

## Thermoregulation in pronghorn antelope (*Antilocapra americana* Ord) in the summer

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### Summary

We have used thermistor/data logger assemblies to measure temperatures in the brain, carotid artery, jugular vein and abdominal cavity, and subcutaneously, in five pronghorn antelope over a summer in Wyoming. Globe and air temperature varied by up to ~50°C daily during the summer and maximum solar radiation was ~900 W m<sup>-2</sup>. Brain temperature (38.9±0.3°C) was consistently ~0.2–0.5°C higher than carotid blood temperature (38.6±0.3°C), which was the same as abdominal temperature (38.8±0.4°C). Jugular blood temperature (38.0±0.4°C) varied, probably because of changes in Respiratory Evaporative Heat Loss (REHL), and was lower than other temperatures. Subcutaneous temperature (38.3±0.6°C) varied, probably because of peripheral vasoactivity, but on average was similar to other temperatures. Carotid blood temperature had a circadian/nycthemeral rhythm weakly but significantly ( $r=0.634$ ) linked to the time of sunrise, of amplitude

0.8±0.1°C. There were daily variations of up to 2.3°C in carotid body temperature in individual animals. An average range of carotid blood temperature of 3.1±0.4°C over the study period was recorded for the group, which was significantly wider than the average variation in brain temperature (2.3±0.6°C). Minimum carotid temperature (36.4±0.8°C) was significantly lower than minimum brain temperature (37.7±0.5°C), but maximum brain and carotid temperatures were similar. Brain temperature was kept relatively constant by a combination of warming at low carotid temperatures and cooling at high carotid temperatures and so varied less than carotid temperature. This regulation of brain temperature may be the origin of the amplitude of the average variation in carotid temperature found, and may confer a survival advantage.

Key words: pronghorn, brain warming, thermoregulation.

### Introduction

Pronghorn antelope are the only surviving member of the Antilocapridae, a unique artiodactyl family that first appeared in the late Miocene between 23.9 and 32.5 Mya (Hassanin and Douzery, 2003). Neither a deer nor a true antelope, its closest extant relative is the giraffe (Mitchell and Skinner, 2003). It was first brought to the attention of science by Meriwether Lewis and William Clark during their exploration of the newly acquired Louisiana territory (Lewis and Clark, 1904). In the early autumn of 1804 (September 14) Lewis wrote:

“— in my walk I Killed a Buck Goat of this Country, about the hight of the Grown Deer, its body Shorter the Horns which is not very hard forks  $\frac{2}{3}$  up one prong short the other round & Sharp arched, – Verry actively made, has only one pair of hoofs to each foot, his brains on the back of his head, his Nostrals large, his eyes like a Sheep he is more like the Antelope or Gazella of Africa than any other Species of Goat.”

In the intervening 200 years very few studies of their anatomy and physiology have been done. Perhaps the most famous study is that of Lindstedt et al. (Lindstedt et al., 1991),

who investigated the capacity of pronghorns for aerobic exercise. They found it to be remarkable, with a  $V_{O_2}$  of 300 ml kg<sup>-1</sup> min<sup>-1</sup>, the highest recorded so far for any vertebrate except perhaps for hovering hummingbirds (Suarez et al., 1991) and bats (Lindstedt et al., 1991). To support this oxygen consumption Lindstedt et al. showed that, compared to goats, pronghorn have a greater capacity for oxygen diffusion across the lung, a greater capacity to deliver oxygen to muscles, and a greater total volume of mitochondria in skeletal muscle (Lindstedt et al., 1991). These results confirmed aspects of an earlier study by McKean and Walker (McKean and Walker, 1974) who, also comparing pronghorns to goats (*Capra hircus*), found that pronghorns have a greater heart weight:body weight ratio and a lower airway resistance. Dhindsa et al. (Dhindsa et al., 1974) found that pronghorn blood was unremarkable compared to several species of deer, while McKean and Walker (McKean and Walker, 1974) found 50% more haemoglobin, a higher haematocrit, and larger blood volume compared to goats. From the data provided by Dhindsa et al. ( $N=4$ ) (Dhindsa et al., 1974) and from the eight individual

animals on which McKean and Walker based their report (McKean and Walker, 1974), however, it is also clear that pronghorns have very different blood indices compared to those calculated from average data for nine different species of southern African antelope (Rhodes, 1975). Pronghorns have significantly higher haematocrit ( $44.5 \pm 3.4$  vs  $39.8 \pm 3.7\%$ ;  $t=2.76$ ), haemoglobin concentration ( $16.8 \pm 2.0$  vs  $13.4 \pm 1.4$  g dl<sup>-1</sup>;  $t=4.36$ ), and red blood cell number ( $11.8 \pm 1.1$  vs  $6.9 \pm 1.6 \times 10^{12}$  l<sup>-1</sup>;  $t=7.42$ ), and significantly smaller red cells (mean corpuscular volume, MCV= $37.1 \pm 2.7$  vs  $60.9 \pm 14.2$  fl;  $t=4.76$ ) containing a lower absolute amount of haemoglobin (MCH= $14.1 \pm 1.1$  vs  $20.7 \pm 5.1$  pg;  $t=3.70$ ) but with a higher concentration of haemoglobin per cell (MCHC= $38.8 \pm 3.1$  vs  $33.5 \pm 1.3\%$ ;  $t=4.34$ ). All these attributes allow them to run at 100 km h<sup>-1</sup> for 3–4 min (McCabe et al., 2004) and 65 km h<sup>-1</sup> for 10 min (McKean and Walker, 1974). The anatomy of their cranial vasculature has been described (Carlton and McKean, 1977) and it is similar to that in other artiodactyls, specifically in that it has a well developed carotid rete-cavernous sinus system, one of the functions of which is to cool arterial blood destined for the brain (Maloney and Mitchell, 1997).

Two measurements of their body temperatures have been reported. Average temperature “under the deep musculature of the paralumbar fossa” was 40.7°C (range 36.2–42.2°C) in a mature doe and 39.0°C (range 36.8–42.2°C) in a yearling doe (Thorne, 1975). Both animals were semi-tame. Mean rectal temperature was 40.8°C (range 38.5–43.3°C) in 41 wild, hand-captured animals (Barrett and Chalmers, 1977).

We report here a further study of pronghorn physiology, with an emphasis on thermoregulation, using techniques we have developed for use in southern hemisphere animals (Fuller et al., 2005). The climatic conditions in the northern hemisphere are different to those of the southern hemisphere and thermoregulatory challenges experienced by pronghorn could, therefore, be expected to be different. The aims of the study were to record body temperatures in free-living pronghorn, to describe the thermoregulatory mechanisms used by pronghorn during a 3-month period in the summer in Wyoming, and to compare these with temperatures and mechanisms we have found in a free-living, similar-sized, South African antelope (springbok *Antidorcas marsupialis*) during a southern hemisphere summer (Fuller et al., 2005). We show that thermoregulatory patterns in pronghorn and springbok are similar, but pronghorns also seem to have evolved a mechanism for warming their brains, not seen in southern hemisphere artiodactyls.

### Materials and methods

This study was done at the Tom Thorne/Beth Williams Wildlife Research Center at Sybille, southeastern Wyoming (Lat. 41.7789°N, Long. -105.3394°W). The study lasted from 12 May 2005 to 11 August 2005.

#### Animals

Five adult pronghorn *Antilocapra americana* Ord (one male and four females, body mass 40–50 kg) were captured by darting (thiafentanil, 5 mg; Wildlife Pharmaceuticals, Inc., Fort Collins, CO, USA) at Warren Air Force Base, and transported to the research center. 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 hectare enclosure with four other pronghorns where they were free-living for the 3-month recording period. During the study one of the females gave birth to twins.

#### Measurement of body temperatures

Temperatures were measured and recorded from five body sites (brain, carotid artery, jugular vein, abdominal cavity and subcutaneous) using small bead thermistors (GE Thermometrics ABOE3-BR11KA 103K-L10). Data were stored on data loggers (Onset, Pocasset, USA; XITC 32+34+36) connected to the thermistors by a flexible coax cable (#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/Elyax, 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 quartz thermometer (Quat 100, Heraeus, Hanau, Germany).

#### Surgical procedures

At the time of surgery the animals were re-darted using thiafentanil and anesthetized 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 (mean=3.9±2.1%) in oxygen. The effects of thiafentanil were reversed with nalterzel (5 ml; Wildlife Pharmaceuticals, Inc.). Using aseptic surgical techniques, thermistors were implanted into the five body sites and the loggers were buried subcutaneously nearby.

#### Brain

To measure brain temperature a thermistor was encased in a rigid guide tube (cellulose acetate butyrate tubing; World Precision Instruments, Savarola, FL, USA; o.d. 3.2 mm, i.d. 1.98 mm, length 34 mm) 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. The guide tube was attached to a head plate (22 mm×15 mm×9 mm, L×W×H), which was fixed to the skull by two 6-gauge, 15 mm long, self-tapping, stainless steel screws. No neurological sequelae arose from this procedure.

#### Blood vessels

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 the direction of the flow of blood 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.

*Subcutaneous and abdominal measurements*

Thermistors used to measure subcutaneous and abdominal temperatures were encased in a wax cylinder 40 mm long and 5 mm in diameter. The abdominal thermistor was inserted into the abdominal cavity at the paralumbar fossa using a trocar and cannula, and the logger buried subcutaneously above the site of insertion. This method resulted in consistent placement of the thermistor tip in the abdomen within small intestine folds and posterior to the rumen (confirmed at autopsy). The logger assembly was placed subcutaneously in the paralumbar fossa on the opposite side to the abdominal assembly.

All animals were given 5 ml dexamethasone (Vedco, St Joseph, MO, USA) and 2 ml penicillin (GC Hanford Mfg. Co., Syracuse, NY, USA) intramuscularly at the start of surgery. Baytril tablets (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). Six variables were measured: black globe temperature, ambient air temperature, solar radiation, relative humidity, wind speed and wind direction. Black globe temperature is an integral of air temperature, solar radiation and wind speed, and is the best measure of total heat load.

*Data analysis*

Temperature data were obtained from all loggers in three animals and from three loggers in a fourth animal. Insufficient data was collected from the fifth animal. We obtained measurements for 6 days from one animal, 12 from another, 92 from a third and 97 from the fourth. Most data analysis was based on the measurements obtained from the latter two animals. The data were consolidated 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 four animals were, in turn, averaged to produce a 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 temperatures throughout the recording period. For example, a second consolidation was to average daily means into a week, to produce 14 separate weekly temperature profiles for each body site over the study period. These 14 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 (CBF)*

The amount of heat lost from the brain (or any other tissue) by convection to blood flow can be calculated from the convective heat loss equation:

$$W = BF \times \text{specific heat} \times \Delta T,$$

where  $W$  ( $\text{J s}^{-1} 100 \text{ g}^{-1}$ ) is watts of heat produced by 100 g brain tissue and removed by the blood,  $BF$  ( $\text{g s}^{-1} 100 \text{ g}^{-1}$ ) in the case

of the brain is cerebral blood flow (CBF) per 100 g brain tissue, specific heat ( $\text{J g}^{-1} \text{ }^\circ\text{C}^{-1}$ ) is constant and assumed to be  $3.6 \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$  for blood (Jessen, 2001), and  $\Delta T$  is the temperature gradient ( $^\circ\text{C}$ ) between  $T_{\text{brain}}$  and  $T_{\text{carotid}}$ .  $W$  varies with  $T_{\text{brain}}$ , which we assumed to have a  $Q_{10}$  of 2.3 (Yablonskiy et al., 2000). In order to account for the effects of changes in  $T_{\text{brain}}$  on oxygen consumption and CBF this value for  $Q_{10}$  was used to calculate a correcting function to take into account the  $Q_{10}$  effect on brain heat production, and was  $W = 0.0448 \cdot e^{(T_{\text{brain}}/11.7)}$ .  $W$  was then calculated for each brain temperature. Using this estimate of  $W$  at each brain temperature and rearranging the equation, CBF was calculated from:

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

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

**Results***Weather conditions*

Over the study period daily mean black globe temperature ( $T_{\text{globe}}$ ) was  $22.5 \pm 5.3^\circ\text{C}$  ( $\pm$  s.d.) with a lowest measured temperature of  $-5.0^\circ\text{C}$  and a highest  $52.0^\circ\text{C}$ . Minimum  $T_{\text{globe}}$  occurred at  $\sim 06:00$  h and maximum  $T_{\text{globe}}$  at  $\sim 13:00$  h each day. Mean air temperature ( $T_{\text{air}}$ ) was  $18.3 \pm 5.0^\circ\text{C}$  with a range of  $2.0^\circ\text{C}$  to  $37.8^\circ\text{C}$ . Mean daily solar radiation was  $256 \pm 34 \text{ W m}^{-2}$  and maximum solar radiation was  $870 \pm 71 \text{ W m}^{-2}$ . Mean photoperiod was 14 h,  $45 \pm 24$  min, with sunrise occurring at 05 h,  $41 \pm 0.13$  min and sunset at 20 h,  $27 \pm 0.11$  min. Wind speed was  $1.1 \pm 0.3 \text{ m s}^{-1}$ . No rainfall was recorded.

*Body temperatures**Mean temperatures*

The mean temperatures for the different body locations are shown in Table 1. These data were derived by pooling hourly mean temperatures over the study period for each temperature site in the four animals. On average brain temperature ( $T_{\text{brain}} = 38.9 \pm 0.3^\circ\text{C}$ ) was significantly higher than carotid artery blood temperature ( $T_{\text{carotid}} = 38.6 \pm 0.3^\circ\text{C}$ ;  $t = 5.23$ ) and jugular vein temperature ( $T_{\text{jugular}} = 38.0 \pm 0.4$ ;  $t = 3.21$ ).  $T_{\text{carotid}}$  was also significantly higher than  $T_{\text{jugular}}$  ( $t = 2.14$ ) but not different from abdominal temperature ( $T_{\text{abdominal}}$ ;  $t = 0.71$ ) or subcutaneous temperature ( $T_{\text{subcut}}$ ;  $t = 0.81$ ).  $T_{\text{jugular}}$  was lower than  $T_{\text{abdominal}}$  ( $t = 8.00$ ). Subcutaneous temperatures showed the largest

Table 1. Weighted mean hourly temperatures at each of the body sites in four animals

	Temperature ( $^\circ\text{C}$ )				
	$T_{\text{carotid}}$	$T_{\text{brain}}$	$T_{\text{jugular}}$	$T_{\text{abdominal}}$	$T_{\text{subcut}}$
Mean	$38.6 \pm 0.3$	$38.9 \pm 0.3$	$38.0 \pm 0.4$	$38.8 \pm 0.4$	$38.3 \pm 0.6$
Range	35.8–40.3	37.4–40.7	36.1–39.9	35.0–40.4	34.1–40.3
<i>N</i>	4902	4037	2935	4902	4779

Temperatures are means  $\pm$  s.d.; *N*, number of measurements.

Table 2. Individual variation in carotid and brain temperatures

Gender	$T_{\text{carotid}}$ (°C)			$T_{\text{brain}}$ (°C)		
	Minimum	Maximum	Max–min	Minimum	Maximum	Max–min
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
Group mean $\pm$ s.d.	36.4 $\pm$ 0.8	39.5 $\pm$ 0.7	3.1 $\pm$ 0.4	37.7 $\pm$ 0.5	40.1 $\pm$ 0.5	2.3 $\pm$ 0.6

$N=4$ ; each animal had a single highest or lowest  $T_{\text{carotid}}$  or  $T_{\text{brain}}$  during the study period.

difference between maximum and minimum temperature (6.2°C) and as a result on average were similar to  $T_{\text{abdominal}}$  ( $t=0.75$ ),  $T_{\text{brain}}$  ( $t=1.62$ ) and  $T_{\text{abdominal}}$  ( $t=1.25$ ).  $T_{\text{abdominal}}$  did not differ from  $T_{\text{brain}}$  ( $t=0.36$ ).

#### Individual variation in temperatures

Table 2 summarizes the variation in carotid and brain temperatures in each of the four animals over the study period. The male pronghorn had a minimum carotid temperature 1.6°C higher than the female average and a maximum  $T_{\text{carotid}}$  1.0°C higher than the female average. The male's minimum brain temperature was 0.9°C higher than the female average but its maximum brain temperature was the same as the female average. These differences suggest that the male regulated its body temperatures in a higher range than did the female pronghorn. The minimum  $T_{\text{carotid}}$  for all four animals was significantly less than the minimum  $T_{\text{brain}}$  ( $P<0.05$ ;  $t$ -test) and the range of  $T_{\text{carotid}}$  was significantly wider than the range of  $T_{\text{brain}}$  ( $P<0.05$ ;  $t$ -test). Maximum  $T_{\text{carotid}}$  and  $T_{\text{brain}}$  were not significantly different.

These conclusions are supported by a slightly different analysis of  $T_{\text{abdominal}}$  shown in Table 3. To obtain these data, hourly means for each hour of each day were averaged. Thereafter the minimum and maximum temperature for each day for each animal was calculated. These data support the idea that the male pronghorn regulated his body temperatures within a narrower and higher range than did the females, although with data on only one male we cannot draw firm conclusions on gender differences.

#### Frequency distribution of $T_{\text{carotid}}$ , $T_{\text{brain}}$ and $T_{\text{jugular}}$

Arithmetical means of temperatures are not necessarily the same as preferred or mode temperatures, which are the physiological, 'set-point' temperatures. The three most important temperatures from a thermoregulatory point of view are  $T_{\text{brain}}$ ,  $T_{\text{carotid}}$  and  $T_{\text{jugular}}$ , indicative of hypothalamic

Table 3. Daily variation of  $T_{\text{abdominal}}$  over the summer

Gender	$T_{\text{max}}$ (°C)	$T_{\text{min}}$ (°C)	$T_{\text{max}}-T_{\text{min}}$ (°C)
F593	38.4 $\pm$ 0.4	36.4 $\pm$ 0.8	2.0 $\pm$ 0.9
F594	39.3 $\pm$ 0.3	38.1 $\pm$ 0.3	1.2 $\pm$ 0.4
F598	39.2 $\pm$ 0.4	37.0 $\pm$ 0.7	2.3 $\pm$ 0.7
M595	39.6 $\pm$ 0.3	38.9 $\pm$ 0.2	0.7 $\pm$ 0.2
Group mean	39.1 $\pm$ 0.5	37.6 $\pm$ 1.1	1.6 $\pm$ 0.7

Temperatures are means  $\pm$  s.d.;  $N=207$  days.

temperature, core body temperature and the capacity for respiratory convective and evaporative heat loss (REHL), respectively. To determine the 'set point' temperatures for  $T_{\text{brain}}$  and  $T_{\text{carotid}}$ , frequency distributions of these two temperatures were constructed for each animal by pooling measurements made for each of them into 0.1°C intervals, combining these intervals for all four animals and plotting the frequency (%) occurrence of each 0.1°C interval. These analyses, derived from over 52 000 paired data points (mainly from two animals) for

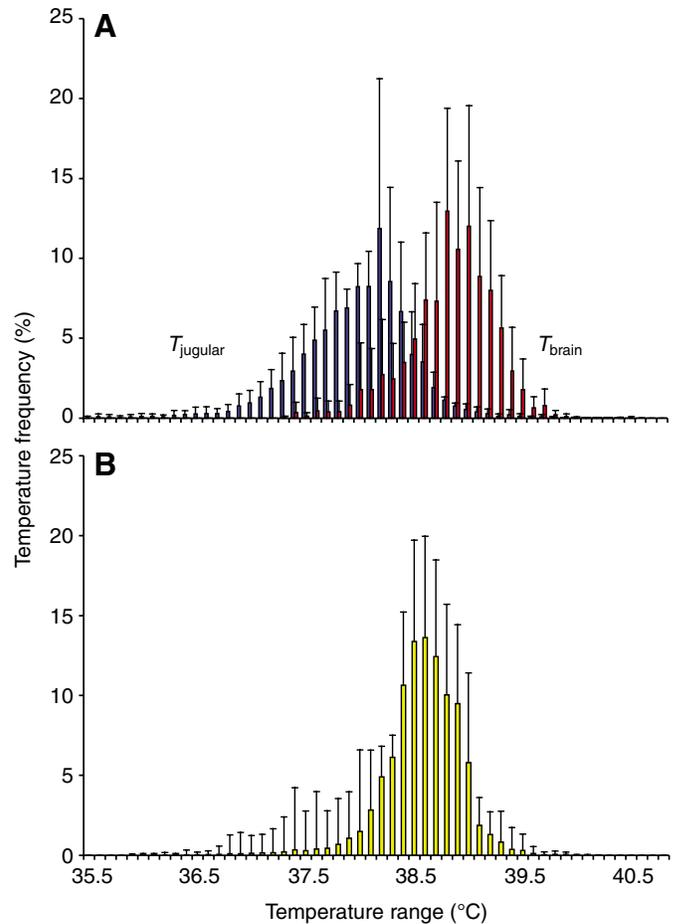


Fig. 1. Frequency of occurrence of 0.1°C intervals of (A) brain and jugular temperatures and (B) carotid temperature. The brain distribution is narrower and its mode temperature occurs to the right of the carotid and jugular distributions. The frequency of occurrence of jugular temperatures is to the left of the carotid distribution and is characterized by a long tail. Values are means  $\pm$  s.d. (see text for  $N$ ).

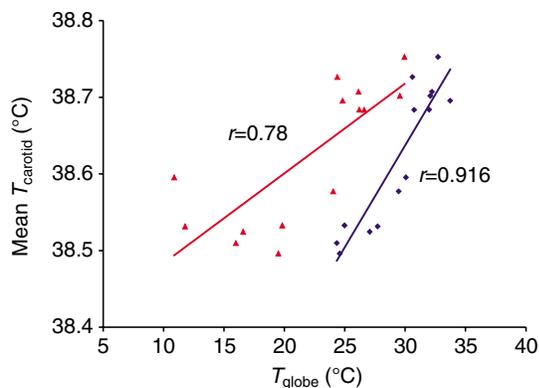


Fig. 2. The weekly mean pronghorn carotid blood temperature plotted against the weekly mean (red) black globe temperature and the weekly range (blue) of black globe temperature. As mean and variability of globe temperature increases and decreases over the summer, mean pronghorn temperature tends to change in the same direction.

$T_{\text{brain}}$ ,  $T_{\text{jugular}}$  and  $T_{\text{carotid}}$  are shown in Fig. 1A,B. For all animals,  $T_{\text{carotid}}$  varied from 35.8°C to 40.3°C.  $T_{\text{brain}}$  frequency distribution was narrower (37.4–40.7°C). Fig. 1 also shows the position of the distribution of jugular temperatures in relation to carotid and brain temperatures.  $T_{\text{jugular}}$  is significantly left-shifted to both (Table 1) and is characterized by a long tail.

#### Factors affecting body temperatures

##### $T_{\text{globe}}$

The weekly range and mean of  $T_{\text{globe}}$  had no significant influence on the weekly variation in  $T_{\text{carotid}}$ . The product–moment correlation between the weekly means of  $T_{\text{globe}}$  and the weekly ranges of  $T_{\text{carotid}}$  was 0.104 ( $F=0.132$ ) and for the weekly ranges of  $T_{\text{globe}}$  and the weekly ranges of  $T_{\text{carotid}}$  it was 0.203 ( $F=0.516$ ). However, mean  $T_{\text{carotid}}$  (as opposed to its range) was correlated with the mean ( $r=0.78$ ,  $F=18.19$ ) and the range of  $T_{\text{globe}}$  ( $r=0.916$ ,  $F=62.56$ ) found in each week over the course of the summer (Fig. 2).

##### Circadian/nycthemeral rhythm

Consecutive maximum and minimum  $T_{\text{carotid}}$  occurred on average  $24.0 \pm 1.5$  h and  $23.8 \pm 1.2$  h apart, respectively. Using the method of Nelson et al. (Nelson et al., 1979), variations (over a period of three consecutive but randomly selected days in each month) in  $T_{\text{globe}}$ , and  $T_{\text{carotid}}$  over the same days, in each of the four animals were analyzed for the occurrence of a circadian rhythm and evidence for daily adaptive heterothermy. This analysis showed that throughout the summer, maximum  $T_{\text{globe}}$  occurred around 13:00 h (13 h  $24 \pm 30$  min), coinciding with maximum solar radiation (12:24  $\pm 0:24$  h), while  $T_{\text{carotid}}$  peaked 7 h later between 18:00 h and 21:00 h (18:47  $\pm 1:05$  h). The amplitude of the circadian change was  $0.8 \pm 0.1$ °C (Fig. 3). To determine if the circadian rhythm was adjusted by photoperiod, the mean time of occurrence of maximum  $T_{\text{carotid}}$  and minimum  $T_{\text{carotid}}$  in each of the 14 weekly periods was correlated with mean time of sunrise or sunset in each epoch. This analysis showed that the time of minimum  $T_{\text{carotid}}$  was positively correlated with the time of sunrise ( $r=0.634$ ) and negatively with the time of sunset ( $r=0.726$ ). The time of

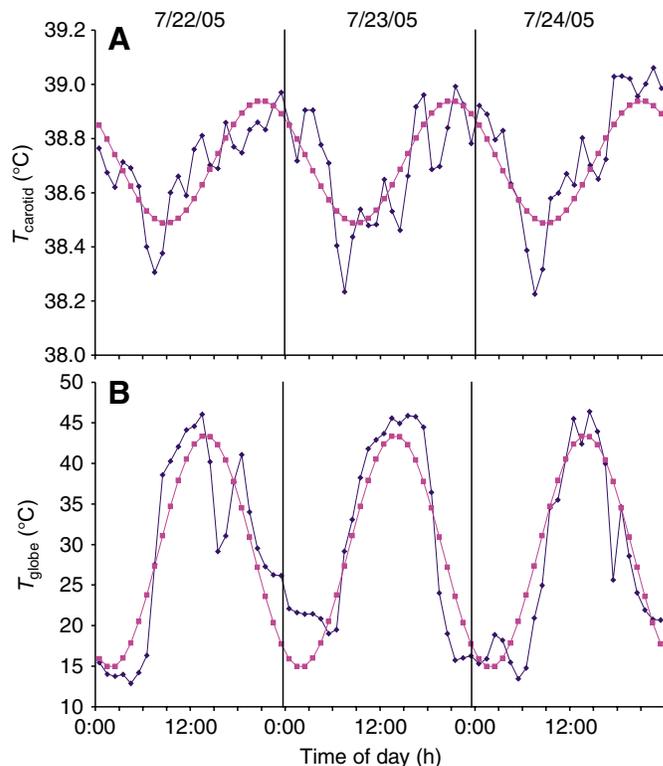


Fig. 3. (A) Circadian rhythms over 3 days for one animal (22–24 July 2005) shown by cosinor analysis (Nelson et al., 1979), which fits a ‘best fit’ cosine wave to the actual data if such a wave form exists. Raw data are represented by the blue line and the best-fit curve is in pink. (B) Changes in  $T_{\text{globe}}$  over the same 3 days. Note that the time of maximum  $T_{\text{globe}}$  occurs about 7 h before the time of maximum  $T_{\text{carotid}}$ . In both A and B, actual data points (diamonds) and their corresponding calculated points (squares) are shown.

maximum  $T_{\text{carotid}}$  was not significantly correlated with either sunrise ( $r=0.019$ ) or sunset ( $r=0.139$ ).

##### Subcutaneous temperature

$T_{\text{subcut}}$  follows  $T_{\text{globe}}$  quite closely throughout a day (Fig. 4), although at night it separates from  $T_{\text{globe}}$ . To assess if peripheral vasoactivity could be a factor contributing to this pattern,  $T_{\text{subcut}}$  was compared to  $T_{\text{carotid}}$ . Assuming that the pelage minimizes radiant heating of the subcutaneous space, then when  $T_{\text{globe}}$  is low vasoconstriction will result in an increase in the difference between  $T_{\text{subcut}}$  and  $T_{\text{carotid}}$ , and a decrease in the difference, resulting from vasodilation, will occur when  $T_{\text{globe}}$  is increasing.

Fig. 4 shows an analysis of the difference between  $T_{\text{subcut}}$  and  $T_{\text{carotid}}$  plotted against time of day. At night (19:00 h to 05:00 h) when  $T_{\text{globe}}$  is falling,  $T_{\text{carotid}}$  on average is 1.0°C warmer than  $T_{\text{subcut}}$ . During the day (05:00 h to 19:00 h) when  $T_{\text{globe}}$  is increasing, the two temperatures become similar, suggesting vasodilation and increased flow of warm blood to the periphery in a typical heat loss mechanism.

##### Respiratory evaporative heat loss

The occurrence of REHL in pronghorn can be estimated by analysis of  $T_{\text{jugular}}$ . If heat is being lost *via* the nasal mucosa then  $T_{\text{jugular}}$  will fall because the temperature of blood returning in

the nasal veins to the jugular vein will fall. Conversely when REHL is reduced  $T_{\text{jugular}}$  will increase.

The relationship between  $T_{\text{carotid}}$  and  $T_{\text{jugular}}$  is shown in Fig. 5A. When  $T_{\text{carotid}}$  is below  $37.5^{\circ}\text{C}$   $T_{\text{jugular}}$  is warmer than  $T_{\text{carotid}}$  by  $0.5\text{--}1.0^{\circ}\text{C}$ , suggesting that REHL is reduced at these  $T_{\text{carotid}}$ . As  $T_{\text{carotid}}$  increases mean and minimum  $T_{\text{jugular}}$  become less than  $T_{\text{carotid}}$  indicating an increase in REHL. At  $T_{\text{carotid}}$  of approximately  $39.5^{\circ}\text{C}$   $T_{\text{jugular}}$  falls sharply, suggesting an increase in REHL to a level greater than it is at lower  $T_{\text{carotid}}$ . The overall trend of this relationship is that as  $T_{\text{carotid}}$  increases so does  $T_{\text{jugular}}$  but at a slower rate with the net effect that the two temperatures diverge.

#### $T_{\text{brain}}/T_{\text{carotid}}$ relationship

The relationship between mean, maximum and minimum  $T_{\text{brain}}$  and  $T_{\text{carotid}}$  (Fig. 5B), shows that  $T_{\text{brain}}$  varies less than  $T_{\text{carotid}}$  over the range of  $T_{\text{carotid}}$  of  $35.8^{\circ}\text{C}$  and  $40.3^{\circ}\text{C}$ . Three phases of  $T_{\text{brain}}$  can be identified: (i) when  $T_{\text{carotid}}$  is less than  $37.8^{\circ}\text{C}$ , (ii) when  $T_{\text{carotid}}$  is between  $37.8$  and  $39.5^{\circ}\text{C}$  and (iii) when  $T_{\text{carotid}}$  is greater than  $39.5^{\circ}\text{C}$ .

Fig. 6 shows these three phases of  $T_{\text{brain}}$  plotted with  $T_{\text{jugular}}$  against  $T_{\text{carotid}}$ . At  $T_{\text{carotid}}$  of  $37.8^{\circ}\text{C}$  or less,  $T_{\text{brain}}$  is remarkably constant and both  $T_{\text{brain}}$  and  $T_{\text{jugular}}$  are warmer than  $T_{\text{carotid}}$ .  $T_{\text{jugular}}$  is approximately  $0.5^{\circ}\text{C}$  warmer and  $T_{\text{brain}}$  can be up to  $2.5$  to  $3.0^{\circ}\text{C}$  warmer.

At  $T_{\text{carotid}}$  of between  $37.8^{\circ}\text{C}$  and  $39.5^{\circ}\text{C}$  (which corresponds

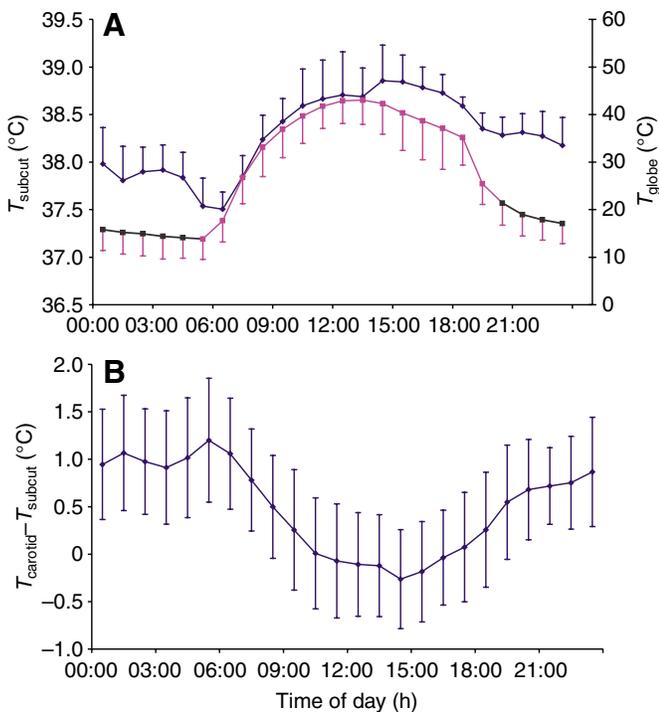


Fig. 4. (A)  $T_{\text{subcut}}$  and  $T_{\text{globe}}$  (means  $\pm$  s.d.) from 2 animals in July over 24 h with means derived from 12 temperatures per hour, showing coincident changes during the day (pink) and the separation of temperatures at night (black). (B) In the mornings and evenings the difference between carotid and subcutaneous temperatures increases suggesting vasoconstriction, but narrows at midday suggesting vasodilation.

to the preferred 'set point' temperature frequency range; Fig. 1A,B)  $T_{\text{brain}}$  approaches  $T_{\text{carotid}}$  with  $T_{\text{brain}}$  being  $0.2$  to  $0.5^{\circ}\text{C}$  warmer than  $T_{\text{carotid}}$ .  $T_{\text{jugular}}$  in this phase falls below  $T_{\text{carotid}}$  but increases in parallel with  $T_{\text{brain}}$ .

The third phase of  $T_{\text{brain}}$  occurs at  $T_{\text{carotid}}$  greater than  $39.5^{\circ}\text{C}$ . This phase is characterized by a sharp decline in both  $T_{\text{brain}}$  and  $T_{\text{jugular}}$  such that  $T_{\text{carotid}}$  becomes  $0.5^{\circ}\text{C}$  warmer than  $T_{\text{brain}}$ , which is characteristic of selective brain cooling (SBC), and implies that brain heat is being removed faster than it is being produced.

SBC constitutes a small part of the regulation of  $T_{\text{brain}}$ . Its onset [as defined by a mean  $T_{\text{brain}}$  less than  $T_{\text{carotid}}$  (IUPS Thermal Commission, 1987)] occurs at a  $T_{\text{carotid}}$  close to  $39.5^{\circ}\text{C}$ , a  $T_{\text{carotid}}$  that occurs infrequently (less than 10% of all recorded  $T_{\text{carotid}}$  in this study). However, brain temperatures lower than  $T_{\text{carotid}}$  can be detected at  $T_{\text{carotid}}$  as low as  $38.0^{\circ}\text{C}$  (Fig. 5B), implying that SBC occurs over a wider range of brain temperatures. The regulation of  $T_{\text{brain}}$  also depends on the amount of convective heat loss by CBF. The relative contributions of CBF and SBC to brain heat removal can be estimated from the  $T_{\text{carotid}}-T_{\text{brain}}$  gradients recorded during the

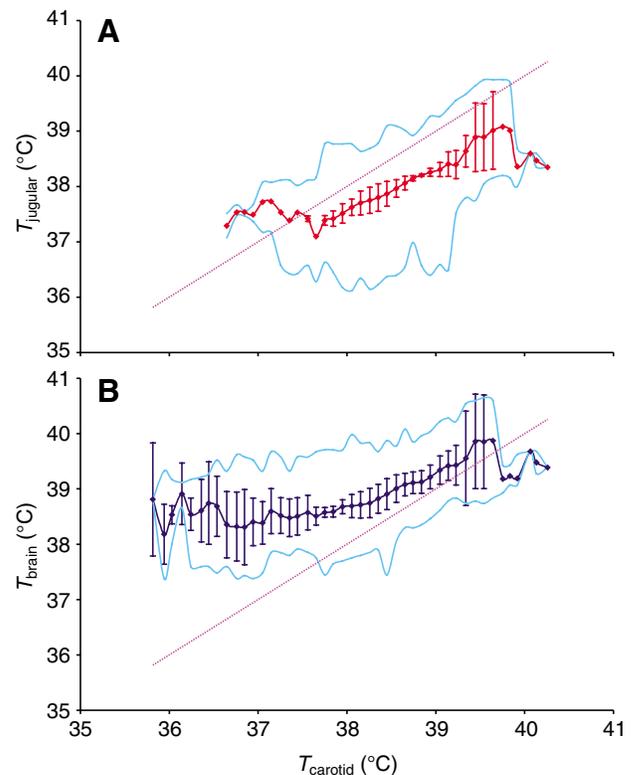


Fig. 5. (A) Minimum and maximum (blue) and mean (red)  $T_{\text{jugular}}$  and (B) minimum and maximum (light blue) and mean (dark blue)  $T_{\text{brain}}$  plotted against  $T_{\text{carotid}}$ . At  $T_{\text{carotid}}$  below  $\sim 38^{\circ}\text{C}$  brain temperature stays constant; between  $38^{\circ}\text{C}$  and  $\sim 39.5^{\circ}\text{C}$  brain and carotid temperatures approach each other as CBF increases and SBC begins. Above  $39.5^{\circ}\text{C}$  typical SBC is evident. At carotid temperatures below  $\sim 37^{\circ}\text{C}$  all three jugular temperatures are above carotid temperature, but at higher carotid temperatures mean and minimum jugular temperatures become cooler reflecting an increase in REHL. Values are means  $\pm$  s.d. but note that error bars disappear from the mean jugular trace at low  $T_{\text{carotid}}$  because data from only two animals could be matched to corresponding data points for  $T_{\text{carotid}}$ .

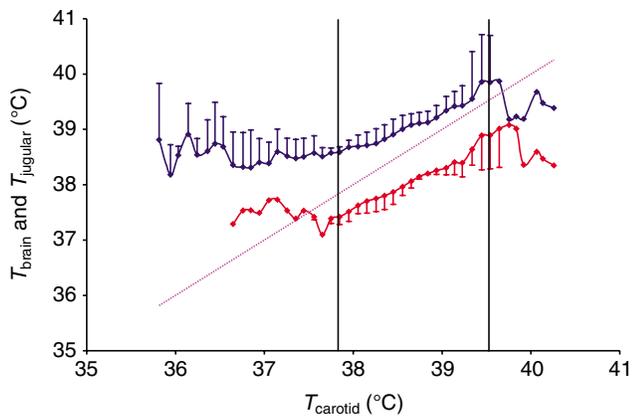


Fig. 6. Mean jugular ( $T_{\text{jugular}}$ ; red) and brain temperatures ( $T_{\text{brain}}$ ; blue) plotted against carotid temperature  $T_{\text{carotid}}$ , with the three phases of brain temperature demarcated (see text).  $T_{\text{jugular}}$  and  $T_{\text{brain}}$  change in parallel as  $T_{\text{carotid}}$  increases, and increase at a slower rate than  $T_{\text{carotid}}$ , showing that  $T_{\text{jugular}}$  and  $T_{\text{brain}}$  are linked.

study. If CBF is able to remove all brain heat then all the temperature gradients between  $T_{\text{carotid}}$  and  $T_{\text{brain}}$  measured should be accounted for by variations in CBF between the minimum ( $\sim 20 \text{ ml } 100 \text{ g}^{-1} \text{ min}^{-1}$ ) (Heckman, 2001) and the maximum ( $\sim 80 \text{ ml } 100 \text{ g}^{-1} \text{ min}^{-1}$ ) (Purves, 1972) possible. Using the modified convection equation to calculate CBF, Fig. 7 shows the results of an analysis of this possibility and it is clear that many of the measured gradients require explanations other than changes in CBF: gradients of less than  $0.2^\circ\text{C}$  (Fig. 7A) would require CBF above the assumed maximum and those greater than  $1^\circ\text{C}$  (Fig. 7C) would require CBF below values necessary to provide oxygen and glucose for brain metabolism.

### Discussion

Only two previous studies have been done on pronghorn body temperatures, with neither of these taking measurements throughout a day or over a long period of time. The data reported here are extensive and were obtained from five different body sites in four animals, allowing some interpretation of the thermoregulatory mechanisms used generally by pronghorn in a Wyoming summer.

The pronghorns were exposed to weather conditions typical of a hot and arid environment, characterized by large variations in ambient temperature. They are dissimilar to southern African climatic conditions. In the southern hemisphere summer days are longer, wind speed lower and, while mean globe and air temperatures are similar, in Wyoming the variation in globe and air temperatures is 2–3-fold higher. Despite these conditions our data show that the pronghorns in our study were able to maintain a remarkably constant body temperature ( $38.6 \pm 0.3^\circ\text{C}$ ) over a period of 3 months. These temperatures are lower than those reported previously (Thorne, 1975; Barrett and Chalmers, 1977), and lower than those recorded in springbok in the southern hemisphere summer ( $39.5 \pm 0.1$ ) (Fuller et al., 2005). In the former studies (Thorne, 1975; Barrett and Chalmers, 1977) the difference can be attributed to the method of measurement and the fact that the animals in those studies were subjected to stress, while in ours they were free-living and able to use the

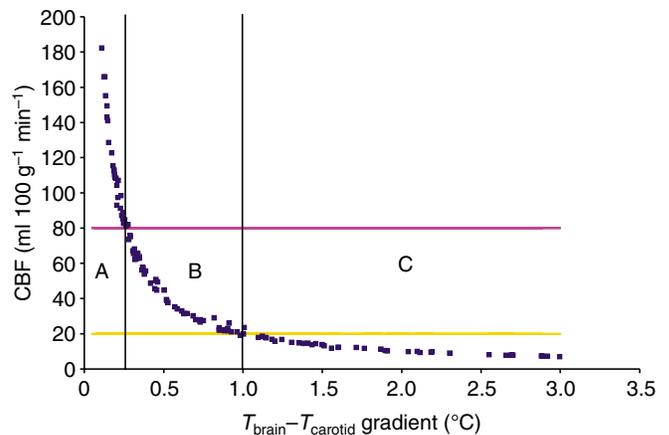


Fig. 7. Calculated CBFs for 130 brain minus carotid temperature gradients ( $T_{\text{brain}} - T_{\text{carotid}}$ ). The gradients were calculated by obtaining a mean  $T_{\text{carotid}}$  for  $0.1^\circ\text{C}$  intervals of  $T_{\text{carotid}}$  and subtracting that mean value from the corresponding average  $T_{\text{brain}}$ . (A) CBFs above the upper maximum CBF (pink line) cannot be achieved and the gradients associated with these CBFs are produced by SBC. (C) CBFs below the minimum CBF (yellow line) are unable to provide adequate oxygen and glucose to the brain (see text) and the gradients measured cannot be the result of reduced CBF. They must result from a brain warming mechanism. (B) Gradients between A and C can be produced by changes in CBF alone but are likely to be the result of changes in both CBF and SBC.

full array of thermoregulatory mechanisms available to them from behavioral to physiological.

We did not make formal observations of behavior nor correlate behavior with body temperature. However it was obvious that pronghorn used the landscape, for example, to avoid excess solar radiation (midday) and exposed themselves to solar radiation in the mornings and evenings. It can also be inferred that they used typical thermoregulatory mechanisms associated with homeothermy, such as peripheral vasoactivity (Fig. 3) and REHL (Fig. 5). The mechanisms underlying REHL depend on an adequate surface area for heat loss. We analyzed the anatomy of the pronghorn nasal cavity and found that the turbinate bones are scrolled, the length of the nasal cavity is short (approximately 20 cm in the four animals in this study) and the nasal mucosa surface area was  $14.4 \text{ cm}^2$  for each cm of length. Total surface area was  $280 \text{ cm}^2$ , and this surface area is similar to that in similar sized antelope (Kamau, 1992). During REHL cardiac output is redistributed to the head, anastomotic channels are opened between nasal mucosal arteries and veins to enlarge the surface area for cooling, respiratory rate increases, and finally, but not necessarily, there is panting (Maloney and Mitchell, 1997). Our data provide some evidence that the same mechanisms function in pronghorn.

Our data also reveal, however, that individual pronghorn show a much greater variation in body temperatures than has been found in southern hemisphere artiodactyls. Mean  $T_{\text{abdominal}}$  was almost  $1^\circ\text{C}$  lower than it is in springbok and the difference between maximum and minimum  $T_{\text{abdominal}}$  of  $5.4^\circ\text{C}$  was fivefold greater than that reported in springbok (Fuller et al., 2005). There was a degree of heterothermy of more than  $2.0^\circ\text{C}$  in a day in one animal and it seems that the females in the group

varied more than the male, although as there was only a single male this latter conclusion is speculative. The females had an average daily variation in  $T_{\text{abdominal}}$  of  $1.8^{\circ}\text{C}$ , which also is higher than we have found in southern hemisphere artiodactyls. We have previously attributed variation in body temperatures to episodes of exercise or fever and a nycthemeral rhythm. Our animals in this study were not subjected to exercise and data that could be attributed to fever were excluded. They did show a well-developed circadian rhythm linked to sunrise as it is in southern hemisphere animals (Fuller et al., 2005) but its amplitude was about  $1^{\circ}\text{C}$ , and can explain neither the wider daily variation nor the much larger range of temperatures ( $4.5^{\circ}\text{C}$ ) found over the study period.

A variation in mean body temperature that correlates with variations in mean and range of  $T_{\text{globe}}$  over the course of the summer are suggestive of a longer term heterothermy that could account for another  $0.25^{\circ}\text{C}$  of the variation over the study period. The amplitude of this variation is larger than that found in springbok (Fuller et al., 2005).  $T_{\text{carotid}}$  does not appear to be affected by changes in day length as in southern hemisphere animals (Fuller et al., 2005), and  $T_{\text{globe}}$  was not significantly correlated with day length ( $r=0.14$ ). The dissociation between  $T_{\text{globe}}$  and  $T_{\text{carotid}}$  in pronghorn as revealed by this analysis confirms the presence of a circadian rhythm, and suggests that pronghorn, even if they allow their body temperature to fluctuate over a summer, do not have a daily adaptive heterothermic response to environmental heat load.

To account for the remaining variation and the much wider range of temperatures found than we have reported for springbok, we think that another possible contributor is a lower variation in  $T_{\text{brain}}$  than in  $T_{\text{carotid}}$ . The maximal thermoregulatory responses to body temperature changes and, therefore, the smallest variation in body temperature occur when both  $T_{\text{brain}}$  and  $T_{\text{core}}$  were changed in the same direction (Jessen and Feistkorn, 1984). In our study this association is shown by the very high proportion of core body (carotid) temperatures that were recorded within the temperature range of  $38.1\text{--}39.0^{\circ}\text{C}$  (Fig. 1). Within this range changes in  $T_{\text{carotid}}$  and  $T_{\text{brain}}$  are correlated significantly (Fig. 6;  $r=0.979$ ), resulting in maximal thermoregulatory responses to temperature challenges, and defence of body temperature.

Outside of this 'set point' range  $T_{\text{brain}}$  was dissociated from  $T_{\text{carotid}}$  and as a result thermoregulatory responses should be blunted. The dissociation is achieved by what appears to be a unique combination of mechanisms that combine to regulate  $T_{\text{brain}}$  rather than  $T_{\text{body}}$ . Three factors influence  $T_{\text{brain}}$ : its metabolism, the rate of blood flow through it, and the temperature of the blood perfusing it. Of these only the latter two can be controlled. Brain metabolic rate is related to its temperature. CBF is controlled to some extent by nerves but mostly by metabolic rate and the demands for glucose and oxygen and, we think, for heat removal. The temperature of cerebral blood can be altered by the carotid rete-cavernous sinus system which exists in all artiodactyls including pronghorns (Carlton and McKean, 1977).

We calculate that changes in CBF can account for the heat removed from the brain and the gradient between  $T_{\text{brain}}$  and  $T_{\text{carotid}}$  in the range of  $0.2\text{--}1.0^{\circ}\text{C}$ , i.e. those which exist at preferred  $T_{\text{carotid}}$ . This conclusion is conservative. Our estimates

are based on the assumption that  $T_{\text{carotid}}$  is the temperature of blood entering the brain. In fact internal carotid artery (post-rete) blood is cooler than  $T_{\text{carotid}}$  when SBC is occurring (Maloney et al., 2007) so the curve shown in Fig. 7 is likely to be left shifted, SBC will account for a larger proportion of the gradients below  $0.8^{\circ}\text{C}$ , and changes in CBF for a smaller proportion. Nevertheless gradients smaller than  $0.2^{\circ}\text{C}$  and indeed negative gradients ( $T_{\text{carotid}} > T_{\text{brain}}$ ), which occur at  $T_{\text{carotid}} > 39.5^{\circ}\text{C}$ , cannot be explained by changes in CBF and must depend on the onset of SBC. Gradients above  $1^{\circ}\text{C}$  (and some as high as  $3^{\circ}\text{C}$ ), which occur at  $T_{\text{carotid}}$  less than  $\sim 38^{\circ}\text{C}$ , similarly cannot be explained by a reduction in CBF. They are not conspicuous in the southern hemisphere artiodactyls we have studied and therefore are so far unique to pronghorn. The only way they can be achieved is by a brain warming mechanism. As  $T_{\text{jugular}}$  is greater than  $T_{\text{carotid}}$  at these  $T_{\text{carotid}}$ , it follows that REHL is reduced during this phase of brain temperature regulation, but in addition there is the possibility that warm blood leaving the brain is being re-circulated past the cavernous sinus before entering the jugular vein. Warming of blood entering the brain, rather than cooling it as is the more usual function of the carotid sinus-cavernous sinus system, will result. However no anatomical basis for this sort of re-circulation has been described in any animal. A second possibility is that glial cell metabolism is activated at low  $T_{\text{carotid}}$  as it is in dolphins (Manger, 2006). Again, however, no anatomical basis for this possibility has been described in any artiodactyl.

Whatever the mechanism of brain warming, it, and SBC at high  $T_{\text{carotid}}$ , produces a relatively constant  $T_{\text{brain}}$  over the whole range of  $T_{\text{carotid}}$  temperatures we recorded during this study (Fig. 5). If  $T_{\text{brain}}$  is kept constant then a consequence should be blunted thermoregulatory responses. The result will be an increase in variation in  $T_{\text{carotid}}$ , until  $T_{\text{carotid}}$  and/or  $T_{\text{abdominal}}$  are themselves sufficiently low or high to activate thermoregulatory responses (Jessen and Feistkorn, 1984). This effect could be the origin of the significantly lower minimum  $T_{\text{carotid}}$  and  $T_{\text{abdominal}}$  than  $T_{\text{brain}}$ , and the long tail of  $T_{\text{carotid}}$  shown in Fig. 1.

These data show that pronghorns are homeotherms that have some typical thermoregulatory mechanisms found in southern hemisphere artiodactyls. They also, and specifically females, seem to have evolved some heterothermic characteristics that have made them well adapted to an arid environment in which ambient temperature can vary by over  $50^{\circ}\text{C}$  during the course of a typical summer day. While some of the temperature variations can be attributed to a well-developed circadian/nycthemeral rhythm, and to a longer term, direct effect of changes in  $T_{\text{globe}}$ , our data also strongly suggest that there may also be a contribution arising from divergence between  $T_{\text{brain}}$  and  $T_{\text{carotid}}$ . Our data show that at low body temperatures  $T_{\text{brain}}$  is maintained by a warming mechanism. At high body temperatures  $T_{\text{brain}}$  is cooled by SBC as in southern hemisphere artiodactyls. The relatively constant brain temperature that results is likely to result in the conservation of water and energy needed by pronghorn to survive in their typical habitat in the summer, when daily temperature fluctuations are high and the availability of water is low.

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