body temperature was least in rats, which reduced their body temperature by 2°C, and greatest in ground squirrels, which reduced their body temperature by 10°C (Fig. 5.1B). All adults, except for rats, exhibited a greater hypoxia-induced reduction in body temperature than their newborn counterparts (Fig. 5.1B).

5.3.2 Newborn and adult rodents do not appear to exhibit an HVR in the cold

All newborn and adult rodents maintained constant levels of ventilation as inspired air was reduced down to 7% O_2, except for adult rats (P=0.06; Fig. 5.2A). Unlike all other animals, adult rats progressively increased their ventilation, but only by 40% as inspired O_2 levels fell to 7% O_2 (Fig. 5.2A). Although most rodents did not exhibit an HVR in the cold, breathing pattern varied among species and age during progressive hypoxia (Fig. 5.2B&C). All newborns, except for rats, maintained constant levels of breathing frequency and tidal volume during progressive hypoxia (Fig. 5.2B&C). Unlike the other newborns, rats decreased breathing frequency and increased tidal volume as inspired O_2 fell to 7% O_2 (Fig. 5.2B&C). In adults, rats and mice maintained their breathing frequency and tidal volume constant during progressive hypoxia in the cold (Fig. 5.2B&C) whereas hamsters and ground squirrels increased breathing frequency and maintained (hamsters) or decreased (ground squirrels) tidal volume (Fig. 5.2B&C). These trends produced a significant 3-way interaction between O_2 level, species, and age on breathing frequency ($F_{9,129}$=4.3, P<0.0001; Fig. 5.2B) and a 2-way interaction between O_2 level and species ($F_{9,129}$=2.9, P<0.01; Fig. 5.2C), and O_2 level and age on tidal volume ($F_{3,129}$=3.3, P<0.05; Fig. 5.2C). Regardless of these changes in breathing pattern, ventilation was unaffected by hypoxia in newborn and adult heterothermic rodents (Fig. 5.2A).

5.3.3 Newborn and adult rodents differ in how they match O_2 supply and O_2 demand during progressive hypoxia in the cold

No rodent, except for the adult rat, increased their ventilation when exposed to decreasing O_2 levels in the cold (Fig. 5.3A). As a result of this maintenance of ventilation, there was a

significant, 50-70% reduction in the amount of O_2 delivered to their lungs ($F_{9, 129}$=2.0, P<0.05; Fig. 5.3B). However, because of the significant reduction in O_2 consumption rate, ventilation increased progressively relative to O_2 consumption rate (the ventilatory equivalent) in all rodents when inspired O_2 was reduced down to 7% O_2, with a significant interaction between O_2 level, species, and age on their ventilatory equivalent ($F_{9,129}$=2.1, P<0.05; Fig. 5.4A). Newborns, rats and ground squirrels exhibited a 200-250% increase in their ventilatory equivalent at 7% O_2, whereas mice and hamsters exhibited a modest 55% increase in their ventilatory equivalent at 7% O_2, compared to normoxic values (Fig. 5.4A). All rodents exhibited age-related differences in their ventilatory equivalent, with all but the hamster exhibiting a lower hypoxia-induced increase in the ventilatory equivalent as adults than as newborns (Fig. 5.4A). Conversely, hamsters exhibited a greater hypoxia-induced increase in their ventilatory equivalent as adults than as newborns (Fig. 5.4A).

The interaction between O_2 level, species, and age was significant for lung O_2 extraction efficiency ($F_{9,129}$=5.1, P<0.0001; Fig. 5.4B). Compared to normoxic values, all groups but adult hamsters, and newborn rats and ground squirrels exhibited a significant increase in lung O_2 extraction efficiency when exposed to a progressive reduction in inspired O_2 (Fig. 5.4B). Of the groups that significantly increased lung O_2 extraction efficiency during progressive hypoxia, adult rats exhibited a modest 50% increase in lung O_2 extraction efficiency at 7% O_2, compared to normoxic values, whereas all other groups exhibited increases of 100-150% (Fig. 5.4B). Unlike all other animals, adult hamsters and newborn rats and ground squirrels maintained their lung O_2 extraction efficiency during exposure to progressive hypoxia in the cold (Fig. 5.4B).

5.4 Discussion

In this study I examined how newborn and adult homeothermic and heterothermic rodents match O_2 supply and O_2 demand when exposed to progressive hypoxia in the cold, and evaluate the interaction between the HMR and HVR. All species of both ages exhibited a fall in O_2 consumption rates (HMR) with little or no ventilatory response (HVR) to progressive hypoxia in the cold. When exposed to hypoxia and cold, adult heterotherms exhibited a greater reduction in their O_2 consumption rate and body temperature, and a greater attenuation of their HVR than adult homeotherms. These effects of cold on the HMR and the HVR were

even greater in newborns due to their poor thermogenic capacity and low body temperatures. Taken together, my data indicate that the HVR is affected by body temperature, age, and the degree of heterothermic expression, but that the relative magnitude of the depression of the HVR does not correlate directly with normoxic mass-specific O_2 consumption rate.

5.4.1 The effects of hypoxia in the cold on O_2 consumption rate and body temperature

Based on data from Chapter 2, all adults in this study should have been well below their TNZ at 15°C (the ambient temperature at which adults were exposed to in this study). To maintain a high body temperature at these ambient temperatures they should all have required an increase in thermogenesis, and an associated increase in O_2 consumption rate. However, the rat and hamster did not, and thus these species appear not to have been below their TNZ (Fig. 5.7A). In the rat, this likely reflects its broad TNZ (see Chapter 2). In the hamster, it possibly reflects the abnormally high normoxic O_2 consumption rates recorded in this group at thermoneutrality in Chapter 4 (see Chapter 2 for comparison).

In hypoxia small mammals often reduce body temperature, through behavioural and physiological thermoregulatory responses, leading to reductions in their O_2 consumption rate (the HMR; Hill 1959; Taylor 1960; Mortola et al. 1989; Frappell et al. 1992; Mortola and Dotta 1992; Clark and Fewell 1996; Mortola and Feher 1998; Barros et al. 2001; Tattersall and Milsom 2003b; Tattersall and Milsom 2009). This was true of the adult rodents in the present study and, with the exception of the rat, the hypoxia-induced depression in body temperature was greater in the cold than at thermoneutrality (Figure 5.8B). A blunted thermogenic response, and thus lowered body temperature, is believed to be a desirable response in conditions of reduced O_2 availability because it would reduce the high energetic demands associated with thermogenesis. Furthermore, a reduction in body temperature would (i) improve O_2 extraction at the lungs, due to the increased affinity of hemoglobin for O_2 in the cold; (ii) lower O_2 demands directly through the Q_{10} effect on O_2 consumption rate; and (iii) reduce energetically costly responses to hypoxia such as increases in ventilation and cardiac output (for a review see Wood 1991; Wood 1995; Boutilier 2001).

Previous studies found that mammals with higher mass-specific O_2 consumption rates in normoxia exhibited an enhanced HMR (Hill 1959; Mortola 2004), suggesting that the relative

magnitude of the HMR depends on normoxic mass-specific O_2 consumption rate, which is a function of size, age, and ambient temperature (Hill 1959; Mortola 2004). If the relative magnitude of the HMR depends on normoxic mass-specific O_2 consumption rate it should be particularly prominent in the cold, where animals are metabolically challenged and O_2 consumption rate is increased above values observed at thermoneutrality (Hill 1959; Blatteis 1964; Saiki et al. 1994; Barros et al. 2001; Cadena and Tattersall 2014). In the present study, while we found a greater absolute reduction in O_2 consumption rates in animals with higher normoxic mass-specific O_2 consumption rates, when expressed in relative terms, the drop in the rate of O_2 consumption (~30 to 50%) was similar at thermoneutrality and in the cold (Fig. 5.8A). The discrepancies in the effects of ambient temperature on the HMR between our study and Hill's most likely reflects differences in protocols, as Hill (1959) used milder levels of hypoxia (10% O_2 vs. 7% O_2) that failed to produce an HMR within the TNZ, while her use of a more extreme cold exposure (10°C vs. 15°C) would have produced a much greater rise in normoxic O_2 consumption rates, resulting in an enhanced HMR in the cold.

In contrast to their adult counterparts, newborn rodents have a poor thermogenic capacity (Chapter 3). Because physiological thermogenesis is only partially functional in newborn rodents, behavioural thermoregulation is more important for maintaining body temperature in the cold (Hull 1973; Fowler and Kellogg 1975; Fewell et al. 1997). Thus, it is no surprise that the newborns in my study exhibited lower normoxic mass-specific O_2 consumption rates and body temperatures in the cold than that at thermoneutrality (Fig. 5.5A&B). It should be noted that I restricted newborns from using behavioural means to thermoregulate, as each newborn was restrained, and studied in isolation. As a result, their body temperatures in the cold remained within a few degrees of ambient temperature, restricting or eliminating their ability to depress body temperature when exposed to hypoxia. Even though there was little capacity for their body temperature to fall in hypoxia, all newborns exhibited significant, temperature-independent HMRs that were similar to the HMRs at thermoneutrality (Figure 5.6A). Thus, my data indicate that animals with higher mass-specific O_2 consumption rates in normoxia exhibit an enhanced HMR in absolute terms but not in relative terms.

5.4.2 The effects of cold and the HMR on the HVR

Ambient temperature affects how rodents match O_2 supply and O_2 demand in hypoxia (Saiki et al. 1994; Mortola and Gautier 1995; Wood and Gonzales 1996; Barros et al. 2001; Mortola and Maskrey 2011). Previous studies on newborn and adult rodents have found that in hypoxia, rodents rely more on the HVR to match O_2 supply and O_2 demand at high ambient temperatures, whereas use of the HMR prevails at low ambient temperatures (Saiki et al. 1994; Mortola and Gautier 1995; Wood and Gonzales 1996; Barros et al. 2001; Mortola and Maskrey 2011). What causes this temperature-dependent switch in strategies remains unknown, but what these previous data do suggest is that the greater the HMR the smaller the HVR. Exposure to cold in the present study did attenuate the robust HVR that was observed in newborn and adult rodents at thermoneutrality all but eliminating it (Fig. 5.6C; Fig. 5.8C, respectively). However, there was no correlation between the relative magnitude of the HMR and the degree of attenuation of the HVR, as the relative magnitude of the HMR was similar in warm and cold conditions (Fig. 5.6A; Fig. 5.8A). Other factors sensitive to temperature that could have contributed to the attenuation of the HVR in the cold include a decrease in the responsiveness of chemoreceptors (McQueen and Eyzaguirre 1974; Alcayaga et al. 1993), and an increase in the release of inhibitory neurotransmitters (Waters and Gozal 2003).

Since the hypoxia-induced increase in ventilation at thermoneutrality was attenuated in the cold, while the relative magnitude of the HMR was similar under both conditions, rodents in the cold were breathing less for each volume of O_2 they consumed (their ventilatory equivalent was reduced), and thus they extracted more O_2 from each breath than at thermoneutrality. The increase in lung O_2 extraction efficiency in the cold could be due to longer resident time of inspired air in the lungs due to a lower breathing frequency and greater tidal volume in the cold, relative increases in heart rate, stroke volume, hematocrit, or hemoglobin-O_2 affinity of the blood. Regardless of mechanism, the reduced HVR in the cold represents a beneficial adaptation, since an increase in ventilation is an energetically expensive response to hypoxia.

5.4.3 Newborns and adult heterotherms exhibit an enhanced HMR and a greater depression of their HVR than homeotherms in the cold

I found that the effects of cold on the HMR and HVR were similar between newborn homeotherms and heterotherms, with all newborns exhibiting an HMR that was similar in relative magnitude to that at thermoneutrality (Fig. 5.6A). However, cold exposure attenuated the prominent HVR that was observed at thermoneutrality (Fig. 5.6C). On the other hand, I found that as adults, heterotherms exhibited a greater depression of body temperature and attenuation of their HVR than homeotherms, complementing the findings of Frappell et al. (1992).

Unlike the other animals, the adult rat maintained a high body temperature when exposed to hypoxia in the cold. As a result of the energetic costs required to maintain a high body temperature in the cold, rats did not depress their rate of O_2 consumption as much as newborns and adult heterotherms. Instead they increased their ventilation in order to match O_2 supply and O_2 demand when exposed to hypoxia in the cold. Thus, adult heterotherms behaved more like the newborns than the homeothermic rat.

5.4.4 Conclusions

In contrast to the available literature, the present study indicates that neither cold exposure, nor normoxic mass-specific O_2 consumption rates alter the relative magnitude of the HMR in newborn or adult homeothermic and heterothermic rodents. However, exposure to hypoxia and cold does attenuate the HVR that is observed in newborn and adult rodents at thermoneutrality.

In the previous chapter, I found that in hypoxia, homeothermic and heterothermic rodents matched O_2 supply and O_2 demand in a similar fashion, both as newborns and as adults. During progressive hypoxia all rodents reduced O_2 demand by reducing their O_2 consumption rate, and increased O_2 supply by increasing ventilation, while generally maintaining their lung O_2 extraction efficiency. In the present chapter, I found that when metabolically challenged by cold, all rodents of both ages when made hypoxic reduced O_2 demand by reducing O_2 consumption rate more, and increased O_2 supply by increasing ventilation less. The enhanced HMR and reduced HVR were not due to Q_{10} effects of reduced body temperature, as they occurred in the newborns even though hypoxia produced no fall in body temperature.

It appears that when exposed to hypoxia and cold, adult heterotherms exhibit a greater reduction in their O_2 consumption rates and body temperature, and a greater attenuation of

their HVR than adult homeotherms. These effects of cold on the HMR and the HVR are similar between newborns and adult heterotherms, but greater than in adult homeotherms. The mechanistic bases of these changes remain to be explored.

Table 5.1. Species, number of individuals per treatment, sex, age, and body mass of newborn and adult homeothermic and heterothermic rodents exposed to progressive hypoxia in the cold (newborns: 21°C; adults: 15°C). Age and body mass are presented as mean ± s.e.m.

Species	Degree of heterothermy	Age group	Sample size		Age (days)	Body mass (g)
			female	male		
Rattus norvegicus rat	homeotherm	newborn	3	1	3.3 ± 0.3	11.7 ± 1.1
		adult	0	5	>70	732.7 ± 42.3
Mus musculus mouse	facultative heterotherm	newborn	4	2	1.5 ± 0.2	2.0 ± 0.1
		adult	3	3	>70	23.6 ± 0.5
Mesocricetus auratus hamster	facultative heterotherm	newborn	5	3	3.6 ± 0.5	6.5 ± 0.5
		adult	5	2	>70	199.4 ± 4.2
Ictidomys tridecemlineatus ground squirrel	obligate heterotherm	newborn	3	4	2.4 ± 0.5	6.5 ± 0.8
		adult	5	4	>70	249.5 ± 7.6

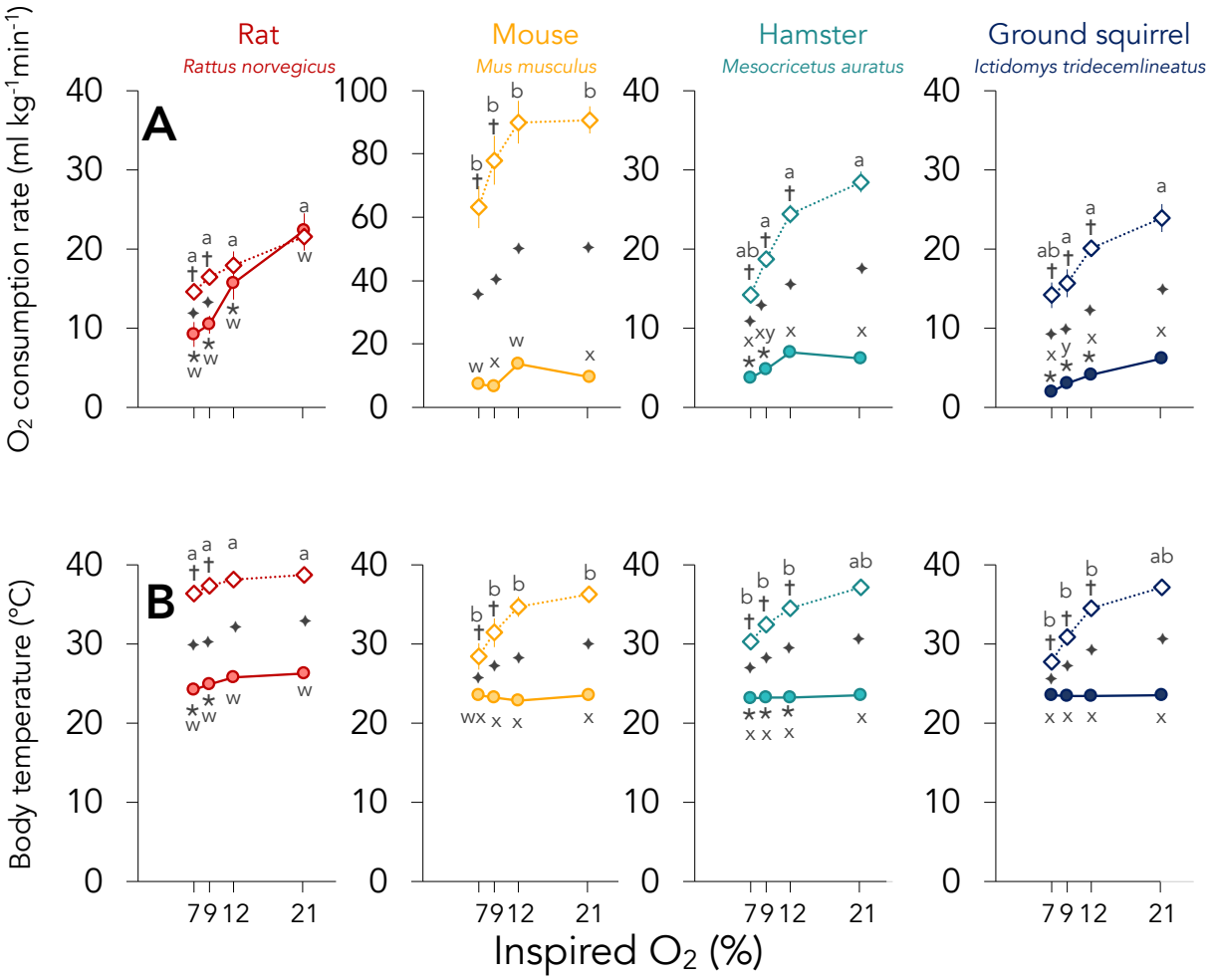

Figure 5.1. Effects of progressive hypoxia on **(A)** O_2 consumption rate (ml $kg^{-1}min^{-1}$), and **(B)** body temperature (°C) of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents in the cold, 13°C below their thermoneutral zone (an ambient temperature of 21°C for newborns and 15°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦indicates a significant difference between newborns and adults of each species at each level of O_2. Note the y-axis in panel **(A)** for mouse O_2 consumption rate is different from that of the other species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=4, adults- n=5. Facultative heterotherms: mice (*Mus musculus*): newborns- n=6, adults- n=6; hamsters (*Mesocricetus auratus*): newborns- n=8, adults- n=7. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=7, adults- n=9.

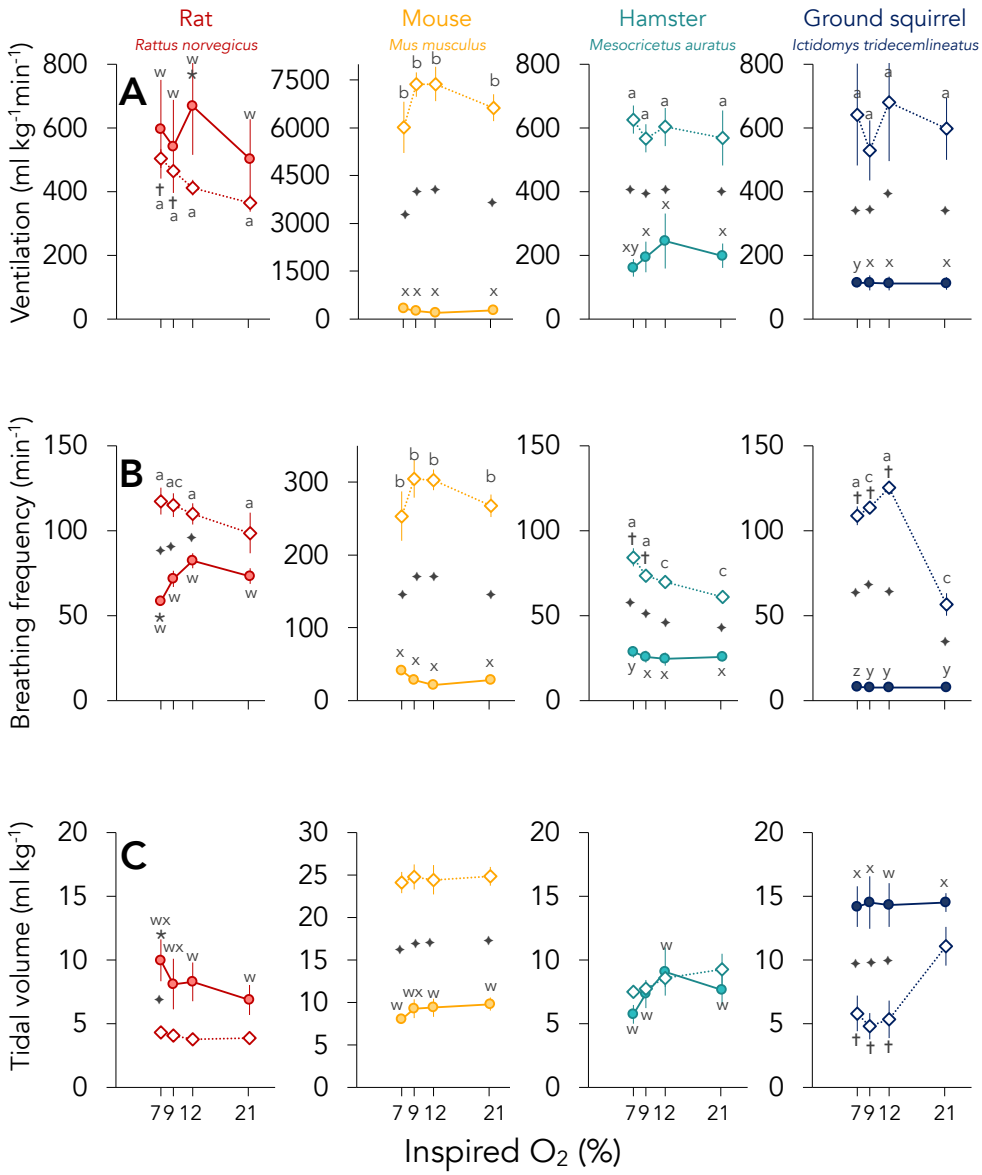

Figure 5.2. Effects of progressive hypoxia on **(A)** ventilation (ml $kg^{-1}min^{-1}$), **(B)** breathing frequency(min^{-1}), and **(C)** tidal volume (ml kg^{-1}), of newborn (filled circles) and adult (open diamonds) rodents in the cold, 13°C below their thermoneutral zone (an ambient temperature of 21°C for newborns and 15°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦ indicates a significant difference between newborns and adults of each species at each level of O_2. Note the y-axis of the mouse is different from that of the other species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=4, adults- n=5. Facultative heterotherms: mice (*Mus musculus*): newborns- n=6, adults- n=6; hamsters (*Mesocricetus auratus*): newborns- n=8, adults- n=7. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=7, adults- n=9.

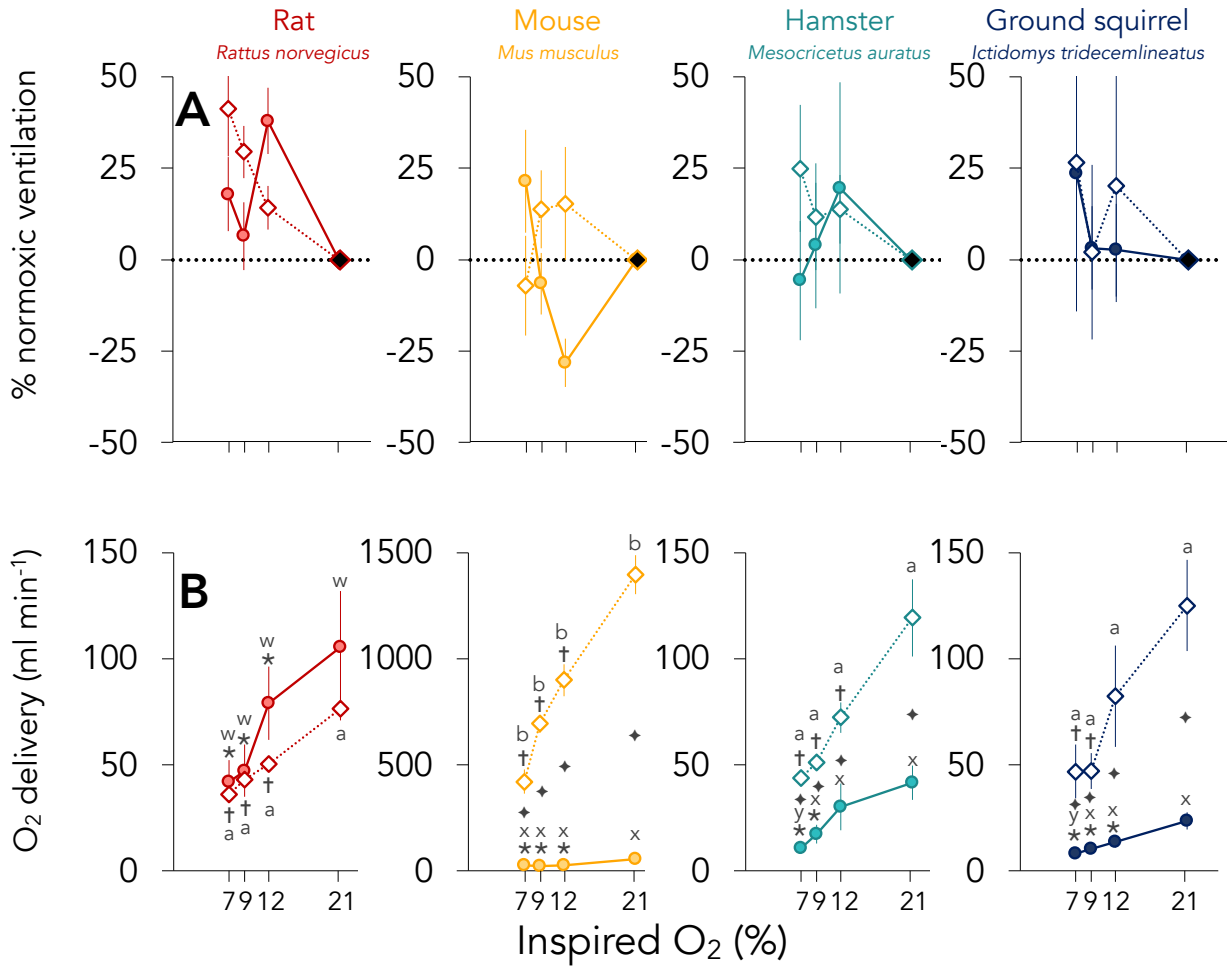

Figure 5.3. Effects of progressive hypoxia on **(A)** percent normoxic ventilation, and **(B)** O_2 delivery (ml min^{-1}) of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents in the cold, 13°C below their thermoneutral zone (an ambient temperature of 21°C for newborns and 15°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦ indicates a significant difference between newborns and adults of each species at each level of O_2. Note the y-axis in panel **(B)** for mouse O_2 delivery is different from that of the other species. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=4, adults- n=5. Facultative heterotherms: mice (*Mus musculus*): newborns- n=6, adults- n=6; hamsters (*Mesocricetus auratus*): newborns- n=8, adults- n=7. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=7, adults- n=9.

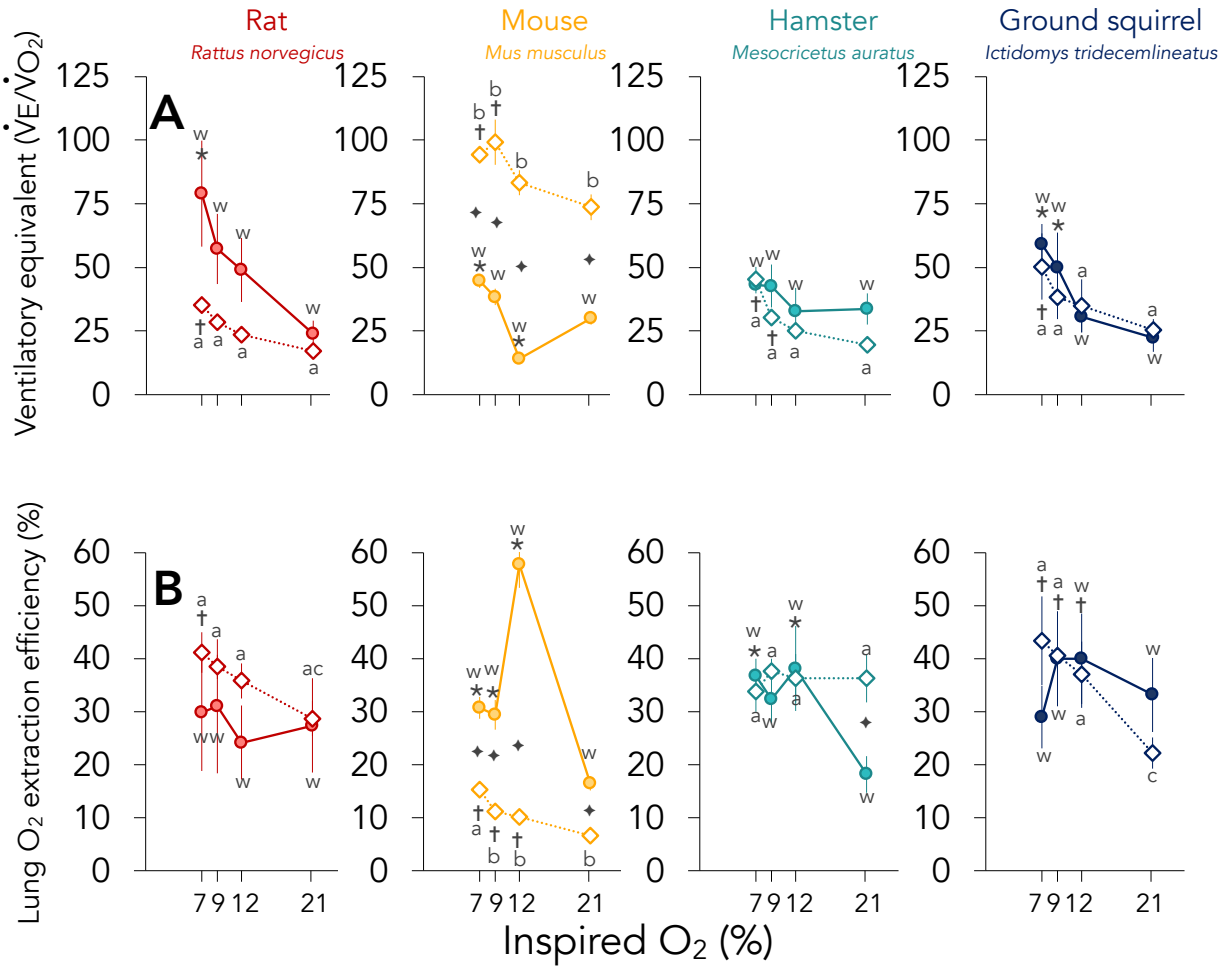

Figure 5.4. Effects of progressive hypoxia on **(A)** the ventilatory equivalent, and **(B)** lung O_2 extraction efficiency (%) of newborn (filled circles) and adult (open diamonds) homeothermic and heterothermic rodents in the cold, 13°C below their thermoneutral zone (an ambient temperature of 21°C for newborns and 15°C for adults). Significant differences between species at any given O_2 level within an age group are indicated by different letters (newborns: x-z; adults: a-c). Within each species, a significant difference from normoxia is indicated by an asterisk (*) for newborns and a cross (†) for adults. ✦indicates a significant difference between newborns and adults of each species at each level of O_2. Data are expressed as means ± s.e.m, and $P \leq 0.05$ for all significant comparisons. Homeotherms: rats (*Rattus norvegicus*): newborns- n=4, adults- n=5. Facultative heterotherms: mice (*Mus musculus*): newborns- n=6, adults- n=6; hamsters (*Mesocricetus auratus*): newborns- n=8, adults- n=7. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): newborns- n=7, adults- n=9.

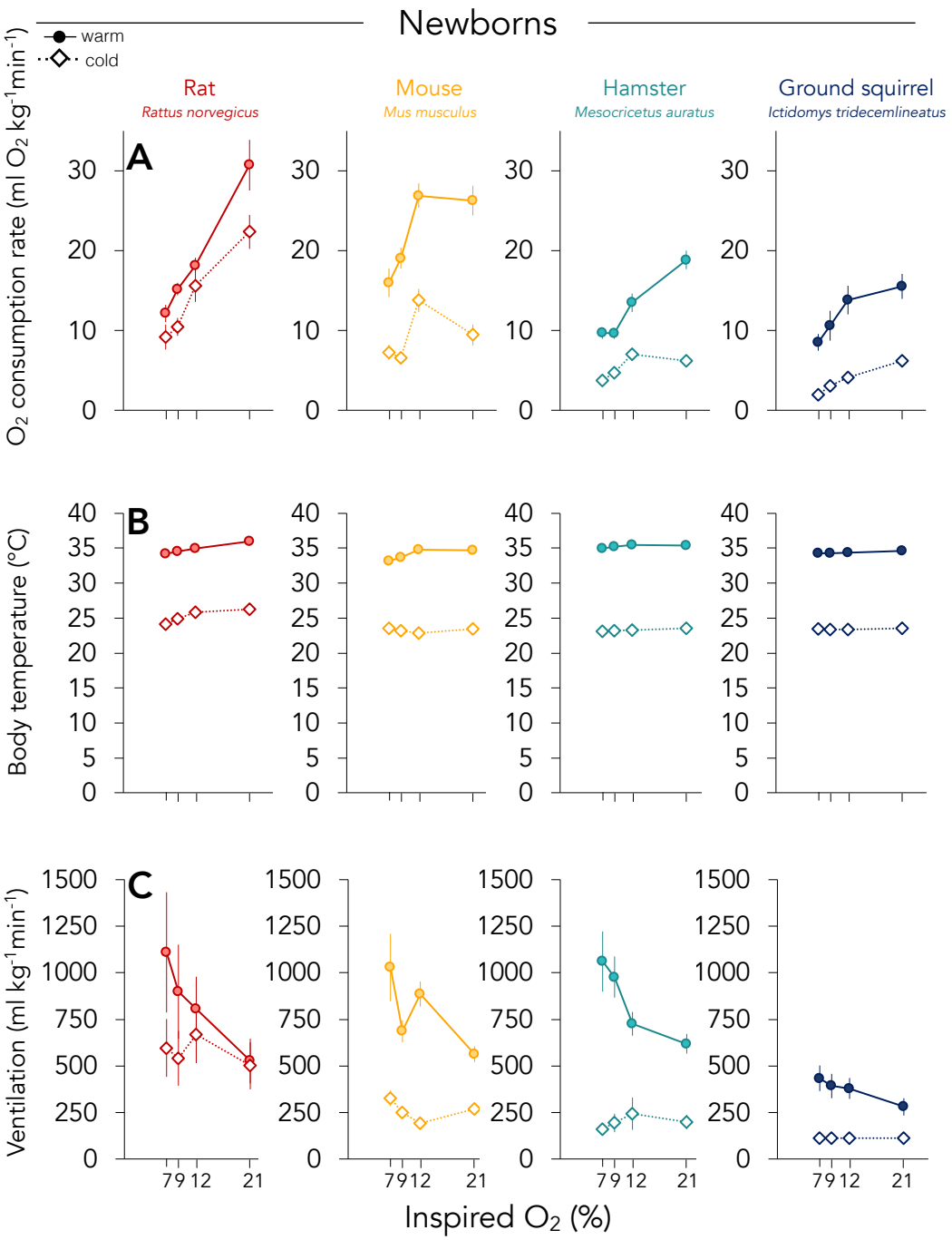

Figure 5.5. Comparison of the effects of progressive hypoxia on **(A)** O_2 consumption rate (ml $kg^{-1}min^{-1}$), **(B)** body temperature (°C); and **(C)** ventilation (ml $kg^{-1}min^{-1}$) of newborn homeothermic and heterothermic rodents at their preferred ambient temperature (33°C; filled circles) and in the cold (21°C; open diamonds). Data are expressed as means ± s.e.m. Homeotherms: rats (*Rattus norvegicus*): preferred ambient temperature- *n*=8, cold- *n*=4. Facultative heterotherms: mice (*Mus musculus*): preferred ambient temperature- *n*=15, cold- *n*=6; hamsters (*Mesocricetus auratus*): preferred ambient temperature- *n*=12, cold- *n*=8. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): preferred ambient temperature- *n*=11, cold- *n*=7.

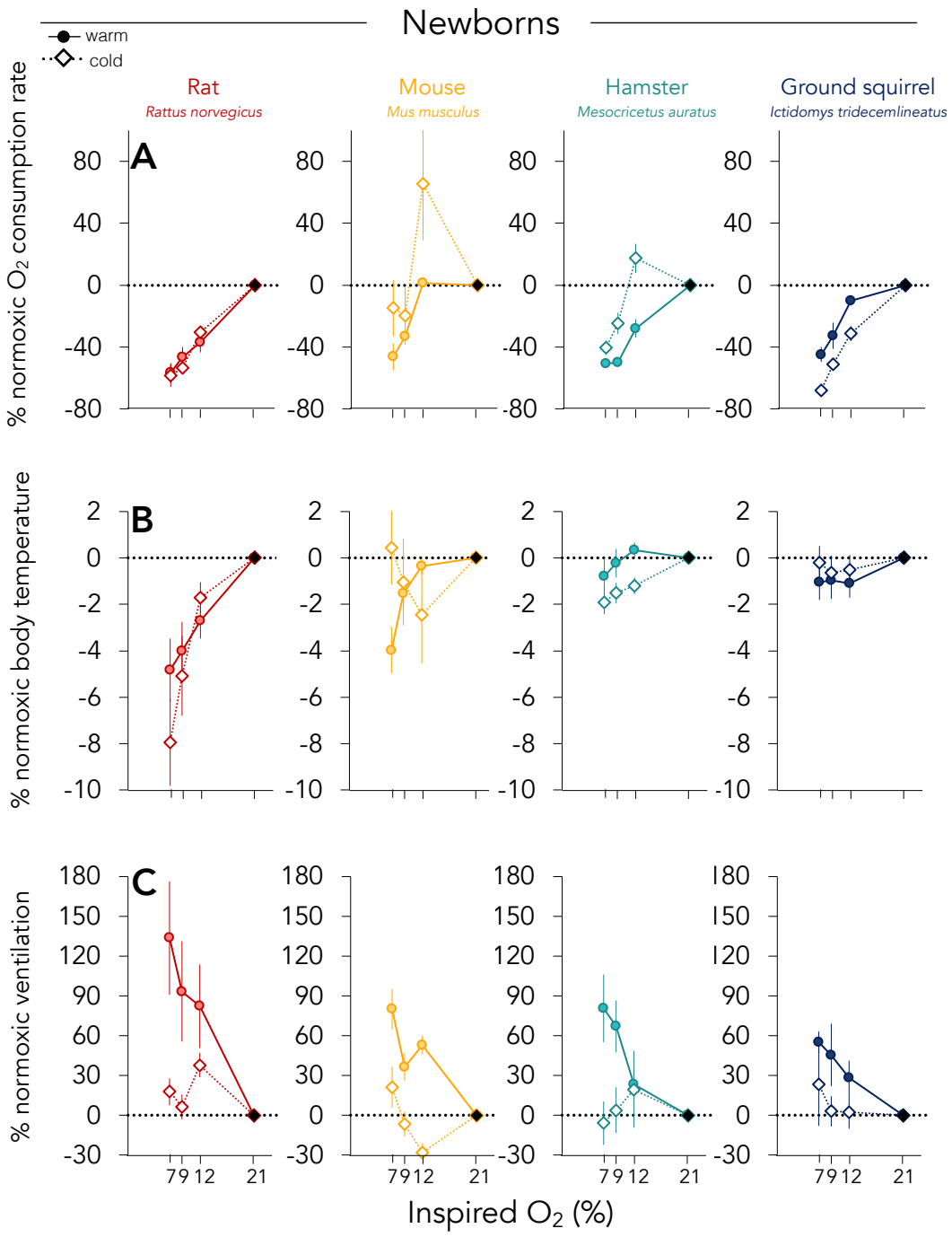

Figure 5.6. Comparison of the effects of progressive hypoxia on percent normoxic: **(A)** O_2 consumption rate, **(B)** body temperature; and **(C)** ventilation of newborn homeothermic and heterothermic rodents at their preferred ambient temperature (33°C; filled circles) and in the cold (21°C; open diamonds). Data are expressed as means ± s.e.m. Homeotherms: rats (*Rattus norvegicus*): preferred ambient temperature- *n*=8, cold- *n*=4. Facultative heterotherms: mice (*Mus musculus*): preferred ambient temperature- *n*=15, cold- *n*=6; hamsters (*Mesocricetus auratus*): preferred ambient temperature- *n*=12, cold- *n*=8. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): preferred ambient temperature- *n*=11, cold- *n*=7.

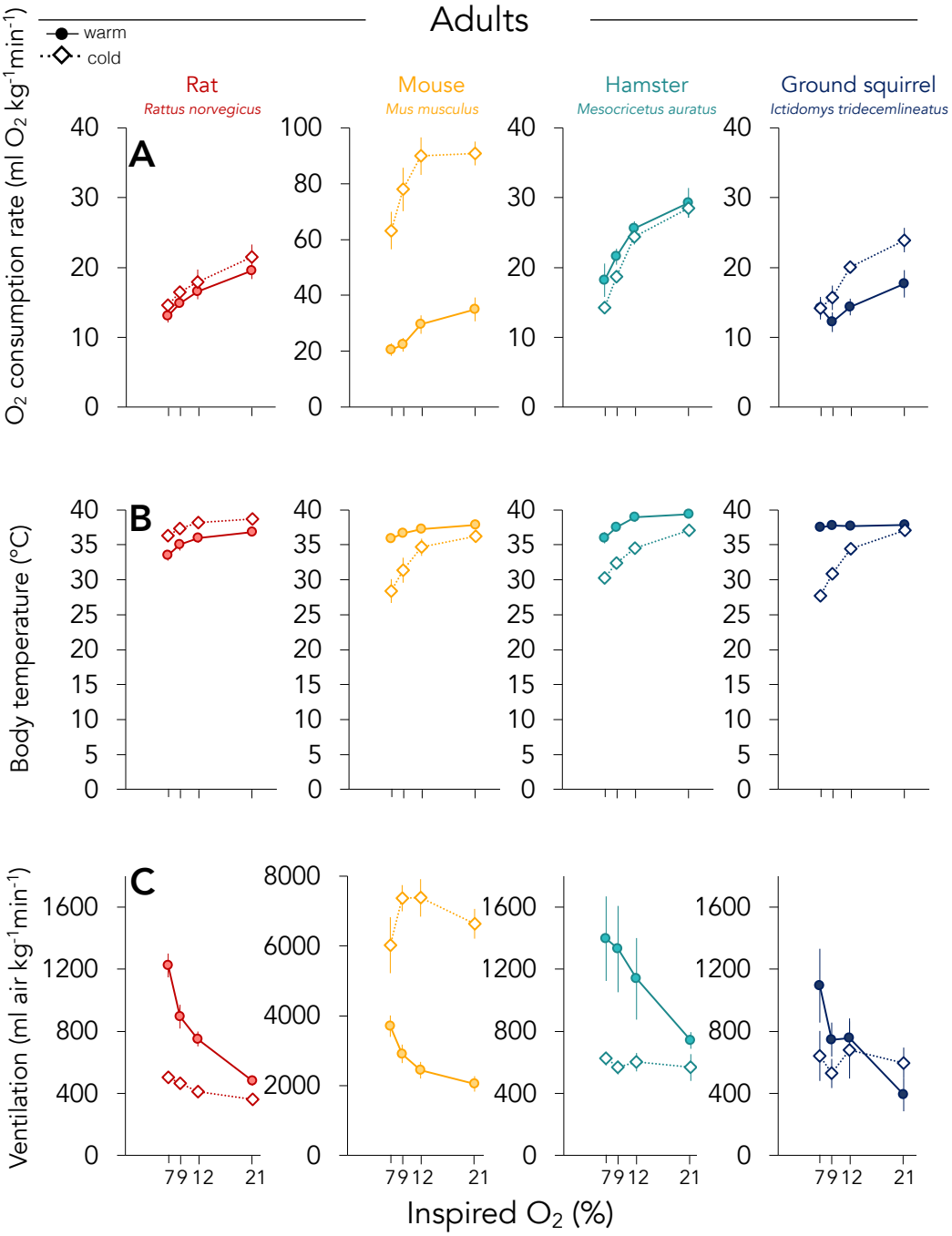

Figure 5.7. Comparison of the effects of progressive hypoxia on **(A)** O_2 consumption rate (ml $kg^{-1}min^{-1}$), **(B)** body temperature (°C); and **(C)** ventilation (ml $kg^{-1}min^{-1}$) of adult homeothermic and heterothermic rodents at thermoneutrality (26°C; filled circles) and in the cold (15°C; open diamonds). Note the y-axis in panels **(A & C)** for the mouse are different from that of the other species. Data are expressed as means ± s.e.m. Homeotherms: rats (*Rattus norvegicus*): thermoneutrality- *n*=6, cold- *n*=5. Facultative heterotherms: mice (*Mus musculus*): thermoneutrality- *n*=7, cold- *n*=6; hamsters (*Mesocricetus auratus*): thermoneutrality- *n*=6, cold- *n*=7. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): thermoneutrality- *n*=7, cold- *n*=9.

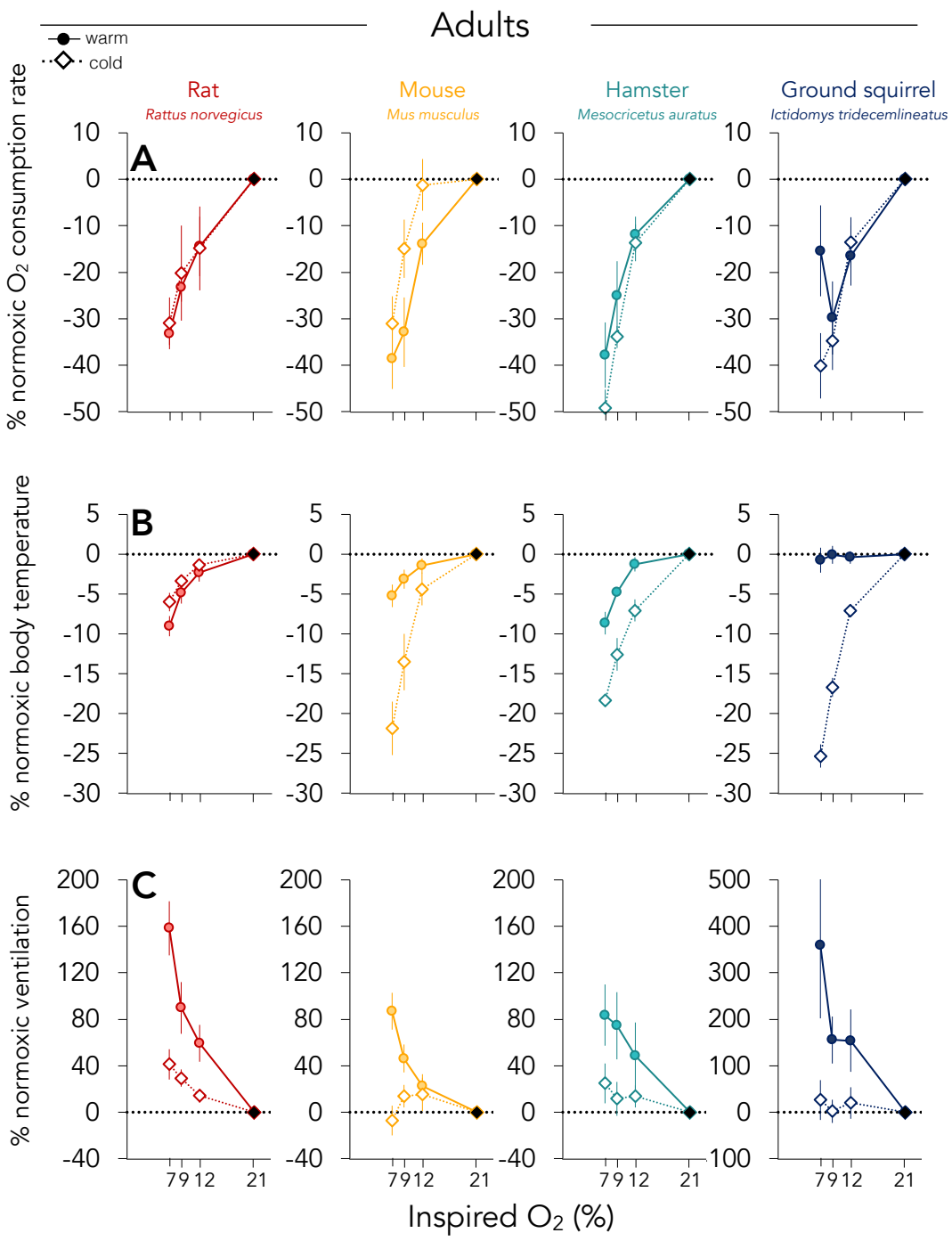

Figure 5.8. Comparison of the effects of progressive hypoxia on percent normoxic: **(A)** O_2 consumption rate, **(B)** body temperature; and **(C)** ventilation of adult homeothermic and heterothermic rodents at thermoneutrality (26°C; filled circles) and in the cold (15°C; open diamonds). Data are expressed as means ± s.e.m. Homeotherms: rats (*Rattus norvegicus*): thermoneutrality- *n*=6, cold- *n*=5. Facultative heterotherms: mice (*Mus musculus*): thermoneutrality- *n*=7, cold- *n*=6; hamsters (*Mesocricetus auratus*): thermoneutrality- *n*=6, cold- *n*=7. Obligate heterotherms: ground squirrels (*Ictidomys tridecemlineatus*): thermoneutrality- *n*=7, cold- *n*=9.

Chapter 6: General Discussion and Conclusions

The overarching objective of this thesis was to understand how newborn and adult rodents that range in their degree of heterothermic expression match O_2 supply and O_2 demand in response to cold and hypoxia. I hypothesized: (1) that the basis of enhanced cold and hypoxia tolerance in newborn and adult heterothermic rodents lies in their inherent ability to suppress their metabolic demand for O_2 and reduce body temperature, and (2) that heterothermic rodents match O_2 supply and O_2 demand through the retention of traits common to all newborn mammals. Homeothermic rodents, however, lose these neonatal traits and thus follow a different developmental trajectory than heterotherms. My results provide insight into how the metabolic, thermoregulatory, and ventilatory responses used by rodents when exposed to cold and hypoxia vary with age, heterothermic expression, and ambient temperature.

6.1 Matching O_2 supply and O_2 demand in the cold and hypoxia

6.1.1 Are the changes in O_2 supply and O_2 demand in response to cold, hypoxia, and cold and hypoxia similar in newborn homeothermic and heterothermic rodents?

The available literature suggests that adult homeotherms and heterotherms differ in how they match O_2 supply and O_2 demand in response to cold and hypoxia. It is conceivable that these differences underlie the variation in cold and hypoxia tolerance reported for homeotherms and heterotherms. Whether these differences reflect the different developmental trajectories of homeotherms and heterotherms or whether these differences are already present at birth has never been ascertained prior to the work presented here.

6.1.1.1 Matching O_2 supply and O_2 demand in the cold

When exposed to the cold, I found that the body temperatures of all newborn rodents fell in parallel with that of the external environment, accompanied by falls in O_2 consumption rates, and ventilation (Chapters 3 to 5). The relative magnitude of these responses, however, varied between newborn homeotherms and heterotherms. I found that newborn homeothermic rats

exhibited a greater thermogenic response, maintained a higher body temperature, and did not depress their rates of O_2 consumption or ventilation as much as newborn heterotherms (Chapters 3 to 5). These findings indicate that elements of the adult homeothermic phenotype may already be present at birth.

6.1.1.2 Matching O_2 supply and O_2 demand in hypoxia at thermoneutrality

When exposed to 7% O_2 at thermoneutrality (their preferred ambient temperature) all newborns exhibited a significant HMR, reducing their O_2 consumption rate by 30-60% compared to normoxic values (Chapters 3 and 4). However, they only depressed their body temperature by 5% (2-4°C), suggesting that a reduction in body temperature is not a large component of their HMR at thermoneutrality (Chapters 3 and 4). All newborns exhibited significant differences in the relative magnitude of their HVR (Chapters 3 and 4). For example, the homeothermic rat exhibited a robust 140% increase in ventilation (Chapters 3 and 4) while the facultative heterotherms exhibited a more modest 75-90% increase in ventilation (Chapters 3 and 4). The ground squirrel, an obligate heterotherm, exhibited a blunted HVR, increasing ventilation by only 30-60% (Chapters 3 and 4). While all newborn species decreased their metabolic demand for O_2 in hypoxia, and increased O_2 supply, there was an apparent shift in their reliance on the HVR to match O_2 supply and O_2 demand, correlated with heterothermic expression; with the reliance on the HVR decreasing as we progress from the homeothermic rat to the most heterothermic ground squirrel.

6.1.1.3 Matching O_2 supply and O_2 demand when exposed to both cold and hypoxia

When exposed to hypoxia in the cold, newborn homeothermic rats maintained a higher body temperature than all other species (Chapters 3 and 5). Newborn ground squirrels, on the other hand, maintained the lowest body temperature among newborns, and exhibited the greatest depression in their O_2 consumption rate (Chapters 3 and 5). The HVR that was observed at thermoneutrality was blunted in newborn rats, mice and hamsters and was eliminated in newborn ground squirrels (Chapters 3 to 5). In summary, it appears that all newborns depress O_2 consumption rate, body temperature, and their HVR is blunted when challenged with cold and

hypoxia. These responses are greatest in the newborn ground squirrel, an obligate heterotherm, least in the newborn homeothermic rat, with the facultative heterotherms falling somewhere in between these two phenotypes.

6.1.2 Are the changes in O_2 supply and O_2 demand in response to cold, hypoxia, and cold and hypoxia different in adult homeothermic and heterothermic rodents?

6.1.2.1 Matching O_2 supply and O_2 demand in the cold

Depending on the pattern of cold exposure (sustained vs. progressive), adult homeotherms and heterotherms exhibited slightly different thermogenic strategies. During sustained cold exposure (15^{o}C) both adult homeotherms and heterotherms maintained high body temperatures (Chapter 5) but during progressive cooling, while adult homeotherms maintained their body temperature at this same ambient temperature, the body temperatures of the adult heterotherms deceased, by 12 to 28% (Chapter 2). Associated with this, all adult species maintained or increased their O_2 consumption rate, supported by parallel changes in ventilation (Chapters 2 and 5). It is not clear why the body temperatures of the adult heterotherms fell when the cold was applied progressively at an average rate of 0.15 ± 0.01°C min^{-1}, but not one hour after being placed directly into the cold, but this does suggest that given time adult heterotherms respond to cold alone in a similar fashion as adult homeotherms.

6.1.2.2 Matching O_2 supply and O_2 demand in hypoxia at thermoneutrality

Because of their inherent ability to reduce body temperature and their rate of O_2 consumption, I predicted that adult heterotherms would rely on a reduction in O_2 demand (the HMR) instead of an increase in O_2 supply (the HVR) when they were exposed to hypoxia at thermoneutrality. However, adult heterotherms did not exhibit an enhanced HMR, nor a blunted HVR compared to that of the adult homeothermic rat within their TNZ (Chapters 2 and 4). Thus, it appears that when adult homeotherms and heterotherms are exposed to hypoxia within their TNZ they match O_2 supply and O_2 demand by both decreasing their O_2 consumption rates (the HMR), and increasing ventilation (the HVR).

6.1.2.3 Matching O_2 supply and O_2 demand when exposed to both cold and hypoxia

Adult homeotherms and heterotherms both exhibited a significant reduction in their rate of O_2 consumption (the HMR), accompanied by a severely blunted HVR when exposed to hypoxia in the cold (Chapters 2, 4, and 5). These changes were proportionately similar during progressive cooling (down to 15°C), but when the cold exposure was sustained at 15°C, species differences in the relative magnitude of the HMR and HVR emerged (Chapters 2 and 5). In hypoxia during sustained cold exposure, adult heterotherms exhibited a greater reduction in body temperature and their rate of O_2 consumption (an enhanced HMR) than the adult homeothermic rat (Chapter 5). Furthermore, the rat was the only species to exhibit a HVR in the cold (Chapter 5). Thus, although adult homeotherms and heterotherms both reduce O_2 demand rather than increase O_2 supply in hypoxia in the cold, adult heterotherms reduce their body temperature and O_2 consumption rate more than the rat when cold exposure is sustained.

6.1.3 Are adult heterotherms more similar to newborns than adult homeotherms in how O_2 supply and O_2 demand change in response to cold, hypoxia, and cold and hypoxia?

6.1.3.1 Matching O_2 supply and O_2 demand in the cold

As heterotherms develop from newborns into adults, they transition from significantly decreasing body temperature, O_2 consumption rate, and ventilation when exposed to cold, to maintaining a high body temperature by increasing or maintaining their O_2 consumption rate and ventilation (Chapters 2, 3, and 5). This developmental trajectory is similar to that of homeotherms (Chapters 2, 3, and 5). Thus, it appears that adult heterothermic rodents are more similar to adult homeothermic rodents than they are to newborns in the manner in which they respond to cold. As adults have a fully developed thermogenic capacity, all species attempt to maintain their body temperatures in the cold during the active euthermic season.

6.1.3.2 Matching O_2 supply and O_2 demand in hypoxia at thermoneutrality

I found that newborn and adult heterotherms both reduced O_2 demand and increased ventilation (Chapters 2-4) when exposed to hypoxia at thermoneutrality. However, this was also true of newborn and adult homeotherms (Chapters 2-4). Thus, these data do not support the "hibernator as a neonate" hypothesis, and indicate that the basis of enhanced hypoxia tolerance in newborns and adult heterothermic rodents is not due to the differential expression of the HMR rather than the HVR.

6.1.3.3 Matching O_2 supply and O_2 demand when exposed to both cold and hypoxia

When exposed to hypoxia in the cold, homeotherms and heterotherms of both ages exhibit a fall in O_2 demand, with little or no ventilatory response (Chapters 2, 3, and 5). However, the changes exhibited by adult heterotherms were more similar to those of newborns than they were to those of adult homeotherms. These data indicate that ambient temperature is an important factor in determining how rodents match O_2 supply and O_2 demand in hypoxia and support the "hibernator as a neonate" hypothesis.

6.2 Conclusions

Collectively, my data indicate that differences in cold and hypoxia tolerance between newborns and adults reflect developmental changes in the way O_2 supply and O_2 demand change under thermal and hypoxic challenges. This is true of both homeotherms and heterotherms. During thermal and hypoxic challenges, newborns depressed their O_2 consumption rate more, and increased ventilation less than their adult counterparts, albeit the relative magnitude of their responses were similar when exposed to hypoxia at thermoneutrality. Thus, it appears that enhanced cold and hypoxia tolerance of newborns stems from their ability to significantly reduce body temperature and their O_2 consumption rate, which is dependent on ambient temperature.

My data support the hypothesis that heterotherms match O_2 supply and O_2 demand through the retention of traits common to all newborn mammals, but only when exposed to hypoxia and cold in combination, as: there were apparent differences between newborn

homeotherms and heterotherms, indicating that newborn rodents differ in how they match O_2 supply and O_2 demand during thermal and hypoxic challenges, even at birth; and (2) homeothermic and heterothermic rodents did not exhibit different developmental trajectories, as adults of both homeotherms and heterotherms responded in a similar fashion when exposed to either cold or hypoxia alone. Thus, my data provide evidence for the "hibernator as a neonate" hypothesis, but only when animals are exposed to environmental hypoxia in the cold. Collectively, these data provide insight into the basis of enhanced cold and hypoxia tolerance of adult heterotherms and newborns.

6.3 Future Directions

My thesis has defined the manner in which changes occur in O_2 supply and O_2 demand when newborn and adult homeothermic and heterothermic rodents are exposed to thermal and/or hypoxic challenges. As an initial approach to understanding the basis of enhanced cold and hypoxia tolerance in newborn and adult heterothermic rodents I focused on the role of the suppression of O_2 consumption rates, possibly resulting from a reduction in the thermoregulatory set-point, in matching O_2 supply and O_2 demand in the cold and in hypoxia, either alone or in combination. With only a fragmentary understanding of the mechanisms underlying enhanced cold and hypoxia tolerance of newborns and adult heterotherms there is much potential for future research.

First, we know that newborns are more cold and hypoxia tolerant than their adult counterparts, and that adult heterothermic rodents are typically more tolerant of cold and hypoxia than adult homeothermic rodents. This raises the possibility that postnatal changes occur in both homeotherms and heterotherms. I have not characterized the extent to which these rodents may modify how they match O_2 supply and O_2 demand throughout postnatal development as I have only concentrated on rodents at two developmental stages, newborns (0-5 days old) and adults (greater than 70 days old). Daily monitoring of metabolic, thermoregulatory, and ventilatory responses during normoxia and hypoxia are required in order to identify: (1) how animals that vary in cold and hypoxia tolerance change the way in which they match O_2 supply and O_2 demand with development; (2) at what age changes occur; (3) what other changes occur during this time; and (4) whether homeotherms and heterotherms differ in their developmental

trajectory. In an impressive set of work, Wong-Riley and her colleagues have uncovered the dynamic developmental and functional changes that occur in the homeothermic rat (Wong-Riley 1989; Liu and Wong-Riley 2002; Liu and Wong-Riley 2002; Liu and Wong-Riley 2003; Liu and Wong-Riley 2005; Wong-Riley and Liu 2005; Liu et al. 2006). These changes abruptly occur within a day or two, first appearing at postnatal day 12. Collectively, her work on the homeothermic rat has identified this period as a critical window during which respiratory regulation is distinctly different from that at other ages, and renders the rat less capable of matching O_2 supply and O_2 demand when exposed to thermal and hypoxic challenges. More importantly, these changes correspond with a severe reduction in cold and hypoxia tolerance in this species (Hiestand et al. 1950; Hiestand et al. 1953; Mortola 2004; Fong 2010). Because homeotherms undergo extreme reductions in cold and hypoxia tolerance with postnatal development it begs the question if this critical window is also present in heterothermic species, who do not exhibit a reduction in cold and hypoxia tolerance with age to the same extent as homeothermic species.

Second, because physiological thermogenesis is only partially functional in newborn rodents, they may rely more on behavioural rather than physiological thermoregulatory mechanisms to favour heat dissipation, and reduce body temperature, O_2 consumption rate, and thus, O_2 demand in hypoxia (the HMR). As all animals in each of my studies were confined to a chamber and studied in isolation, my thesis defines the whole-animal physiological responses of homeothermic and heterothermic rodents to cold and hypoxia independent of their behavioural responses. However, activation of behavioural thermoregulatory mechanisms in hypoxia may represent an important defensive response used by both newborn and adult rodents in the wild. Interestingly, the effects of hypoxia on the behavioural thermoregulatory mechanisms of newborn and adult homeothermic and heterothermic rodents remains largely unknown. The limited studies that do exist indicate that in hypoxia, adult rodents placed within a thermocline select an ambient temperature lower than that in normoxia (Gordon and Fogelson 1991; Wood 1991; Dupré and Owen 1992), whereas newborn rats adopt a stretched and extended posture to favour heat dissipation (Mortola and Feher 1998). The function of behavioural thermoregulatory mechanisms in facilitating the HMR has not been unequivocally established. Thus, future studies should concentrate on whether hypoxia alters behavioural thermoregulatory mechanisms, how

these responses change with postnatal development, and whether these responses diverge in animals that vary in hypoxia tolerance.

Third, the studies of Tattersall and Milsom (2003a; 2003b; and 2009) have been instrumental for laying out the foundation and hypotheses for each of the chapters in this thesis. In an intricate set of experiments, they demonstrated that the hypoxia-induced reductions in O_2 consumption rates and body temperature (the HMR) are due to a controlled reduction in the thermoregulatory set-point, and not a limitation of O_2 supply (Tattersall and Milsom 2009). To do so they directly heated and cooled the central thermoregulatory control center of two obligate heterotherms in normoxia and hypoxia. They found that in hypoxia heterotherms lowered their thermoregulatory set-point, which resulted in a downward shift in the threshold temperature for thermogenesis (the LCT), and a widening of the TNZ. Furthermore, they found that thermogenesis could still be recruited in hypoxia by cooling the hypothalamus, albeit at reduced levels. Their findings strongly support the hypothesis that in hypoxia the mammalian thermoregulatory system is not O_2 limited, but is regulated and functions properly. However, these experiments have only been carried out on adult obligate heterotherms during the active euthermic season. Data from Chapter 2 indicate that the extreme hypoxia-induced reductions in rate of O_2 consumption and body temperature of the homeothermic rat likely resulted from a limitation of O_2 supply and failure of thermoregulatory mechanisms in hypoxia. Thus, it does not appear that all mammalian thermoregulatory systems maintain function in hypoxia. This possibility needs to be directly tested. Furthermore, whether a lowering of the thermoregulatory set-point is responsible for the extreme depression of O_2 consumption rates typical of newborn rodents in hypoxia remains unknown. Experiments examining the central regulation of body temperature and its role in the HMR of rodents that range in their degree of heterothermic expression, throughout postnatal development, during the active and hibernating season warrant investigation. These experiments are vital for furthering our understanding of the mechanisms behind the striking HMR exhibited by most small rodents.

Fourth, my thesis has concentrated on the hypoxic responses of adult heterotherms during the active-euthermic season. I have not accounted for seasonal changes that may affect the heterotherm's HMR. However, while we know that heterotherms have an immense capacity to lower their body temperature and decrease O_2 consumption rates, hypoxic exposure does not induce the same reduction as that observed during cold exposure in the hibernation season.

Indeed, obligate heterotherms demonstrate seasonal changes in their propensity to reduce body temperature and O_2 consumption rate, as they typically only do so during cold exposure in the winter. Furthermore, obligate heterotherms also demonstrate seasonal changes in their hypoxia tolerance, as tolerance during hibernation exceeds that during the active-euthermic season (Biörck et al., 1956; Bullard et al. 1960; Frerichs and Hallenbeck 1998). Thus, it is conceivable that seasonal changes in the heterotherm's reliance on the HMR to match O_2 supply and O_2 demand coincide with this reduction in tolerance. In one of the only studies to look at seasonal changes in the HMR and HVR of mammals, Levesque and Tattersall (2009) found that in the active-euthermic season the heterothermic Eastern chipmunk (*Tamias striatus*) exhibited a hypoxic response similar to that of homeothermic mammals. Yet during the hibernating season homeotherms and heterotherms exhibited divergent hypoxic responses. While, in a study comparing the HVR of the heterothermic Columbian ground squirrel (*Spermophilus columbius*) during the summer and winter, McArthur and Milsom (1991) found that there were no seasonal effects on the robust HVR exhibited by this species. However, their study was conducted during the early winter months (October) when animals were preparing for hibernation. The contrasting findings of McArthur and Milsom (1991), and Levesque and Tattersall (2009) begs the question: do seasonal changes affect how obligate heterotherms match O_2 supply and O_2 demand in hypoxia? And if so, are hibernating obligate heterotherms more similar to newborn mammals than they are during the active-euthermic season or when they are preparing for hibernation? Addressing these questions will shed great insight into the underlying mechanisms of hypoxia tolerance in heterotherms, and will be essential for truly appreciating the striking physiological similarities between newborn mammals and those mammals capable of hibernation.

Lastly, I did not examine the effects of chronic exposure to cold and hypoxia on the strategies that newborn and adult homeothermic and heterothermic rodents use to match O_2 supply and O_2 demand. The overarching hypothesis of my thesis was that enhanced cold and hypoxia tolerance of newborns and adult heterotherms stems from their remarkable propensity to reduce body temperature and O_2 consumption rate. However, a reduction in O_2 consumption rate is not a viable option for animals that are chronically exposed to these stressors, owing to the fact that they must grow, forage, reproduce, and remain active in a low O_2 environment. Although the acute responses (minutes to hours) have been reported in multiple species of newborn and adult rodents (Mortola et al. 1989; Frappell et al. 1992), studies on their chronic responses are limited

(but see Frappell and Mortola 1994; Beaudry and McClelland 2010; Cadena and Tattersall 2014; Ivy and Scott 2017). This begs the questions: do heterotherms use the same strategies to match O_2 supply and O_2 demand when they are exposed to cold and hypoxia acutely and chronically? And do the strategies they use to match O_2 supply and O_2 demand in chronic cold and hypoxia vary from that of homeotherms? In hypoxia, responses are known to vary depending on when during development the hypoxic exposure occurs, and the duration of the exposure (Powell et al. 1998). For example, Frappell and Mortola (1994) found that adult rats raised in hypoxia were smaller in size, had lower O_2 consumption rates, and increased ventilation compared to rats raised in normoxia. Whereas adult hamsters raised in hypoxia were similar in size, maintained comparable O_2 consumption rates, but had higher ventilation rates than hamsters raised in normoxia. Whether these species differences manifest themselves when homeotherms and heterotherms are raised in cold and hypoxic environments, or when these environmental challenges are introduced at other stages in development remain unknown. What we do know is that acclimation to cold and hypoxia alters the initial hypoxia-induced drop in body temperature and O_2 consumption rate of adult mice and rats (Beaudry and McClelland 2010; Cadena and Tattersall 2014). With sustained cold and hypoxic exposure, initial decreases in body temperature and O_2 consumption rate are followed by their partial reestablishment, as physiological adjustments take place that increase O_2 uptake and delivery to the tissue to deal with hypoxia (Ivy and Scott 2017). Whether the gradual replacement of the HMR with an enhanced HVR is also true of adult heterotherms acclimated to cold and hypoxia is an interesting avenue of research. Studies examining and comparing the time-course of the metabolic, thermoregulatory, and ventilatory responses to hypoxia, and how acute responses are modified by acclimation to cold and hypoxia are needed to provide further insight into the mechanisms underlying the enhanced cold and hypoxia tolerance of heterotherms.

These are just a few of the promising avenues for future research aimed at better understanding the enhanced cold and hypoxia tolerance of newborns and adult heterothermic rodents. The strategies animals use to match O_2 supply and O_2 demand are complex, and with the ultimate need to survive the strategies employed reflect a species and context dependent balance. Despite previous investigations, the mechanisms underlying cold and hypoxia tolerance of newborns and adult heterothermic rodents are unknown presenting novel and exciting opportunities for future studies.

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