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2   **Selective brain cooling in Arabian oryx (*Oryx leucoryx*):**

3   **a physiological mechanism for coping with aridity?**

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20 Running page head: Selective brain cooling in Arabian oryx

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1 <u>Abstract</u>

2 Selective brain cooling is a thermoregulatory effector proposed to conserve body water and, as

3 such, may help artiodactyls cope with aridity. We measured brain and carotid blood temperature,

4 using implanted data loggers, in five Arabian oryx (*Oryx leucoryx*) in the desert of Saudi Arabia.

5 On average, brain temperature was $0.24 \pm 0.05°C$ lower than carotid blood temperature for four

6 oryx in April. Selective brain cooling was enhanced in our Arabian oryx compared to another

7 species from the same genus (gemsbok *Oryx gazella gazella*), exposed to similar ambient

8 temperatures but less aridity. Arabian oryx displayed a lower threshold ($37.8 \pm 0.1°C$ vs $39.8 \pm$

9 $0.4°C$), a higher frequency (87 ± 6 % vs $15 \pm 15\%$) and a higher maximum magnitude ($1.2 \pm$

10 $0.2°C$ vs $0.5 \pm 0.3°C$) of selective brain cooling than did gemsbok. The dominant male oryx

11 displayed less selective brain cooling than did any of the other oryx, but selective brain cooling

12 was enhanced in this oryx as conditions became hotter and drier. Enhanced selective brain

13 cooling in Arabian oryx supports the hypothesis that selective brain cooling would bestow

14 survival advantages for artiodactyl species inhabiting hot hyper-arid environments.

15

16 Keywords: brain temperature, artiodactyl, biologging, desert

17

1 Introduction

2 Artiodactyla, an evolutionarily successful and hugely speciose order of large ungulates, with 90

3 extant genera, evolved during the Eocene and speciated during a warming period in the Mid-

4 Miocene Climatic Optimum (Barnosky et al., 2003). Since they evolved in conditions that were

5 hotter and drier than present, artiodactyls may be pre-adapted to cope with warmer and drier

6 environments than those currently experienced (Barnosky et al., 2003; Mitchell and Lust, 2008).

7 Traditionally, the success of artiodactyls has been attributed to the evolution of improved

8 locomotion and the ruminant digestive tract, adaptations which allowed these ungulates to use

9 widely dispersed forage with a high fibre content (Janis, 1989; Codron et al., 2008). Recently,

10 however, another potential contributor to their success has been proposed: the evolution of the

11 carotid rete and the use of selective brain cooling (Fuller et al., 2008; Mitchell and Lust, 2008).

12 The carotid rete consists of an intertwining network of arterioles that lies within a venous sinus at

13 the base of the brain. Venous blood cooled by evaporation from the nasal mucosa drains into the

14 sinus and so cools the arterial blood destined for the brain, generating selective brain cooling

15 (Baker, 1982; Mitchell et al., 1987) - the reduction of brain temperature below arterial blood

16 temperature (IUPS Thermal Commission, 2003). The rete is absent in the closest relatives to the

17 artiodactyls, the perissodactyls, absent in the oldest ruminant group, the Tragulidae (Fukuta et al.,

18 2007), but is present in each of the artiodactyl clades: Ruminantiamorpha, Suinamorpha, and

19 Camelidamorpha. Recent systematic analysis of the Artiodactyla places the Ruminantiamorpha /

20 Suinamorpha / Camelidamorpha divergence before the appearance of the Tragulidae within the

21 Ruminantiamorpha (Spaulding et al., 2009), making it likely that the absence of the rete in

22 Tragulidae has been a secondary loss. Thus the carotid rete in the Artiodactyla lineage evolved

23 probably at about the same time as the rumen, during the mid-Miocene, about 20 Mya.

24

25 Selective brain cooling originally was hypothesized to primarily protect the brain during rises in

26 body temperature (Baker, 1979; Mitchell et al., 1987). However, recent studies support an

27 alternative concept, namely that mammals possessing a carotid rete may employ selective brain

28 cooling to attenuate the drive for evaporative cooling in arid conditions. Selective brain cooling

29 reduces the drive from temperature-sensitive neurones in the hypothalamus on evaporative heat

30 dissipation and conserves body water by switching body heat loss to non-evaporative avenues

31 (Jessen et al., 1994; Mitchell et al., 1997; Fuller et al., 1999; Maloney et al., 2002; Mitchell et al.,

32 2002). Abolition of selective brain cooling, which can occur rapidly, allows evaporative heat loss

33 to be restored in thermal emergencies, such as flight from a predator (Jessen, 1998; Mitchell et

1 al., 2002). So selective brain cooling would have survival advantages for artiodactyls, not

2 available to perissodactyls, in environments in which water resources are limited and

3 unpredictable (Mitchell et al., 2002).

4

5 If the evolution of the carotid rete indeed promoted thermoregulatory flexibility and thus

6 facilitated artiodactyl invasion of arid zones during the highly seasonal post-Eocene world

7 (Mitchell and Lust, 2008), selective brain cooling may be a key adaptation allowing artiodactyls

8 to cope with the increasing aridity and heat stress that is occurring with climate change (Fuller et

9 al., 2008). However, not all artiodactyls species exhibit the same capacity for selective brain

10 cooling. The absence of the rete in primitive artiodactyls, such as the mouse deer (*Tragulus*

11 *javanicus*, *Tragulus napu*; (Fukuta et al., 2007), might be expected to decrease the water

12 economy of these species and restrict them to habitats where water scarcity is not an issue.

13 Indeed, the mouse deer is restricted to climatically-stable forest environments (Whittow et al.,

14 1977). By contrast, enhanced selective brain cooling may be expected in artiodactyl species

15 inhabiting hot hyper-arid environments. Arabian oryx (*Oryx leucoryx*) inhabit one of the hottest

16 deserts in the world, in Saudi Arabia, and survive most of the year, including the hottest summer

17 months, without access to drinking water. To investigate the status of their selective brain

18 cooling, we employed implanted data loggers to measure brain and carotid arterial blood

19 temperatures of five free-living Arabian oryx in their natural habitat. Brain and carotid blood

20 temperature have not been measured in Arabian oryx previously, but have been measured in

21 another member of the same genus, the gemsbok (*Oryx gazella gazella*; Maloney et al., 2002).

22 Gemsbok inhabit hot dry environments in southern Africa, and indeed were studied at ambient

23 temperatures similar to those experienced by our Arabian oryx, but the rainfall experienced by

24 our Arabian oryx was one third of that experienced by the gemsbok.

25

26 Materials and methods

27 *Animals and habitat*

28 The study took place between March and July 2006 within the 2200 km^2 Mahazat as-Sayd

29 Protected Area (22°25'N, 41°48'E) in the open steppe desert in Saudi Arabia that is both the

30 historical and the current habitat for Arabian oryx (*Oryx leucoryx*, Pallas 1777). Adult, wild-born

31 oryx (two male and two female) were captured in the Protected Area in mid-March 2006. The

32 oryx were habituated in outdoor pens for two weeks to reduce potential peri-operative stress. An

1 additional male oryx was obtained from the breeding herd at the National Wildlife Research

2 Center in Taif (21°15'N, 40°42'E). This male oryx remained in Taif in a partially shaded pen

3 throughout the study period and had lucerne and water available *ad libitum*. All experimental

4 procedures were approved by the Animal Ethics Screening Committee of the University of the

5 Witwatersrand (protocol no. 2005/87/5).

6

7 *Surgery*

8 The oryx, at both locations, were darted and anaesthetized in their holding pens with etorphine

9 hydrochloride (2.5 mg intramuscularly (IM), M99, C-Vet, Leyland, UK) and, once recumbent,

10 were transported to a temporary operating theatre within 200 m of the pens. At this theatre, the

11 animals were placed in sternal recumbency, with their heads elevated. The animals were

12 intubated and anaesthesia was maintained with 2-6% isoflurane (Aerrane, Astra Zeneca,

13 Johannesburg, South Africa) administered in 100% oxygen. Respiratory rate, heart rate, arterial

14 oxygen saturation and rectal temperature were monitored throughout the surgery, which lasted

15 about two hours.

16

17 Under sterile surgical conditions, we implanted temperature-sensitive data loggers (see below)

18 with sensors in the brain and carotid artery. The loggers were dry sterilized in formaldehyde

19 vapour before implantation. After we had administered a local anaesthetic (3 ml 2% lignocaine

20 hydrochloride, Bayer Animal Health (Pty) Ltd, Isando, South Africa) subcutaneously (SC) at the

21 incision sites, the sites were shaved and sterilized with povidone iodine antiseptic (Vetedine,

22 Vetoquinol, Lure, France). For the measurement of carotid arterial blood temperature, a

23 thermistor inserted in a blind-ended and thin-walled polytetrafluoroethylene (PTFE) catheter (o.d.

24 1.35 mm, i.d. 0.97 mm; Straight Aortic Flush 4F Catheter, Cordis, the Netherlands) was

25 advanced 60 mm into the left common carotid artery towards the heart, at a position midway

26 along the length of the neck, and secured with a purse-string suture in the arterial wall. Outside

27 the artery, the PTFE tube was sealed on a Teflon-coated co-axial cable (150 mm long, o.d. 3 mm,

28 Belden, Richmond, USA) connecting the thermistor to the temperature-sensitive data logger (see

29 temperature measurements). The data logger was positioned subcutaneously, dorsal to the artery.

30 For measurement of brain temperature, a second data logger was positioned subcutaneously,

31 caudal to the base of the left ear. Its Teflon-coated cable was advanced subcutaneously over the

32 skull, where it was connected to a thermistor housed in a head plate and guide tube. The guide

1 tube, constructed from cellulose acetate butyrate tubing (40 mm long, o.d. 1.95 mm, i.d. 1 mm;
2 World Precision Instruments, Sarasota, Florida, USA) sealed at the tip by a steel cap, was
3 inserted through a 2 mm diameter burr hole, which was drilled through the cranium, at co-
4 ordinates pre-determined from head sections of dead oryx of similar size, so that the probe tip
5 would be positioned near the hypothalamus. The polyvinyl chloride head plate ($20 \times 10 \times 5$ mm)
6 was secured to the skull by two bone screws and covered by skin.

7

8 The wounds were treated with a topical antiseptic spray (Necrospray, Centaur Labs,
9 Johannesburg, South Africa). Each oryx received a long-acting antibiotic (450 mg IM, penicillin,
10 Norocillin La, Norbrook Laboratories Ltd., Newry, Northern Ireland), a non-steroidal anti-
11 inflammatory analgesic (100 mg IM, phenylbutazone, Dexaphenylarthrite injectable solution,
12 Vetoquinol Veterinary Pharmaceuticals, Cedex, France), a long-acting parasiticide (2 ml SC,
13 Ivermectin, Noromectin, Norbrook Laboratories Ltd., Newry, Northern Ireland) and a
14 multivitamin injection (9 ml I.M., Multivit injectable solution, Univet Ltd., Ireland). Before
15 anaesthesia was terminated, we fitted a neck collar containing a tracking radio transmitter (MOD-
16 500 Telonics, Inc. Mesa, AZ, USA).

17

18 Following surgery, the oryx were transported back to their pens, where they became ambulatory
19 within ~10 minutes after the effects of etorphine were reversed with diprenorphine hydrochloride
20 (7.5 mg intravenously (IV), M5050, C-Vet, Leyland, UK). The oryx at Taif remained in its pen
21 for the duration of the study. Two weeks after surgery, the four oryx in the Mahazat as-Sayd
22 Protected Area were released into a 2 km^2 fenced enclosure with natural forage and water
23 available *ad libitum.* Ten days later they were allowed to enter and range freely within the
24 Mahazat as-Sayd Protected Area, where they were left undisturbed, apart from the occasional
25 serendipitous visual contact by rangers. The four oryx separated from each other shortly after
26 their release, with some joining other oryx in the reserve.

27

28 A year after surgery, the four oryx were radio tracked, captured and transported back to the
29 holding pens. Those oryx, and the one at Taif, were anaesthetized again and the data loggers were
30 removed under a surgical procedure similar to that used for the original implantation. The
31 surgical wounds had healed and there were no signs of infection. After two weeks of recovery in
32 pens, the four free-living oryx were re-released into the Mahazat as-Sayd Protected Area.

33

1   *Temperature measurements*

2   The miniature thermometric data loggers (StowAway XTI, Onset Computer, Pocasset,

3   Massachusetts, USA) had outside dimensions of ~ $50 \times 45 \times 20$ mm and a mass of ~ 40 g when

4   covered in inert wax (Sasol, Johannesburg, South Africa). Temperature sensors used to measure

5   brain and carotid blood temperatures were constructed from ruggedized glass-coated bead

6   thermistors with insulated extension leads (bead diameter 0.3 mm; AB0E3-BR11KA103N,

7   Thermometrics, Edison, New Jersey, USA). The loggers had a resolution of 0.04°C and a

8   measurement range from + 34°C to + 46°C. The scan interval of the loggers was set at 5 minutes.

9   Each sensor assembly was calibrated against a high-accuracy thermometer (Quat 100, Heraeus,

10   Hanau, Germany) in an insulated water bath. After calibration, the loggers and their sensors

11   measured blood and brain temperatures to an accuracy better than 0.05°C.

12

13   *Climatic data measurements*

14   We collected climatic data from a portable weather station erected near the Mahazat as-Sayd

15   Protected Area, at the Saja/Umm ar-Rimth Protected Area (23°22'N, 42°45'E), and also at the

16   National Wildlife Research Center in Taif. We recorded wind speed (m.s^{-1}), solar radiation

17   (W.m^{-2}), dry-bulb temperature (°C) and relative humidity (%). We also recorded black globe

18   temperature (°C) and rainfall on site at the Mahazat as-Sayd Protected Area for the duration of

19   the study period. Black globe temperature provides an index of the thermal load on an animal as

20   it integrates the effects of radiant and convective heat exchange.

21

22   *Data analysis*

23   Although we recovered the data a year after surgery, we analysed responses for a period when we

24   had the most complete data set. The data loggers that measured carotid blood temperature in three

25   of the oryx (identified as Oryx 1, Oryx 2, and Oryx 3) malfunctioned within a month after

26   surgery (after a minimum of 4400 simultaneous measurements of brain and carotid blood

27   temperature). The data logger that measured brain temperature in the captive male oryx in Taif

28   (identified as Taif oryx) malfunctioned within a month after surgery (after ~7900 simultaneous

29   measurements of brain and carotid blood temperatures). We obtained brain temperature and

30   carotid blood temperature data from the other free-living male oryx (identified as Oryx 4) for

31   four months after surgery (~16500 simultaneous measurements of brain and carotid blood

1 temperatures), but his brain temperature probe exhibited an intermittent fault that restricted data
2 analysis to two months. Despite the fault, the data from this animal allowed us to compare
3 selective brain cooling over two seasonal periods. Furthermore, the opportunity presented itself to
4 compare a dominant male with a subordinate male exposed to similar environmental conditions.
5
6 We compared variables of the nychthemeral rhythm of brain and carotid blood temperatures with
7 paired t-tests. We analyzed the relationship between brain temperature and carotid blood
8 temperature by sorting all 5-minute measurements of carotid blood temperature into 0.1°C
9 classes, and determining the mean, standard deviation, maximum and minimum brain
10 temperature at each class of carotid blood temperature. We determined the threshold for selective
11 brain cooling as the point at which the regression line of brain temperature against carotid blood
12 temperature intercepted the line of identity (y = x). Selective brain cooling was calculated as the
13 difference between carotid blood and brain temperature, with positive values indicating selective
14 brain cooling. The maximum positive difference was defined as maximum selective brain cooling
15 capacity. Mean selective brain cooling was calculated as the average positive difference and the
16 frequency of selective brain cooling was calculated as the proportion of positive values.
17
18 We used mixed effects linear models (function 'lme' using the statistical package 'R', R
19 Development Core Team 2008) to investigate the effect of air temperature, water vapour
20 pressure, water availability and carotid blood temperature on the average 24-hour selective brain
21 cooling (difference between carotid blood and brain temperatures). Water availability was a
22 categorical predictor and divided our data into four periods: water was freely available and the
23 oryx drank daily, water was freely available and the oryx drank weekly, limited free-standing
24 water (7 mm rain in May) and no free-standing water (0 mm rain in June). We controlled for
25 repeated measurements by including "individual" and "Julian day" as random factors. To account
26 for potential autocorrelation between our variables, we included a first-order autoregressive
27 process (AR1) in our model structure. We generated 16 models (1 full model, 1 null model and
28 14 reduced models) based on all additive terms combinations and determined the best-fit model
29 based on the Akaike's Information Criterion (AIC). Models presenting with Δ_{AIC} greater than two
30 were not considered plausible models (Burnham and Anderson, 2002).
31
32 Values are expressed as mean ± s.d. and $P < 0.05$ was considered significant.

1

2 <u>Results</u>

3 *Climate*

4 Air and black globe temperatures varied as a function of the time of day, peaking just after solar

5 noon (12:00) and reaching a minimum just before sunrise (Fig. 1). Air and black globe

6 temperatures increased progressively from April (when all rain fell) to June (when no rain fell,

7 Table 1). Despite a difference in the number of days over which selective brain cooling was

8 recorded for each oryx in April, there was no difference in the 24-hour air temperatures

9 experienced by the four oryx ($F_{3.84} = 2.0$, $P = 0.12$). Solar radiation showed the expected bell-

10 shaped distribution and peaked at 1034 ± 64 W.m^{-2} around solar noon in the month in which we

11 had the most complete selective brain cooling data set (April). Wind speed increased to a

12 maximum of 9.4 ± 2.1 m.s^{-1} in the late afternoon and averaged 4.6 ± 1.5 m.s^{-1} over the 24-hour

13 periods during April. Mean 24-hour vapour pressure and relative humidity decreased

14 progressively from April to June (Table 1). Rainfall totalled 31 mm during 2006, substantially

15 lower than the ten-year average of 100 ± 60 mm. April was the wettest month (17 mm) but some

16 rain fell during March (7 mm) and May (7 mm). Because surgery was completed in late March,

17 we have excluded data for March from our analysis to eliminate any possible post-surgical

18 abnormalities in responses.

19

20 *Selective brain cooling*

21 Brain and carotid blood temperatures exhibited a nychthemeral rhythm with a trough between

22 6:00 and 7:00 (following sunrise) and a peak between 17:00 and 18:00 (shortly before sunset,

23 Fig. 2). During April, in four animals (i.e. excluding Oryx 4), the mean 24-hour brain

24 temperature was lower than carotid blood temperature (38.45 ± 0.06ºC vs 38.70 ± 0.10ºC; $t_3 = $

25 11.5, $P = 0.0014$). Minimum 24-hour brain and carotid blood temperatures were similar ($37.36 \pm $

26 0.28ºC vs 37.53 ± 0.34ºC; $t_3 = 0.99$, $P = 0.39$), while the maximum 24-hour brain temperature

27 was significantly lower than carotid blood temperature (39.34 ± 0.28ºC vs 39.61 ± 0.20ºC; $t_3 = $

28 4.2, $P = 0.024$). On average, the oryx implemented selective brain cooling throughout the day,

29 except for the period after sunrise (Fig. 2). For the four oryx, brain temperature was lower than

30 carotid blood temperature, measured simultaneously, $87 \pm 6\%$ of the time. On average, brain

31 temperature was 0.24 ± 0.05ºC lower than carotid blood temperature for these four oryx in April.

1 Mean selective brain cooling, the average difference between carotid blood and brain temperature

2 when the oryx were implementing selective brain cooling, was 0.32 ± 0.03ºC and the maximum

3 magnitude of selective brain cooling was 1.15 ± 0.17ºC. The threshold for selective brain

4 cooling, defined as the point at which carotid blood and mean brain temperatures were equal, was

5 37.8 ± 0.1ºC (mean $\pm$ s.d., $n = 4$, Table 2).

6

7 Figure 3 (upper panels) shows brain temperature as a function of carotid blood temperature and

8 the frequency distribution of carotid blood temperature in two male oryx while both had access to

9 food and water; the male in Taif drank water daily (Fig. 3 upper right panel) whereas the male

10 (Oryx 3) in the Mahazat as-Sayd Protected Area drank less frequently (Fig. 3upper left panel).

11 The captive male oryx (Taif oryx) employed selective brain cooling less frequently (81% vs

12 94%) than did the free-living male oryx (Oryx 3), despite the two oryx having the same carotid

13 blood temperature and threshold of selective brain cooling (Table 2).

14

15 Oryx 4, a male that frequently was observed pacing the fence while in captivity, initiating fights

16 with other males and squat-defecating, behaviours typical of a dominant male (Stanley Price,

17 1989), displayed less selective brain cooling than did any of the other oryx (Table 2). Compared

18 to Oryx 3, a male that appeared to us to be a subordinate, the dominant male (Oryx 4) showed a

19 higher threshold of selective brain cooling (39.6ºC vs 37.8ºC), a lower maximum magnitude of

20 selective brain cooling (0.93ºC vs 1.35ºC) and implemented selective brain cooling less

21 frequently (11% vs 94%, Table 2). Unlike that of the other oryx (Fig. 2), the brain temperature of

22 the dominant male oryx (Oryx 4) generally was above that of his carotid blood temperature,

23 particularly at low body temperatures in May (Fig. 4 upper panel), when climatic conditions were

24 relatively mild and drinking water was available through ephemeral pools of rainwater. But

25 selective brain cooling was enhanced in Oryx 4 when conditions were hotter and drier in June

26 (Fig. 4 lower panel). Although Oryx 4 spent only 11% of the time with his hypothalamus cooler

27 than carotid blood during May (Fig. 3 lower left panel), this frequency increased to 38% of the

28 time in June (Fig. 3 lower right panel). His brain temperature was an average 0.28 ± 0.25ºC

29 higher than carotid blood temperature during May, and only 0.03 ± 0.32ºC higher during June.

30

31 These differences in selective brain cooling between May and June were not simply the result of

32 a higher carotid blood temperature during June. When analysed over the same carotid blood

1   temperature range (37-40ºC), both the frequency of selective brain cooling (30%) and the average

2   difference between carotid blood and brain temperatures (0.06 ± 0.30ºC) indicated enhanced

3   selective brain cooling during the dry June period compared to May. The threshold for selective

4   brain cooling in Oryx 4 decreased with increasing ambient temperature and aridity, from 39.6°C

5   in May to 39.0°C in June. Above the threshold, the difference between carotid blood and brain

6   temperatures was higher in June (0.28 ± 0.24ºC) than during May (0.16 ± 0.20ºC). Nevertheless,

7   the maximum magnitude of selective brain cooling was similar in May (0.93ºC) and June

8   (1.03ºC), and selective brain cooling seldom exceeded 1ºC at any time.

9

10 The mixed effects linear models allowed us to differentiate the effects of air temperature and

11 aridity and confirmed that water availability (drinking frequency) was the best predictor for mean

12 24-hour selective brain cooling, with the top five models all incorporating water availability

13 (Table 3). The best model incorporated the effects of water availability, carotid temperature and

14 water vapour pressure. Among tested explanatory variables, air temperature had no measurable

15 effect on mean 24-hour selective brain cooling; the addition of air temperature to the model

16 changed the AIC value by more than two (Burnham and Anderson, 2002).

17

18 Discussion

19 Our study provides the first remote and continuous measurement of brain and carotid blood

20 temperatures of Arabian oryx (*Oryx leucoryx*) in their natural habitat, the deserts of Saudi Arabia.

21 Brain temperature generally was below carotid blood temperature near the peak of the

22 endogenous nychthemeral rhythm of body temperature. However, selective brain cooling was not

23 obligatory at high body temperatures; on occasion, brain temperature reached 41.2ºC when the

24 corresponding carotid blood temperature was also 41.2ºC. Despite the amplitude of the

25 nychthemeral rhythm of brain and carotid blood temperature of our oryx being similar to that

26 measured in other species (~ 2ºC), selective brain cooling appeared to be enhanced in our

27 Arabian oryx. The degree of selective brain cooling can be measured in three ways, namely, the

28 magnitude of the difference between carotid blood and brain temperature, the threshold at which

29 selective brain cooling is implemented, and the proportion of time spent using selective brain

30 cooling. Our Arabian oryx, which were exposed to drier natural conditions than any previous

31 animals in which selective brain cooling has been measured, displayed a high maximum

1   magnitude of selective brain cooling (1.15ºC), a low threshold for selective brain cooling

2   (37.8ºC) and a high frequency of selective brain cooling (87%).

3

4   The view that selective brain cooling would be enhanced under dry conditions is supported by

5   our finding that selective brain cooling was observed more frequently in the free-living male oryx

6   (Oryx 3), which drank infrequently, than did the captive male oryx (Taif oryx), which drank

7   daily, despite the two oryx being exposed to similar ambient temperatures and having the same

8   carotid blood temperature. Furthermore, under hot and dry conditions (in June), male Oryx 4

9   decreased the threshold at which selective brain cooling was implemented compared to that in

10   May, thereby increasing the range of body temperatures over which selective brain cooling was

11   implemented and thus increasing the frequency of selective brain cooling (Fig. 3 lower right

12   panel). The enhanced selective brain cooling was driven primarily by water availability (drinking

13   frequency), but carotid blood temperature and water vapour pressure also influenced 24-hour

14   selective brain cooling (Table 3).

15

16   Our conclusions about the enhancement of selective brain cooling by aridity seem robust, but our

17   conclusions about seasonal differences in selective brain cooling in the arid-adapted oryx are

18   tentative, because we obtained data from only a single male oryx (Oryx 4) under hot and dry

19   conditions (June). Since non-thermal factors affecting sympathetic tone may override the thermal

20   regulation of brain temperature (Kuhnen and Jessen, 1994; Maloney et al., 2001), particularly in

21   free-living animals (Jessen et al., 1994; Maloney et al., 2002; Caputa, 2004), high vigilance, and

22   consequent high sympathetic tone, may have acted to decrease the degree of selective brain

23   cooling in the dominant male oryx. His frequency of selective brain cooling was lower, and his

24   threshold of selective brain cooling was higher, than that of all the other oryx measured (Fig. 3

25   upper panels; Table 2). It remains to be seen whether subordinate oryx have the capacity to

26   further increase the frequency of selective brain cooling above 87% or whether they are capable

27   of increasing the magnitude of selective brain cooling as conditions get hotter and drier

28   seasonally. Other species capable of selective brain cooling increase the magnitude of selective

29   brain cooling during conditions of water deprivation and heat stress, as shown in both laboratory-

30   housed sheep (Fuller et al., 2007) and Bedouin goats (Jessen et al., 1997; Jessen et al., 1998).

31   Compared to euhydration, dehydrated goats and sheep increased selective brain cooling with no

32   alteration in the threshold body temperature at which selective brain cooling was implemented.

33   When animals were not heat stressed and were able to lose heat through non-evaporative

1   avenues, two restrained camels at rest showed no obvious difference in brain and blood

2   temperatures between hydrated and dehydrated states (Schroter et al., 1989).

3

4   We conclude that the Arabian oryx used selective brain cooling to facilitate homeostasis in hot

5   and dry environments. Arid-zone mammals possessing a carotid rete may employ selective brain

6   cooling to attenuate thermal drive by reducing brain temperature, which reduces evaporative heat

7   loss and ultimately conserves water by transferring cooling to non-evaporative means (Kuhnen,

8   1997; Jessen, 1998; Jessen, 2001; Mitchell et al., 2002). Our oryx, like free-living eland

9   (*Tragelaphus oryx*; Fuller et al., 1999), gemsbok (Maloney et al., 2002), black wildebeest

10 (*Connochaetes gnou*; Jessen et al., 1994), kudu (*Tragelaphus strepsiceros*; Hetem et al., 2008)

11 and springbok (*Antidorcas marsupialis*; Mitchell et al., 1997), displayed the greatest magnitude

12 of selective brain cooling when at rest, while body temperature was rising, in the late afternoon

13 (Fig. 2). Selective brain cooling appeared to be enhanced in our Arabian oryx compared to that

14 exhibited by gemsbok, another species from the same genus (Maloney et al., 2002), exposed to

15 similar ambient temperatures but less aridity (rainfall was three times higher than that

16 experienced by our oryx). Our oryx displayed a lower threshold of selective brain cooling (37.8 ±

17 0.1°C vs 39.8 ± 0.4°C), a higher frequency of selective brain cooling (87 ± 6% vs 15 ± 15%) and

18 a higher magnitude of maximum selective brain cooling (1.2 ± 0.2°C vs 0.5 ± 0.3°C) than did the

19 gemsbok. Indeed, the threshold for selective brain cooling in Arabian oryx was lower than that

20 observed in any of the African antelope species mentioned above (Jessen et al., 1994; Mitchell et

21 al., 1997; Fuller et al., 1999; Hetem et al., 2008), implying that they spent a higher proportion of

22 time selectively cooling the brain. Whether the apparent enhanced capacity for selective brain

23 cooling in the Arabian oryx reflects differences in the physiology or the anatomy of the carotid

24 rete heat exchanger, remains to be elucidated. Even though the magnitude of selective brain

25 cooling in our oryx was much less than the 2.7°C originally reported for the exercising captive

26 Thomson's gazelle (Taylor and Lyman, 1972), it was double the magnitude that substantially

27 reduced both the respiratory water loss (Kuhnen, 1997) and the metabolic cost of

28 thermoregulation (Kuhnen and Jessen, 1991) in goats.

29

30 <u>Conclusion</u>

31 We have shown that Arabian oryx use enhanced selective brain cooling in the extremely hot and

32 hyper-arid conditions of the Arabian desert. The enhancement took the form of increased

33 frequency of, and lower threshold for, selective brain cooling; the magnitude of selective brain

cooling in the arid-adapted Arabian oryx was similar to that observed in other free-living artiodactyls (Jessen et al., 1994; Jessen and Kuhnen, 1996; Mitchell et al., 1997; Fuller et al., 1999; Maloney et al., 2002), implying that there may be a physiological limit of about 1ºC to selective brain cooling in free-living artiodactyls. Because the hypothalamus is sensitive to changes in temperature of a few tenths of a degree (Kuhnen, 1997), such selective brain cooling would be sufficient to suppress evaporative cooling and therefore reduce water loss. Since selective brain cooling does not disrupt other homeostatic systems, it may provide an economical form of autonomic thermoregulation. The carotid rete may ultimately provide artiodactyls with greater acclimatization potential to the hot and dry conditions predicted to occur in the future.

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17

18

1   Figure captions

2   Figure 1. Average 24-hour pattern of air temperature (mean ± s.d.) during May (light grey line)

3   and June (black line). The shaded grey area represents the range of air temperature to which each

4   of the four oryx were exposed for the period during which we collected selective brain cooling

5   data in April.

6

7   Figure 2. Average 24-hour rhythm of brain (grey line) and carotid blood (black line) temperatures

8   for two female (top panels) and two male (bottom panels) oryx over 15 and 27 days in April. All

9   oryx had *ad libitum* access to food and water. Horizontal bars indicate night-time.

10

11   Figure 3. Brain temperature as a function of carotid blood temperature (top panels) and the

12   frequency distribution of carotid blood temperature (bottom panels), in two male oryx (Oryx 3

13   and the Taif oryx), when both animals had *ad libitum* access to food and water, and a free-living

14   male oryx (Oryx 4), while free ranging in the desert in May and in June. Each 5-minute recording

15   of carotid blood temperature was sorted into 0.1°C classes and the mean (solid black line),

16   minimum (lower grey line) and maximum (upper grey line) brain temperatures were calculated

17   for each 0.1°C class of carotid blood temperature. The dashed line represents the line of identity;

18   points below this line reflect selective brain cooling. Lower panels in each set show the absolute

19   frequencies with which each 0.1°C class of blood temperature occurred; 6491 data points were

20   obtained for the free-living male oryx (Oryx 3), 7968 data points for the captive male oryx in Taif

21   (Taif oryx), 8067 data points for the free-living male oryx (Oryx 4) in May and 8355 data points

22   in June.

23

24   Figure 4. Brain (grey line) and carotid blood (black line) temperatures of the dominant free-living

25   male oryx (Oryx 4) over four-day periods when mean ambient temperature was 27.9 ± 2.0°C

26   (May) and 34.7 ± 0.5°C (June). No rain fell in June.

27
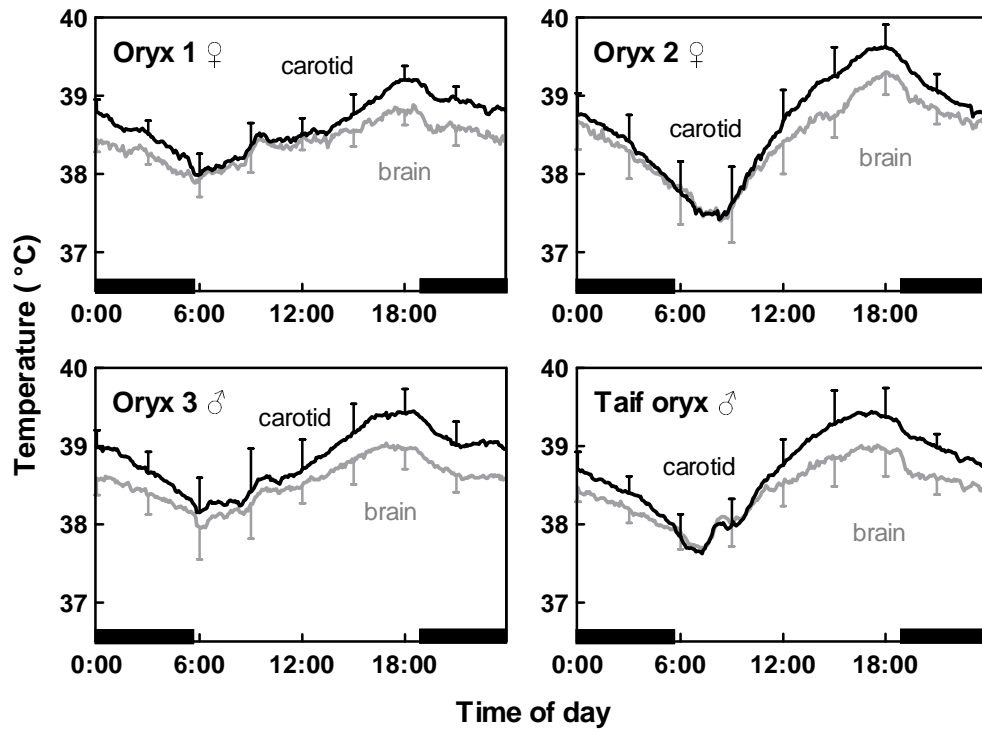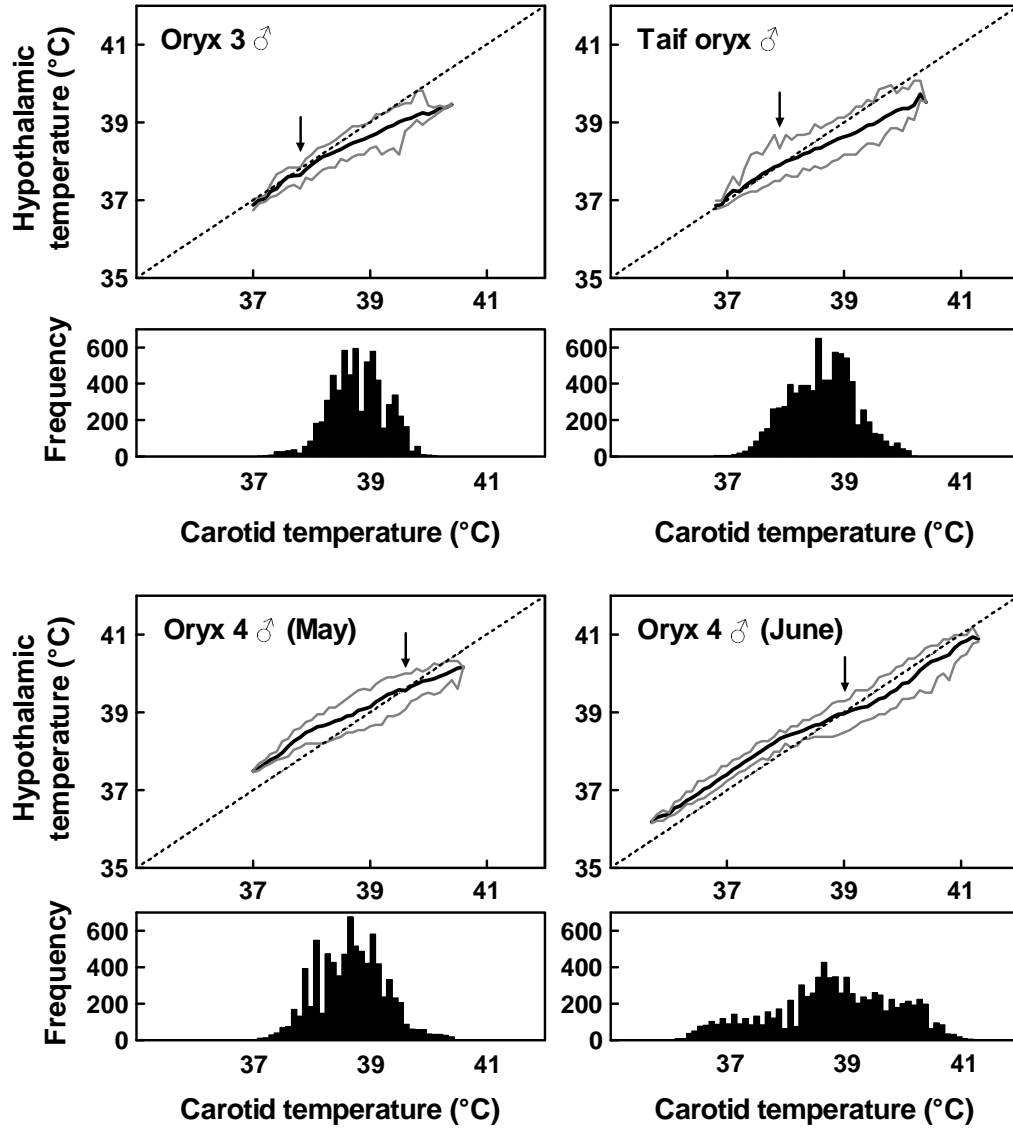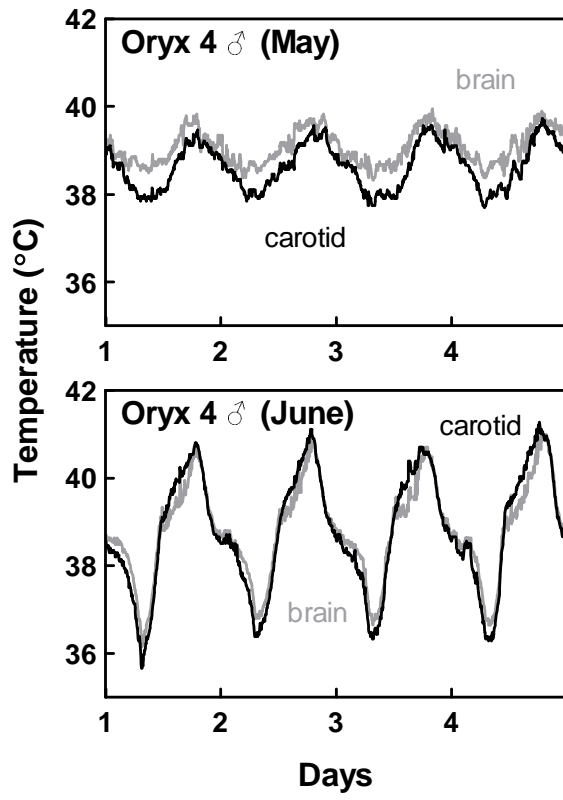
1   Figure 1.

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1   Figure 2.

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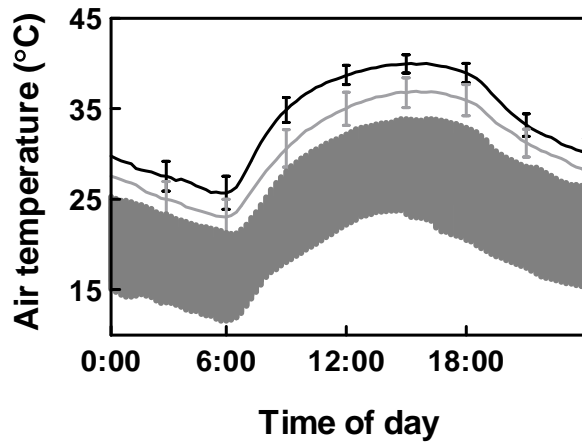
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1   Figure 4.

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3

1   Table 1: Environmental conditions (mean ± s.d.) during the three months in which the oryx were

2   living free in the Mahazat as-Sayd Protected Area

	April	May	June
Air temperature (˚C)			
24-hour mean	25.0 ± 3.6	30.5 ± 1.7	33.7 ± 0.9
24-hour minimum	17.2 ± 4.1	22.7 ± 1.9	25.3 ± 1.7
24-hour maximum	31.4 ± 3.5	36.9 ± 1.9	40.2 ± 1.0
Black globe temperature (˚C)			
24-hour mean	31.7 ± 3.0	35.4 ± 2.9	38.1 ± 1.4
24-hour minimum	16.4 ± 3.2	20.0 ± 2.3	21.5 ± 2.8
24-hour maximum	51.0 ± 5.6	55.0 ± 4.3	57.9 ± 3.3
Mean 24-hour vapour pressure (kPa)	0.81 ± 0.26	0.79 ± 0.28	0.44 ± 0.05
Relative humidity (%)	25 ± 10	20 ± 9	9 ± 1
Mean 24-hour wind speed (m.s^{-1})	4.6 ± 1.5	4.2 ± 1.3	4.1 ± 1.0
Mean 24-hour radiation (W.m^{-2})	286 ± 50	289 ± 46	312 ± 12
Total rainfall (mm)	17	7	0

3

1 Table 2. Average brain and carotid blood temperatures recorded every 5 minutes and the average difference between carotid blood ($T_{carotid}$)

2 and brain (T_{brain}) temperatures, as well as the mean, maximum, frequency and threshold for selective brain cooling in three male and two

3 female oryx. Data are mean ± s.d. and encompass at least 4400 simultaneous measurements of brain and carotid blood temperature in each

4 animal.

Oryx	Month	Mean brain temperature(°C)	Mean carotid temperature(°C)	Mean $T_{carotid}$ - T_{brain} (°C)	Selective brain cooling				N (days)
					Mean (°C)	Max (°C)	Frequency (%)	Threshold (°C)	
Oryx 1 ♀	April	38.42 ± 0.31	38.63 ± 0.39	+0.21 ± 0.19	0.29 ± 0.16	0.95	90	37.9	15
Oryx 2 ♀	April	38.44 ± 0.62	38.64 ± 0.74	+0.20 ± 0.26	0.30 ± 0.19	1.10	82	37.6	24
Oryx 3 ♂	April	38.53 ± 0.38	38.83 ± 0.50	+0.30 ± 0.20	0.34 ± 0.17	1.35	94	37.8	22
Oryx 4 ♂	May	38.99 ± 0.45	38.71 ± 0.60	-0.28 ± 0.25	0.18 ± 0.14	0.93	11	39.6	28
	June	38.87 ± 0.88	38.86 ± 1.11	-0.03 ± 0.32	0.32 ± 0.19	1.03	38	39.0	29
Taif oryx ♂	April	38.41 ± 0.45	38.66 ± 0.60	+0.24 ± 0.26	0.35 ± 0.20	1.19	81	37.9	27

5 N = number of days of data

1 Table 3. Akaike's Information Criterion (AIC) for the five best-fit mixed effects linear models

2 incorporating the main factors which accounted for the variability in average 24-hour selective

3 brain cooling. "Individual" and "Julian day" were included as random factors. d = number of

4 parameters including random factors, Δ_{AIC} = difference between AIC with the best model.

Formula (fixed effects)	d	AIC	Δ_{AIC}
Water availability + water vapour pressure + carotid blood temperature	5	-364	0.00
Water availability + water vapour pressure + carotid blood temperature + air temperature	6	-356.66	7.62
Water availability + carotid blood temperature	4	-351.34	12.95
Water availability + carotid blood temperature + air temperature	5	-349.22	15.07
Water availability + water vapour pressure	4	-296.8	67.49

5