Thermoregulation in pronghorn antelope (*Antilocapra americana*, Ord) in winter

J. Hébert[1], A. Lust[1], A. Fuller[2], S. K. Maloney[3], D. Mitchell[2] and G. Mitchell[1],*

[1]Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA, [2]Department of Physiology, University of the Witwatersrand, Johannesburg, South Africa and [3]Physiology, School of Biomedical and Chemical Sciences, University of Western Australia, Perth, Australia

*Author for correspondence (e-mail: mitchg@uwyo.edu)

SUMMARY

Conservation of energy is a prerequisite thermoregulatory strategy for survival in northern hemisphere winters. We have used thermistor/data logger assemblies to measure temperatures in the brain, carotid artery, jugular vein and abdominal cavity, in pronghorn antelope to determine their winter body temperature and to investigate whether the carotid rete has a survival role. Over the study period mean black globe and air temperature were –0.5±3.2°C and –2.0±3.4°C, respectively, and mean daytime solar radiation was ~186 W m^{-2}. Brain temperature (T_{brain}, 39.3±0.3°C) was higher than carotid blood temperature ($T_{carotid}$, 38.5±0.4°C), and higher than jugular temperature ($T_{jugular}$, 37.9±0.7°C). Minimum T_{brain} (38.5±0.4°C) and $T_{carotid}$ (37.8±0.2°C) in winter were higher than the minimum T_{brain} (37.7±0.5°C) and $T_{carotid}$ (36.4±0.8°C) in summer that we have reported previously. Compared with summer, winter body temperature patterns were characterized by an absence of selective brain cooling (SBC), a higher range of T_{brain}, a range of $T_{carotid}$ that was significantly narrower (1.8°C) than in summer (3.1°C), and changes in $T_{carotid}$ and T_{brain} that were more highly correlated (r=0.99 in winter *vs* r=0.83 in summer). These findings suggest that in winter the effects of the carotid rete are reduced, which eliminates SBC and prevents independent regulation of T_{brain}, thus coupling T_{brain} to $T_{carotid}$. The net effect is that $T_{carotid}$ varies little. A possible consequence is depression of metabolism, with the survival advantage of conservation of energy. These findings also suggest that the carotid rete has wider thermoregulatory effects than its traditional SBC function.

Key words: pronghorn, winter thermoregulation, carotid rete.

INTRODUCTION

In summer, thermoregulation in free-living pronghorn antelope is characterized by an unusually wide variation in body temperature (measured as carotid artery blood temperature, $T_{carotid}$), and an unusually low variation in brain temperature (T_{brain}) (Lust et al., 2007). This combination arises from the regulation of T_{brain} by selective brain cooling (SBC) at $T_{carotid}$ greater than 39.5°C, and brain warming when $T_{carotid}$ is less than 37.8°C. We concluded that the relatively constant T_{brain} was the origin of the variation in $T_{carotid}$. Thermoregulatory effector responses are maximal when $T_{carotid}$ and T_{brain} are changed in parallel (Jessen and Feistkorn, 1984). When they are dissociated, for example by independent regulation of T_{brain}, responses are reduced. A predictable result is that T_{brain} stays relatively constant, $T_{carotid}$ is not as tightly controlled and variation in $T_{carotid}$ will increase. The advantage of variation in $T_{carotid}$ in a hot and arid environment is conservation of water (Jessen, 1998; Mitchell et al., 2002).

The independent regulation of T_{brain} that occurs in summer in pronghorn (Lust et al., 2007) is a function of the carotid rete mirabile (the 'rete'). The rete consists of a plexus of small arteries arising from the external carotid artery. In pronghorn almost all arterial blood that reaches the brain, except for a small supply from the basilar artery, passes through the rete (Carlton and McKean, 1977). The rete is surrounded by a venous cavernous sinus containing blood that is cooler than arterial blood by virtue of its previous passage past the nasal mucosa. The nasal mucosa is cooled by respiratory evaporative heat loss. Heat exchange between the rete carrying warm blood to the brain and the cool venous blood reduces arterial blood temperature and produces SBC. In the years following the discovery of SBC (Baker and Hayward, 1968) it was assumed that its

biological purpose was to protect the brain from thermal damage when body temperatures increased, for example during exercise (Taylor and Lyman, 1972). This function for the rete was, however, dispelled by studies done mostly in southern hemisphere animals exposed to hot and arid conditions (Mitchell et al., 2002), but also in reindeer in the northern hemisphere (Aas-Hansen et al., 2000). These studies showed that brain temperature was not cooled below arterial temperature during exercise. The alternative proposal developed was that the rete modulated thermal responses (Maloney and Mitchell, 1997; Jessen, 2001) especially to achieve water conservation (Jessen, 1998; Mitchell et al., 2002).

The characteristics and mechanisms of thermoregulation in temperate zone winters in large animals and their contribution to survival are less well known. The essential conflict in winter is between increased energy expenditure to maintain body temperature in extreme cold and the need to conserve energy because of food shortages. It is obvious that this conflict is resolved. For example, in two previous studies of thermoregulation in winter in northern hemisphere artiodactyls a relatively constant body temperature has been found. In one study on free-living mule deer, Sargeant et al. (Sargeant et al., 1994) recorded abdominal temperatures ($T_{abdominal}$) from seven mule deer (*Odocoileus hemionus*) by radiotelemetry and found that in winter the range of $T_{abdominal}$ was on average slightly narrower (range 37.8±0.2 to 38.4±0.3°C) than it was in summer (range 38.0±0.2 to 38.8±0.2°C). In the second study, Parker and Robbins (Parker and Robbins, 1984) measured rectal temperature in five mule deer and eight elk (*Cervas elephas*) in captivity. They found that in both species T_{rectal} was constant at ~38.6°C (Parker and Robbins, 1984). It was concluded that this constancy of temperature was achieved partly by growth of a very thick fur

undercoat and partly by behaviour, but in neither of these studies were physiological mechanisms analysed.

A physiological mechanism that could underlie the relatively constant body temperature that has been observed in wintering artiodactyls is reduced cooling of the brain by the rete. The amount of cooling by the rete can be controlled at three sites: at the nasal mucosa, at the veins directing the pathway of returning blood, or at the rete itself. The rete is not innervated so regulation at the last of these sites is unlikely and has not been shown. Regulation of blood flow in the veins supplying the cavernous sinus is an established mechanism (Johnsen et al., 1987; Johnsen and Folkow, 1988) with the input controlled by T_{brain} (Kuhnen and Jessen, 1991). A nasal input has been shown in sheep (Maloney and Mitchell, 1997) and in reindeer (Johnsen et al., 1985). In reindeer, venous blood flow to the rete decreases when low ambient temperatures cause nasal mucosa temperature (T_{nm}) to fall. If so, then in winter conditions rete activity should be minimal, and because changes in T_{brain} and $T_{carotid}$ will then occur together, thermoregulatory responses will be maximal (Jessen and Feistkorn, 1984), and variation in $T_{carotid}$ will be reduced.

We report here the results of a study done specifically to investigate whether the functioning of the rete is reduced in winter, and more generally to provide comprehensive data on winter body temperature in artiodactyls on which to base ideas of how winter thermoregulatory mechanisms might contribute to survival. Our data suggest that rete activity is suppressed in pronghorns in winter, that this suppression is a crucial component of their winter thermoregulatory mechanisms and is a likely prerequisite for their survival. Our data also suggest that the rete has much wider thermoregulatory functions than those outlined above.

MATERIALS AND METHODS

We used the same basic techniques as in our summer study (Lust et al., 2007).

This study, like our summer study, was done at the Tom Thorne/Beth Williams Wildlife Research Center at Sybille, southeastern Wyoming (41°47′N, 105°20′W). The study lasted for 71 days during winter from 9th November 2004 to 18th January 2005.

Animals

Five adult pronghorn antelope (two castrated males and three non-pregnant females), body mass 40–50 kg, were captured by darting at Warren Air Force Base, Cheyenne, WY, USA using thiafentanil at 0.1 mg kg^{-1} body mass (Thiafentanil 10 mg ml^{-1}; Wildlife Pharmaceuticals, Inc., Fort Collins, CO, USA) and transported to the Research Center at Sybille. Prior to the start of the study the animals were kept in a small holding enclosure for 3–4 weeks so they could recover from transport and acclimate to the local environment. For the duration of the experiment they were kept in a 200 ha enclosure with five other pronghorns where they were free-living for the recording period. Throughout the winter they had access to alfalfa hay *ad libitum* supplemented by ~2 kg commercial deer pellets daily (Z+W Mills, Torrington, WY, USA).

Measurement of body temperature

Temperature was measured and recorded from four body sites (brain, carotid artery, jugular vein and abdominal cavity) using small bead thermistors (ABOE3-BR11KA 103K-L10, GE Thermometrics, Edison, NJ, USA). Data were stored on data loggers (XTIC 32+34+36, Onset, Pocasset, MA, USA) connected to the thermistors by a flexible coax cable (no. 83265, Belden, Richmond, IN, USA),

and able to record temperature between 34°C and 46°C, every 5 min, to an accuracy of 0.04°C. The loggers were waxed (paraffin wax/Elvax, Mini-Mitter, Sunriver, OR, USA) to make them waterproof and biologically inert. After waxing, the loggers weighed approximately 55 g and had dimensions of 50 mm× 45 mm×20 mm. Each of the thermistor/logger assemblies was calibrated against a thermometer (Quat 100, Heraeus, Hanau, Germany) that measures temperatures to an accuracy of ±1×10^{-2} K (Arce et al., 2006), and is a recognized reference thermometer (Bock et al., 2005).

Surgical procedures

At the time of surgery the animals were re-darted using thiafentanil and anaesthetized with isoflurane (Abbott Animal Health, Abbot Park, IL, USA) administered via a face mask at a concentration of 8% for induction and 1–2% for maintenance in oxygen. The effects of thiafentanil were reversed with 2 mg kg^{-1} naltrexone (Nalterzel, 50 mg ml^{-1}; Wildlife Pharmaceuticals, Inc.).

Using aseptic surgical techniques, thermistors were implanted into the four body sites. The loggers for the brain and blood vessel thermistors were buried subcutaneously on the side of the neck about half-way between the head and thoracic inlet. The abdominal logger and its thermistor were implanted in the abdominal cavity.

Brain temperature: a thermistor was encased in a rigid guide tube (cellulose acetate butyrate tubing: World Precision Instruments, Sarasota, FL, USA; o.d. 3.2 mm, i.d. 1.98 mm, 34 mm in length) and pushed through a 3.2 mm hole drilled through the skull in the midline, 12.5 mm anterior to the suture between the frontal and parietal bones, so that the thermistor in the tip of the guide tube was near the hypothalamus. These coordinates were determined by prior dissection and analysis of pronghorn heads, and placement was confirmed at autopsy. The guide tube was attached to a head plate (L×W×H 22 mm×15 mm×9 mm) which was fixed to the skull by two 6 gauge, 15 mm long, self-tapping, stainless steel screws. No clinical signs of neurological lesions arose from this procedure.

Blood vessel temperature: thermistors in a blind-ended, thin-walled, polytetrafluorethylene (PTFE) tube approximately 100 mm long, made from a catheter (o.d. 0.9 mm; Straight Flush 4F Catheter, Cordis, The Netherlands), were inserted into the carotid artery and jugular vein about midway along the length of the neck in a direction opposite to that of blood flow so that the thermistor was detecting the temperature of free-flowing blood. The site of insertion in the vessels was closed by a purse string suture using 4/0 nylon.

Abdominal temperature: thermistors used to measure abdominal temperature were encased in the wax surrounding the logger. The abdominal thermistor was inserted into the abdominal cavity through an incision made at the left paralumbar fossa. The loggers were free floating in the abdomen.

All animals were given 5 ml dexamethasone (2 mg ml^{-1}; Vedco, St Joseph, MO, USA) and 2 ml of a combination of penicillin G benzathine plus penicillin G procaine (300 000 units ml^{-1}; Hanford Manufacturing Co., Syracuse, NY, USA) intramuscularly at the start of surgery. Enrofloxacin tablets (Baytril 22.7 mg: Bayer HealthCare LLC, Shawnee Mission, KS, USA) were placed in all surgical sites prior to wound closure.

Climatic conditions

Weather conditions during the study were measured using a 15 channel HOBO weather station (Onset, Pocasset, MA, USA). Six variables were measured: black globe temperature, ambient air temperature, solar radiation, relative humidity, and wind speed and

wind direction. Black globe temperature (T_{globe}) integrates air temperature, solar radiation and wind speed. Snow precipitation was measured separately at the research station and data from three other sites in Wyoming were also obtained, to estimate snowfall in southeastern Wyoming over the study period.

Data analysis

At the end of the study the animals were killed, and the loggers and thermistors removed. Temperatures recorded were calibrated against pre-insertion calibration data. Data were obtained from all five brain assemblies, all five carotid assemblies, four abdominal loggers (one logger failed to launch) and three jugular vein assemblies (one jugular assembly failed to launch and at autopsy it was found that the other had come out of the vein). We obtained over 300 000 temperature measurements in total, over 40 days from pronghorn M476, 52 days from pronghorn F471, 53 days from pronghorn M475, 68 days from pronghorn F473 and 68 days from pronghorn F474.

The data were consolidated first by pooling the 12, 5 min interval data points obtained from each logger for each hour of measurement to produce 24 average hourly temperatures for each animal for each day. These hourly averages for the five animals were, in turn, averaged to produce a weighted mean hourly temperature for them as a group. These averages could be further pooled for all study days or component days of the study period to provide a comprehensive overview of body temperature throughout the recording period.

Specifically, body temperature data were consolidated into three categories consisting of the six warmest days (T_{globe} 5.8±1.9°C), six coldest days (T_{globe} −10.2±2.2°C) and six intermediate days (T_{globe} 0.1±0.2°C) in the study period, to assess whether thermoregulatory mechanisms differed depending on environmental conditions. A second consolidation was to average daily means into a week, to produce nine separate weekly temperature profiles for each body site over the study period. These nine weekly periods were used to establish correlations between the weather variables and body temperatures in each week of the study period. Similar consolidations were made for weather data using the two data points recorded each hour for each of the variables.

Calculation of cerebral blood flow

We calculated cerebral blood flow (CBF) in each animal using a modified convective heat loss equation (Lust et al., 2007):

$$CBF = [0.0448e^{(T_{brain}/11.7)} \div (3.6\Delta T)] \times 60 ,$$

which was simplified to:

$$CBF = 0.75\Delta T e^{(T_{brain}/11.7)} ,$$

where ΔT is the difference between T_{brain} and $T_{carotid}$. The units of CBF are g 100 g^{-1} min^{-1}, which was converted to ml 100 g^{-1} min^{-1} by dividing by the density of blood (1.055 g ml^{-1}). This equation was used to assess the extent to which observed differences between the carotid and brain temperatures that we found in this study could be attributed to increased or decreased removal of heat by CBF.

Statistics

The data were entered into an Excel data analysis spreadsheet. Three statistical tests were used. For comparison of summer and winter temperatures, Student's two-sample t-test assuming unequal variance was used with $P<0.05$ being regarded as significant. For assessing whether body site had an influence on recorded temperatures we used repeated measures ANOVA and an ANOVA single-factor test

to compare temperatures between body sites, with $P<0.05$ being significant. Relationships between temperatures and environmental cues were assessed by linear regression analysis.

RESULTS
Weather

The climatic conditions over the 71 day study period are summarized in Table 1. Compared with summer data, mean T_{air} was ~20°C colder, mean T_{globe} was ~25°C colder, mean daytime solar radiation was halved (186±20 vs 380±107 W m^{-2}), and mean day length was 5 h 45 min shorter. Wind speed was 2.2±0.5 m s^{-1} in winter (vs 1.1±0.3 m s^{-1} in summer). Mean snowfall at four weather stations in SE Wyoming was 668 mm over the study period with 1085 mm being recorded at the Research Center at Sybille.

Body temperature

Mean temperature for the four body sites in each of the animals and the weighted means for all animals are shown in Table 2. For comparison the mean summer temperatures for the same body sites also are shown. Body site had a significant influence on temperature (d.f.=2, F=17.5097, P=0.0003). As in summer, in winter T_{brain} was higher than $T_{carotid}$ (d.f.=1, F=7.3266, P=0.0268) and $T_{jugular}$ (d.f.=1, F=28.1442, P=0.0007). $T_{abdominal}$ was higher than $T_{jugular}$ (d.f.=1, F=15.7443, P=0.0041) but not different from T_{brain} (d.f.=1, F=3.24, P=0.1096) or $T_{carotid}$ (d.f.=1, F=0.8757, P=0.3768). $T_{carotid}$ was significantly higher than $T_{jugular}$ (d.f.=1, F=10.5263, P=0.0118). Mean temperatures for T_{brain}, $T_{carotid}$, $T_{abdominal}$ and $T_{jugular}$ were not significantly different between summer and winter, but minimum T_{brain} (38.5±0.4°C) and minimum $T_{carotid}$ (37.8±02°C) were significantly higher in winter than in summer (t_7=2.718, P=0.017). Maximum T_{brain} and $T_{carotid}$ were not different in summer and winter (t_7=1.1746, P=0.139).

Between individual animals maximum and minimum $T_{carotid}$ and T_{brain} varied (Table 3). The mean values for minimum $T_{carotid}$ in winter were significantly lower than those for T_{brain} (d.f.=1, F=11.0860, P=0.010) but maximum $T_{carotid}$ and T_{brain} were not different (d.f.=1, F=3.751, P=0.0888). Comparing summer with winter, the difference between maximum and minimum $T_{carotid}$ of 1.8±0.7°C was significantly narrower than it was in the summer (3.1±0.4°C; t_7=3.52, P=0.0098). The frequency distribution of 0.1°C temperature intervals for T_{brain}, $T_{jugular}$ and $T_{carotid}$ is shown in Fig. 1. In summer $T_{carotid}$ varied between 35.8 and 40.3°C (ΔT=4.5°C) and T_{brain} varied between 37.4 and 40.7°C (ΔT=3.3°C). In winter $T_{carotid}$ varied between 37.8 and 40.6°C (ΔT=2.8°C) and T_{brain} varied between 38.0 and 40.5°C (ΔT=2.5°C). In addition, in summer the frequency distributions had long tails to the left while in winter the tails were shifted to the right.

Factors affecting body temperature

Unlike in summer, where we established that changes in $T_{carotid}$ over the 14 week study period correlated significantly with T_{globe} mean

Table 1. Summary of winter weather conditions

Variable	Mean±s.d.	Range
T_{air} (°C)	−2.0±3.4	−7.3±4.0 to 4.5±2.6
T_{globe} (°C)	−0.5±3.2	−8.5±4.2 to 13.6±2.9
Daytime solar radiation (W m^{-2})	186.0±20.1	4.2±2.3 to 413.9±29.4
Wind speed (m s^{-1})	2.2±0.5	1.4±0.8 to 3.1±0.5
Day length	9 h 18 min ±18 min	9 h 12 min to 9 h 54 min

T_{air}, air temperature; T_{globe}, black globe temperature (see text).

Table 2. Mean hourly temperature (±s.d.) at each of the body sites

Season	$T_{carotid}$ (°C)	N^*	T_{brain} (°C)	N^*	$T_{jugular}$ (°C)	N^*	$T_{abdominal}$ (°C)	N^*
Summer								
Mean±s.d.	38.6±0.3	4902	38.9±0.3	4037	38.0±0.4	2935	38.8±0.4	4902
Range	35.8–40.3		37.4–40.7		36.1–39.9		35.0–40.4	
Winter								
F471	38.6±0.2	1239	39.6±0. 2	1239	37.9±0.2	1239	–	–
F473	38.4±0.3	1632	38.9±0.3	1632	–	–	38.5±0.3	1632
F474	38.4±0.2	1632	38.9±0.4	1632	–	–	38.6±0.2	1632
M475	39.0±0.4	1272	39.2±0.4	1272	38.5±0.4	1272	39.1±0.4	1272
M476	39.0±0.4	960	39.5±0.5	960	37.2±0.5	960	39.2±0.5	960
Mean±s.d.	38.5±0.4	6735	39.3±0.3	6735	37.9±0.7	3471	38.8±0.3	4496
Range	37.6–40.6		38.1–41.2		36.2–39.9		37.8–40.9	

$T_{carotid}$, carotid blood temperature; T_{brain}, brain temperature; $T_{jugular}$, jugular temperature; $T_{abdominal}$, abdominal temperature. F and M denote female and male, respectively. The number of animals (N) was 4 for summer and 5 for winter. N^*, number of hourly means. Summer and winter mean±s.d. values are weighted means.

Table 3. Individual variation in carotid and brain temperature

	$T_{carotid}$			T_{brain}		
	Min. (°C)	Max. (°C)	Max.–min. (°C)	Min. (°C)	Max. (°C)	Max.–min. (°C)
Summer						
F593	35.9	38.7	2.8	37.4	39.7	2.3
F594	36.4	39.8	3.4	37.6	40.7	3.1
F598	35.8	39.3	3.5	37.5	39.7	2.2
M595	37.6	40.3	2.7	38.4	40.1	1.7
Mean±s.d. ($N=4$)	36.4±0.8	39.5±0.7	3.1±0.4	37.7±0.5	40.1±0.5	2.3±0.6
Winter						
F471	37.8	38.7	0.8	39.2	40.2	1.0
F473	37.8	39.7	1.9	38.0	40.1	2.1
F474	37.6	39.2	1.6	38.5	40.1	1.6
M475	38.1	40.1	2.0	38.4	40.5	2.1
M476	37.9	40.6	2.7	38.6	41.2	2.6
Mean±s.d. ($N=5$)	37.8±0.2	39.7±0.7	1.8±0.7	38.5±0.4	40.4±0.5	1.9±0.6

Min., minimum; max., maximum. F denotes female, M denotes male.

($r=0.78$, $P<0.001$) and range ($r=0.916$, $P<0.001$), no similar relationship was found for winter ($r=0.272$, $P>0.05$ and $r=-0.188$, $P>0.05$, respectively) probably because $T_{carotid}$ varied very little each day ($0.9±0.1$°C vs $1.6±0.7$°C in summer). However, each animal did have an identifiable maximum and minimum daily $T_{carotid}$, and consecutive maximum and minimum $T_{carotid}$ occurred on average 23 h 55 min ±88 min and 23 h 18 min ±102 min apart over the entire study period.

When the times of maximum and minimum $T_{carotid}$ in each of the 9 weeks of the study period were correlated with the external environmental cues of the time of T_{globe} maximum and T_{globe} minimum, day length, and the time of sunrise and sunset, the only significant correlations were between the time of maximum solar radiation and the time of maximum $T_{carotid}$ ($r=0.569$, $P<0.01$), and between the time of minimum $T_{carotid}$ and the time of sunset ($r=0.601$, $P<0.01$). The mean time of maximum $T_{carotid}$ during winter (15 h 11 min ±62 min; range 13 h 45 min to 16 h 43 min) occurred significantly earlier in winter than in summer ($t_{18}=8.24$, $P=1.59e^{-07}$) and closer to the time of maximum solar radiation (11 h 52 min ±25 min). In summer, maximum $T_{carotid}$ occurred at 18 h 47 min ±65 min and the time of solar maximum was 12 h 24 min ±24 min, so body temperature was highest ~6.5 h after solar 'noon'. In winter the delay was ~3.5 h. The time of minimum $T_{carotid}$ in winter was earlier (07 h 36 min ±78 min) than in summer (09 h 18 min ±72 min) but not significantly so.

Respiratory evaporative heat loss

The difference between $T_{carotid}$ and $T_{jugular}$ is an indicator of whether heat loss is occurring from the nasal mucosa. By this measure, during winter nasal cooling occurred continuously (Fig. 2). Mean $T_{jugular}$ in winter was the same as it was in the summer (Table 2), but the pattern of $T_{jugular}$ was different. In summer, maximum $T_{jugular}$ was always higher than the simultaneously measured $T_{carotid}$ and at low $T_{carotid}$ the mean, maximum and minimum $T_{jugular}$ were higher than the simultaneously measured $T_{carotid}$. In winter, maximum, minimum and mean $T_{jugular}$ were always lower than the simultaneously measured $T_{carotid}$ (Fig. 2).

T_{brain} and $T_{carotid}$

In winter there was no evidence of SBC, even though T_{brain} often exceeded the ~39.5°C threshold for SBC observed in summer (Figs 1, 2). Brain warming was minimal (Fig. 2). Minimum T_{brain} consistently was above simultaneously measured $T_{carotid}$, a pattern quite different from the pattern found in summer. Moreover, as Fig. 2 shows, the range of temperatures recorded narrowed as the days became colder. The range of $T_{carotid}$ and T_{brain} was 3.3°C on the warmest days, 2.5°C on intermediate days and 2.3°C on the coldest days.

The absence of SBC implies that in winter the cooling effect of the rete was much reduced, a conclusion supported by comparing the relationship between $T_{carotid}$ and T_{brain} in pronghorn in summer (Fig. 3A) (Lust et al., 2007) and in winter (Fig. 3B),
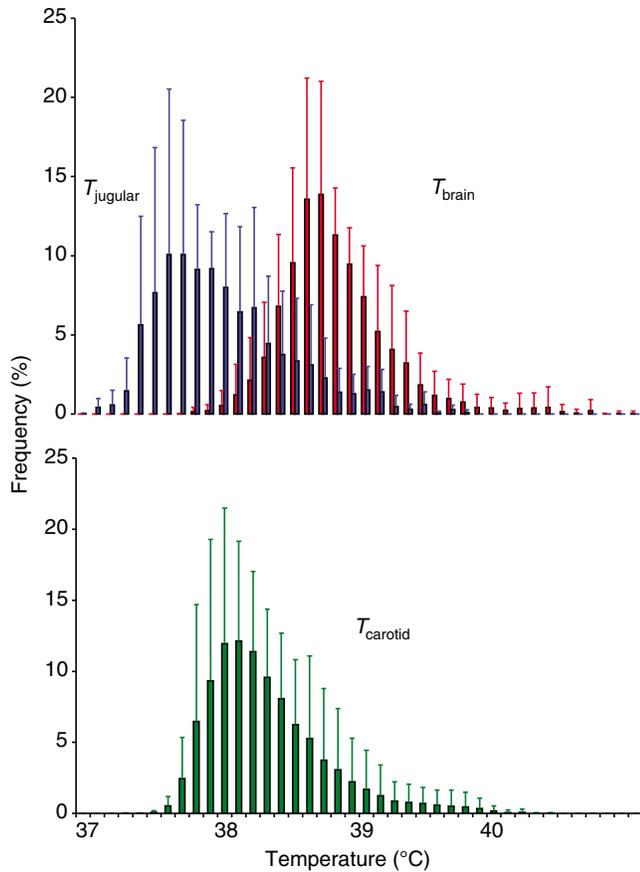
Fig. 1. Frequency of occurrence of 0.1°C intervals of brain (red), jugular (blue) and carotid (green) temperatures. The brain distribution is narrower and mode temperatures occur to the right of the carotid and jugular distributions. The frequency of occurrence of the jugular distribution is to the left of the carotid distribution. All three distributions are characterized by long tails to the right.

Fig. 2. The relationships between T_{brain} (red), $T_{carotid}$ (blue) and $T_{jugular}$ (brown) on the 6 hottest days of winter (A), the 6 days of intermediate temperature (B) and the 6 coldest days (C; see text). As days become colder the range of T_{brain} and $T_{carotid}$ becomes narrower. On hot and intermediate days $T_{jugular}$ and $T_{carotid}$ diverge while on the coldest days $T_{carotid}$ and $T_{jugular}$ converge.

and the relationship we have found in summer in the horse, an animal that has no rete (Fig. 3C) (Mitchell et al., 2006). Fig. 3 also shows the calculated CBF in each case. In winter the relationship between T_{brain} and $T_{carotid}$ was more similar to that of the horse than it was to pronghorn in the summer: there was no SBC, T_{brain} minima rarely were less than contemporaneous $T_{carotid}$, the range of T_{brain} and $T_{carotid}$ was right shifted compared with summer and was higher than it was in the horse by ~0.5°C, and changes in T_{brain} and $T_{carotid}$ were highly correlated. The product-moment correlation (r) between T_{brain} and changes in $T_{carotid}$ in summer was 0.83, in winter it was 0.99, and in horses it was 1.0, over the range of temperatures recorded.

It can also be seen that in summer many of the calculated values for CBF lie above or below the theoretical maxima and minima for CBF (Fig. 3A) (Lust et al., 2007). In winter virtually all of the CBF values lie within these limits (Fig. 3B), while in the horse the range of calculated CBF is very narrow (Fig. 3C). Thus, in winter in pronghorns (and in horses) the changes in T_{brain} could be accounted for by increases or reductions in CBF alone. In contrast, in pronghorn in summer the differences between T_{brain} and $T_{carotid}$ cannot be accounted for by changes in CBF alone, and other mechanisms such as cooling of blood in the rete combined with changes in CBF must be occurring (Lust et al., 2007).
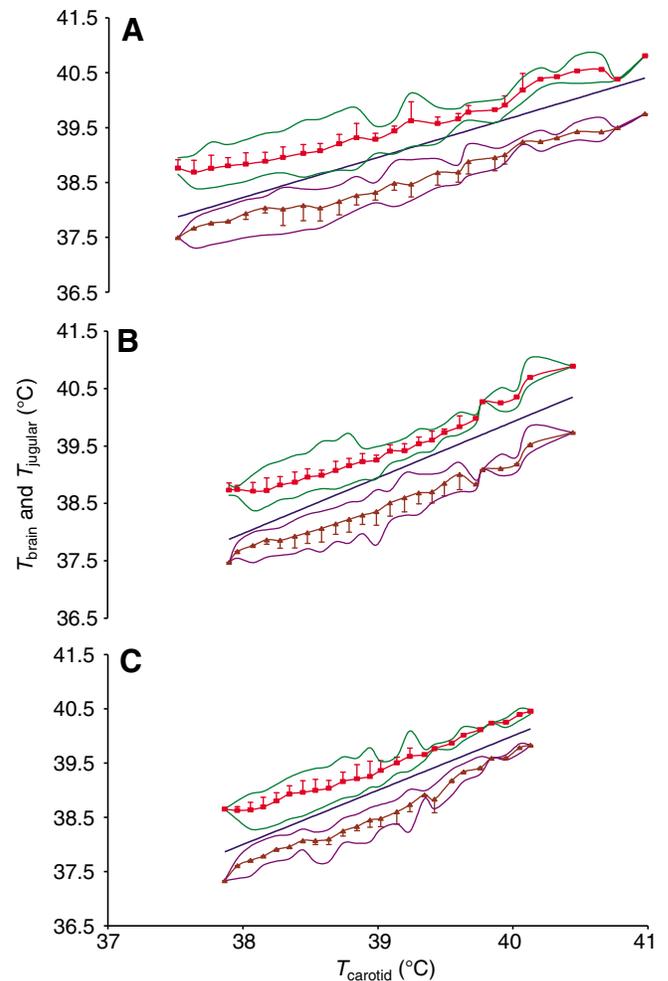
DISCUSSION

Climatic conditions in Wyoming for studying physiological thermoregulatory mechanisms in winter are ideal. Air temperatures are on average below freezing from November to March and arise partly from the average elevation in Wyoming of 2030 m. Day lengths are short (~9 h *vs* ~15 h in summer). Snowfalls are frequent and snow is persistent. In a climate like this pronghorn grow a very thick fur undercoat that is a highly effective insulator (O'Gara and Yoakum, 2004) and/or migrate. If migration is blocked, many thousands die because their body fat content is low [at 3 g 100 g^{-1} *vs* 27–32 g 100 g^{-1} for cattle (Eaton and Konner, 1985; O'Gara and Yoakum, 2004)], and energy depletion leads to hypothermia. If food is available then, as our study shows, despite exposure to very cold environmental conditions they, like mule deer and elk (Parker and Robbins, 1984; Sargeant et al., 1994), can maintain a relatively constant body temperature of around 38.5°C. This constancy, like that of mule deer and elk, is achieved partly by growth of a very thick fur undercoat and partly by behaviour. We did not quantify the contributions of these factors to the regulation of body
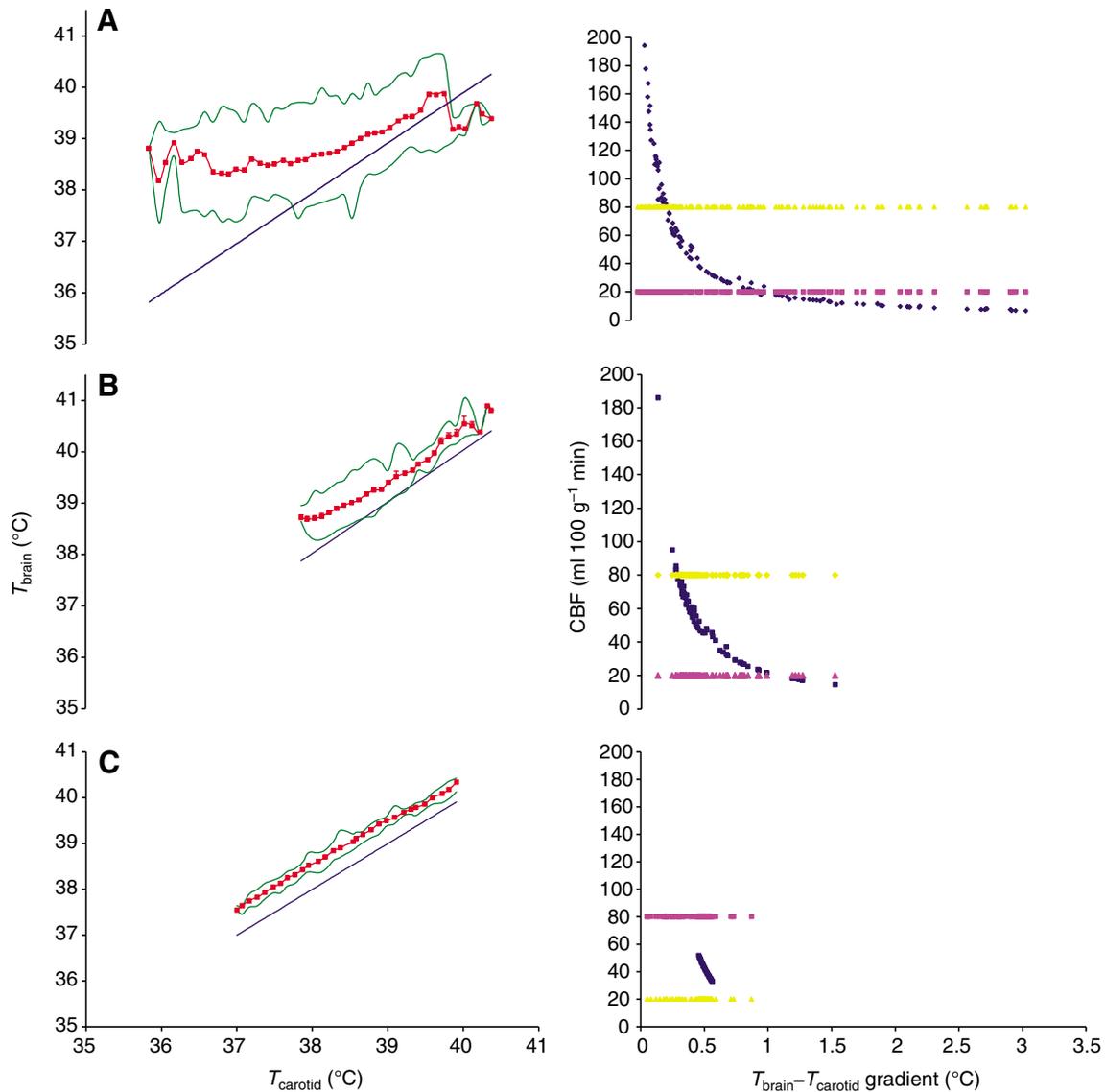
Fig. 3. Comparison of the relationships between $T_{carotid}$, T_{brain} and calculated cerebral blood flow (CBF) in pronghorn in the summer (A) and in winter (B) and in summer in horses (C). In winter the pronghorn temperature profiles are more similar to those in the horse than they are to pronghorn in summer. CBF above the upper maximum CBF (pink) or below the minimum (yellow) occur in pronghorn in the summer, rarely in the winter, and not in horses. In winter, most temperature gradients between T_{brain} and $T_{carotid}$ in pronghorn and all gradients in horses can be achieved by changes in CBF. In summer, low and high gradients depend on SBC or brain warming. Summer pronghorn data are from Lust et al. (Lust et al., 2007). Horse data are from Mitchell et al. (Mitchell et al., 2006).

temperature in pronghorns but it was clear from inspection that they have two hair layers. One layer consists of fine, short and closely packed hairs, which grows in winter and is shed in summer. The other persists throughout the year and consists of thick, long and separated hairs. We do not know whether reduced movement formed part of their winter behaviour pattern as it does in red deer (Arnold et al., 2004) but it was clear that the pronghorns made use of the landscape to minimize the effects of ambient conditions as they do in summer. They thus were able to maintain their body temperature without being able to migrate.

They also seem to shift their daily temperature cycles seasonally to make use of solar radiation in winter and to avoid it in the summer. Maximum $T_{carotid}$ occurred significantly earlier in winter than in summer and the time of maximum $T_{carotid}$ was weakly but significantly correlated with the time of maximum solar radiation.

We also noted that the time of minimum $T_{carotid}$ was linked to sunset but occurred at ~07:30 h. An explanation for this finding is that the longer the length of night (the earlier the sunset) the larger the fall in $T_{carotid}$. Metabolic rate is depressed at night by up to 60% in red deer (Arnold et al., 2004) and in pronghorn early morning $T_{carotid}$ could be up to 2.7°C colder than maximum $T_{carotid}$ suggesting that a similar effect occurred.

We found no SBC in winter even though T_{brain} and $T_{carotid}$ exceeded temperatures at which SBC occurred in summer. There was also a higher and right-shifted range of T_{brain} and $T_{carotid}$ with a frequency distribution (Fig. 1) showing that in winter lower body temperatures occurred less frequently than they did in the summer, while in summer it was high body temperatures that occurred less frequently. T_{brain} and $T_{carotid}$ were also more closely correlated than in summer, and $T_{jugular}$ was consistently lower than $T_{carotid}$. The tight

coupling of T_{brain} and $T_{carotid}$ produced a smaller variation in the range of body temperature in the pronghorns in winter than we found in summer. This result supports the observations made by Parker and Robbins (Parker and Robbins, 1984) in mule deer and elk, and those of Sargeant et al. (Sargeant et al., 1994) in mule deer.

The differences in temperature profiles between summer and winter can be explained by a much reduced functioning of the rete cooling mechanism. In short, in winter, pronghorns appear to thermoregulate in a way very similar to that of a non-rete animal (Fig. 3). This conclusion raises the question of why such a response would be advantageous. The most parsimonious explanation is that the response conserves energy, although this conclusion is counterintuitive. More precise regulation of body temperature should increase energy consumption (Lindstedt and Boyce, 1985). However, energy would be conserved if the result of a higher T_{brain} was depression of appetite and metabolism. These two responses are well established mechanisms for reducing energy use in herbivores in winter (Moen, 1978; Arnold et al., 2004). Both appetite and metabolism are depressed by high body temperature in pigs through suppression of the release of leptin and thyroid hormones (Collin et al., 2002), but a similar link has not yet been evaluated in herbivore artiodactyls. These effects, if they exist, combined with the growth of fur and its consequential lowering of the thermoneutral zone (Nilssen et al., 1984) could significantly reduce energy use.

Rete function is controlled by the regulation of blood flow from the nasal mucosa to the cavernous sinus that surrounds the rete. Johnsen et al. (Johnsen et al., 1985) identified three channels for blood returning from the nasal mucosa. One channel could divert cold nasal blood to the cavernous sinus *via* vasodilatation of the angularis oculi vein (AOV) and constriction of the facial vein to effect SBC, with the opposite occurring to inhibit carotid rete cooling (Johnsen and Folkow, 1988). A second channel directed cold blood to the jugular vein via the dorsal nasal vein (DNV) to effect whole-body cooling, and a third channel was a countercurrent heat exchanger. Johnsen and colleagues (Johnsen et al., 1985) theorized and later showed (Johnsen et al., 1987) that when heat conservation was needed, the DNV and vessels of the nasal mucosa were constricted and warm blood entering the nasal mucosa passed cold venous blood returning from it, warming the venous blood and cooling the arterial blood to the periphery so that heat loss was minimized. The venous effluent entered the sphenopalatine and jugular veins at temperatures similar to arterial temperature. These studies showed that constriction of nasal mucosa vessels was caused by decreases in T_{air} and nasal mucosa temperature (T_{nm}) until at T_{air} and T_{nm}, and below 0°C there was no blood flow in the DNV (Johnsen et al., 1985; Johnsen et al., 1987). When heat dissipation and/or whole-body cooling were required from the brain or body, nasal cooling increased and blood flow in the nasal mucosa vessels was unidirectional with cold effluent blood entering either the AOV en route to the cavernous sinus/rete to effect SBC, or the jugular vein to effect whole-body cooling. In sheep, similar pathways exist (Nijland et al., 1989; Maloney and Mitchell, 1997). In pronghorns the AOV seems to be a minor pathway, if a pathway at all, and they have two main pathways for the return of venous blood from the cooling surfaces of the nose – the DNV and the palatine system (Carlton and McKean, 1977).

Despite these anatomical differences, our data can be explained by the Johnsen et al. (Johnsen et al., 1985) model. For example, if cold environmental temperatures cause T_{nm} to fall and cause increasing constriction of the DNV then it could be predicted that $T_{jugular}$ would not decrease because it too would be deprived of cold blood. Our data show that in pronghorn in winter, $T_{jugular}$ decreased

less, and varied far less, than it did in summer. Constriction of the DNV in pronghorns also would direct blood to the palatine veins and countercurrent warming of blood would occur. If this warm blood entered the CS surrounding the rete, rete cooling would be reduced and the absence of SBC that we found in winter, and the higher average T_{brain}, would be explained. In these circumstances, however, removal of heat from the brain and control of brain temperature must depend on changes in CBF. As Fig. 3B shows, virtually all the temperature gradients we found in pronghorn in winter could be accounted for by changes in CBF alone.

In pronghorn, regulation of the nasal countercurrent system and the rete countercurrent system must be independent because typically in pronghorn $T_{jugular}$ was colder than $T_{carotid}$ while at the same time rete functioning was suppressed. Thus flow of cold blood from the nasal mucosa to the jugular vein was occurring at the same time that flow of cold blood to the veins supplying the cavernous sinus was being reduced. The first and traditional regulator of the direction of nasal venous blood flow is a rising T_{brain}, which increases nasal cooling and, when it reaches ~39.5°C, triggers SBC (Kuhnen and Jessen, 1991). We could only detect this response in the summer. The second regulation is initiated by a low environmental temperature and predominates over the mechanism that regulates SBC. The location of the thermoreceptor that detects low ambient temperature is likely to be the nose and nasal mucosa, which are the only parts of the body that are unprotected from environmental temperature. A thermoreceptor sensitive to cold is present in the nasal cavity of sheep (Bligh, 1963; Maloney and Mitchell, 1997) and in reindeer (Johnsen et al., 1985). In sheep a cold T_{nm} and constriction of the AOV occur only when $T_{carotid}$ is at the low end of its temperature range (Maloney and Mitchell, 1997). At high $T_{carotid}$, a low T_{nm} increases AOV flow and SBC in sheep, whereas in pronghorn and reindeer this latter response appears to be absent. In the sheep study, however, T_{nm} was never less than 15°C, while in reindeer and presumably in pronghorn, T_{nm} approached the low temperatures that inhibit rete cooling at all $T_{carotid}$. Thus our data are consistent with the idea that provided T_{nm} is sufficiently low, nasal thermoreceptors may independently regulate the supply of blood to the cavernous sinus, reducing rete cooling while allowing some whole-body cooling via the jugular vein.

If this interpretation of our data is correct, then our results, apart from providing a mechanism for the conservation of energy in winter, extend our understanding of the functioning of the rete. The rete's traditional role and biological purpose is SBC, and the protection of an allegedly more vulnerable brain from thermal damage. This role has been dispelled by a number of studies that have shown consistently that during exercise, because of increased sympathetic nerve activity, and/or a change from nasal to open mouth breathing that bypasses the nasal mucosa, rete function and SBC are reduced and brain temperature increases. Whole-body cooling results (Jessen, 1998). Jessen proposed therefore that the role of the rete was to modify thermoregulatory responses and conserve water (Jessen, 1998; Jessen, 2001). Our previous study on pronghorns in the summer supports this conclusion. Dissociation of T_{brain} and $T_{carotid}$ in pronghorns resulted in a low variation in T_{brain} and a wide variation in $T_{carotid}$. Our winter data extend this role of the rete. In winter, suppression of rete activity results in a narrow and higher range of T_{brain} and $T_{carotid}$, and we speculate that corollaries are depression of appetite and metabolism and conservation of energy, especially if their pelage provides excellent insulation.

The rete, therefore, seems to be able to cool the brain when T_{body} is high but also to regulate T_{brain} throughout the year to achieve

optimum thermoregulatory responses according to environmental or seasonal needs, even to the extent of differentiating between 'warm' and 'cold' days in winter. It functions over a wide range of body temperatures, and its effects can be adjusted to accommodate high and low environmental temperatures.

REFERENCES

Aas-Hansen, O., Folkow, L. P. and Blix, A. S. (2000). Panting in reindeer (*Rangifer tarandus*). *Am. J. Physiol.* **279**, R1190-R1195.

Arce, A., Rodriguez, H. and Soto, A. (2006). Purification of ethyl *tert*-butyl ether from its mixtures with ethanol by using an ionic liquid. *Chem. Eng. J.* **15**, 219-223.

Arnold, W., Ruf, T., Reimoser, S., Tataruch, F., Onderscheka, K. and Schober, F. (2004). Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). *Am. J. Physiol.* **286**, R174-R181.

Baker, M. A. and Hayward, J. N. (1968). The influence of the nasal mucosa and the carotid rete upon hypothalamic temperature in sheep. *J. Physiol. Lond.* **198**, 561-579.

Bligh, J. (1963). The receptors concerned in the respiratory response to humidity in sheep at high ambient temperature. *J. Physiol. Lond.* **168**, 138-143.

Bock, M., Hohlfeld, U., von Engeln, K., Meier, P. A., Motsch, J. and Tasman, A. J. (2005). The accuracy of a new infrared ear thermometer in patients undergoing cardiac surgery. *Can. J. Anesth.* **52**, 1083-1087.

Carlton, C. and McKean, T. (1977). The carotid and orbital retia of the pronghorn, deer and elk. *Anat. Rec.* **189**, 91-108.

Collin, A., Vaz, M.-J. and le Dividich, J. (2002). Effects of high temperature on body temperature and hormonal adjustments in piglets. *Reprod. Nutr. Dev.* **42**, 45-53.

Eaton, S. B. and Konner, M. (1985). Paleolithic nutrition. *N. Eng. J. Med.* **312**, 283-289.

Jessen, C. (1998). Brain cooling: an economy mode of temperature regulation in artiodactyls. *News Physiol. Sci.* **13**, 218-286.

Jessen, C. (2001). *Temperature Regulation in Humans and Other Mammals*. Berlin: Springer.

Jessen, C. and Feistkorn, G. (1984). Some characteristics of core temperature signals in the conscious goat. *Am. J. Physiol.* **247**, R456-R464.

Johnsen, H. K. and Folkow, L. P. (1988). Vascular control of brain cooling in reindeer. *Am. J. Physiol.* **254**, R730-R739.

Johnsen, H. K., Blix, A. S., Jorgensen, L. and Mercer, J. B. (1985). Vascular basis for regulation of nasal heat exchange in reindeer. *Am. J. Physiol.* **249**, R617-R623.

Johnsen, H. K., Blix, A. S., Mercer, J. B. and Bolz, K.-D. (1987). Selective cooling of the brain in reindeer. *Am. J. Physiol.* **253**, R848-R853.

Kuhnen, G. and Jessen, C. (1991). Threshold and slope of selective brain cooling. *Pflügers Arch.* **418**, 176-183.

Lindstedt, S. L. and Boyce, M. S. (1985). Seasonality, fasting endurance, and body size in mammals. *Am. Nat.* **125**, 873-878.

Lust, A., Fuller, A., Maloney, S. K., Mitchell, D. and Mitchell, G. (2007). Thermoregulation in pronghorn antelope (*Antilocapra americana*, Ord) in the summer. *J. Exp. Biol.* **210**, 2444-2452.

Maloney, S. K. and Mitchell, G. (1997). Selective brain cooling: the role of angularis oculi and nasal thermoreception. *Am. J. Physiol.* **273**, R1108-R1116.

Mitchell, D., Maloney, S. K., Jessen, C., Laburn, H. P., Kamerman, P. R., Mitchell, G. and Fuller, A. (2002). Adaptive heterothermy and selective brain cooling in arid-zone mammals. *Comp. Biochem. Physiol.* **131B**, 571-585.

Mitchell, G., Fuller, A., Maloney, S. K., Rump, N. and Mitchell, D. (2006). Guttural pouches, brain temperature and exercise in horses. *Biol. Lett.* **2**, 475-477.

Moen, A. N. (1978). Seasonal changes in heart rates, activity, metabolism and forage intake of white tailed deer. *J. Wildl. Manage.* **42**, 715-738.

Nijland, M. J. M., Mitchell, D. and Mitchell, G. (1989). Effect of reversible occlusion of the angularis oculi and facial veins on selective brain cooling in sheep. In *Thermal Physiology* (ed. J. B. Mercer), pp. 211-216. Amsterdam: Excerpta Medica.

Nilssen, K. J., Sundsfjord, J. A. and Blix, A. S. (1984). Regulation of metabolic rate in Svalbard and Norwegian reindeer. *Am. J. Physiol.* **247**, R837-R841.

O'Gara, B. W. and Yoakum, J. D. (2004). *Pronghorn Ecology and Management*. Colorado: Wildlife Management Institute, University Press of Colorado.

Parker, K. L. and Robbins, C. T. (1984). Thermoregulation in mule deer and elk. *Can. J. Zool.* **62**, 1409-1422.

Sargeant, G. A., Eberhardt, L. E. and Peek, J. M. (1994). Thermoregulation by mule deer (*Odocoileus hemionus*) in arid rangelands of southcentral Washington. *J. Mammal.* **75**, 536-544.

Taylor, C. R. and Lyman, C. P. (1972). Heat storage in running antelopes: independence of brain and body temperatures. *Am. J. Physiol.* **222**, 114-117.