RESEARCH ARTICLE

Minimum daily core body temperature in western grey kangaroos decreases as summer advances: a seasonal pattern, or a direct response to water, heat or energy supply?

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SUMMARY

Using implanted temperature loggers, we measured core body temperature in nine western grey kangaroos every 5 min for 24 to 98 days in spring and summer. Body temperature was highest at night and decreased rapidly early in the morning, reaching a nadir at 10:00 h, after ambient temperature and solar radiation had begun to increase. On hotter days, the minimum morning body temperature was lower than on cooler days, decreasing from a mean of 36.2°C in the spring to 34.0°C in the summer. This effect correlated better with the time of the year than with proximate thermal stressors, suggesting that either season itself or some factor correlated with season, such as food availability, caused the change. Water saving has been proposed as a selective advantage of heterothermy in other large mammals, but in kangaroos the water savings would have been small and not required in a reserve with permanent standing water. We calculate that the lower core temperature could provide energy savings of nearly 7%. It is likely that the heterothermy that we observed on hot days results either from decreased energy intake during the dry season or from a seasonal pattern entrained in the kangaroos that presumably has been selected for because of decreased energy availability during the dry season.

Key words: mammal, thermoregulation, homeothermy, heterothermy.

INTRODUCTION

The maintenance of a constant core body temperature (T_c), known as homeothermy, requires an animal to use more energy and water than if it displays heterothermy. Torpor and hibernation are forms of heterothermy that involve regulated changes in the set range for body temperature regulation, and are usually ascribed an adaptive role in reducing energy expenditure in small endotherms (Geiser and Turbill, 2009). Torpor has not been reported in large endotherms, but textbooks describe how some large African mammals allow body temperature to rise more than usual on hot days. This heterothermy is often ascribed an adaptive function in reducing water use in an environment where water is in limited supply. Whether kangaroos, macropod marsupials unique to the Australian continent that are as large as many African mammals, exhibit heterothermy is not known. Unlike African mammals, which are mainly active diurnally (Mitchell et al., 2002), kangaroos are active nocturnally, rest during the day (Dawson, 1995) and exhibit a nychthemeral cycle of T_c with a nocturnal maximum (Maloney et al., 2004). A nychthemeral rhythm with a diurnal nadir might impose a larger gradient for environmental heat flow and a larger evaporative cooling burden if the low diurnal T_c was defended actively during the day when ambient temperatures are high. Indeed the mesic-adapted eastern grey kangaroo maintained a lower T_c and used more evaporative cooling during the diurnal period than did the arid-adapted red kangaroo (McCarron et al., 2001).

Heterothermy was originally proposed as a mechanism that reduced water use in hot conditions in tame dehydrated camels (Schmidt-Nielsen et al., 1957). But the concept was applied more generally to euhydrated mammals when Taylor showed an increase in the evening rectal temperature of several euhydrated ungulate species when they were exposed to diurnal heat load in a climate chamber (Taylor, 1970). The original camel data could have been partly explained as dehydration-induced hyperthermia, wherein evaporative heat loss is stimulated only at a higher threshold T_c (Baker and Nijland, 1993; Doris and Baker, 1981; Taylor, 1970). But the camels also exhibited a lower than normal T_c in the morning, which cannot be explained as an effect of dehydration inhibiting the evaporative response to heat load. Louw and Seely recognised the possible important role of this lower T_c in the early morning, in addition to the evening hyperthermia, in the reduced water use in the dehydrated camel and coined the term 'adaptive heterothermy' to describe the increased range of T_c (and hence larger heat storage) (Louw and Seely, 1982). Adaptive heterothermy thus results from a widening of the interthreshold range for T_c at both extremes (Jessen, 2001).

Four criteria have been suggested for qualification as adaptive heterothermy, namely that the excursions of T_c should: (1) be large enough to account for appreciable heat storage, (2) be in phase with circadian changes in environmental thermal load, (3) increase when ambient heat stress increases and (4) be linked demonstrably to the conservation of body water (Mitchell et al., 2002). Two recent

studies, conducted in the Saudi Arabian desert, have shown that the amplitude of T_c in the Arabian oryx and the Arabian sand gazelle is related to the amplitude of environmental temperature and that heterothermy may be important for water saving in that arid environment (Ostrowski and Williams, 2006; Ostrowski et al., 2003). More recently, Hetem et al. showed that the degree of heterothermy for a given environmental temperature varied with season in the Arabian oryx, with the animals maintaining homeothermy in a season in which some rain fell but exhibiting heterothermy on days with similar ambient temperature in the dry season (Hetem et al., 2010). These data imply that heterothermy occurred when the oryx had less access to water, and so may have reflected dehydration-induced hyperthermia. In a further twist to the story, Grigg et al. showed that during the rut, male camels exhibited heterothermy mainly because of a reduced morning T_c, but suggested that the heterothermy advantaged the camel by increasing the amount of heat that could be stored before heat stroke would develop during the strenuous daily contests between rival bulls in rut (Grigg et al., 2009). Thus the proximate cause of the heterothermy was not ambient heat load or water availability, but anticipated heat storage during exertion.

In Western Australia, the western grey kangaroo (*Macropus fuliginosus* Desmarest 1817) (Kirsch and Poole, 1972) is the dominant species in mesic woodlands, but it recently has extended its range to the more arid interior of the continent (Dawson, 1995). The thermoregulatory ability of kangaroos often has been considered primitive (Dawson, 1995), but recent evidence shows that red and eastern grey kangaroos are excellent homeotherms, with T_c increasing only a few tenths of a degree after 4h exposure to 45°C in a climate chamber. Here we report T_c data collected from western grey kangaroos over two spring–summer seasons. Maloney et al. reported that the mean T_c of western grey kangaroos was independent of ambient temperature (T_a), and that the minimum morning T_c was very variable from day to day, with a strong association between morning T_a and minimum T_c (Maloney et al., 2004). Contrary to what would be expected from the physics of heat transfer, minimum T_c was lower on warmer mornings than on cooler mornings. This suggested a pre-emptive core cooling on days when heat load was likely to be high, similar to that observed in dehydrated camels (Schmidt-Nielsen et al., 1957), and consistent with adaptive heterothermy. But in the reserve where the studies were conducted, water was available freely, so presumably the kangaroos had no need to conserve water. There are no data on drinking frequency in western grey kangaroos, but the closely related eastern grey kangaroo drinks at least every second day (Dawson et al., 2006). We have now measured T_c in free ranging kangaroos over two spring–summer seasons and report the structure of the nychthemeral T_c cycle and the effect of changes in environmental conditions on that cycle.

MATERIALS AND METHODS
Animals and habitat

The experiments took place between September and February (Southern Hemisphere spring and summer) over 2 years at the Harry Waring Marsupial Reserve, 30km southeast of Perth, Western Australia. This 254ha fenced reserve represents a typical habitat for *M. fuliginosus*. The habitat includes swamp paper bark (*Melaleuca raphiophylla*), jarrah (*Eucalyptus marginata*) and banksia (*Banksia menziesii* and *B. ilicifolia*) tree cover with an understorey of *Hibbertia hypericoides*, *Pultenaea reticulate* and large areas of grass trees (*Xanthorroea* spp.). The area has a Mediterranean climate with hot and dry summers and cold and wet winters. The dry season usually extends from November to April (Fig. 1), and the availability of natural forage is reduced during this time (Turner, 2004). Water was available at two permanent water troughs within the reserve and also from several ephemeral pools during the wet season (May–October).

Kangaroos were implanted with data loggers to record T_c. In year 1, six kangaroos were implanted and T_c data were obtained from five kangaroos (one female and four males with masses of 25, 61, 69, 32 and 36kg, respectively), and in year 2 five kangaroos were implanted and T_c data were obtained from four kangaroos (one female and three males with masses of 33, 46, 45 and 55kg, respectively). In the other two animals, the data loggers failed to record. Between surgery and recapture at least 4months later, the mean (±s.d.) body mass change was +1±3kg in year 1 and –2±1kg in year 2. Periodic observations of the animals over the course of the study revealed that the animals usually were interacting with conspecifics or engaged in behaviours similar to those of conspecifics, leading us to believe that the study animals behaved normally.

The Animal Ethics Committee of the University of Western Australia (approval no. 01/100/163) approved all experimental procedures.
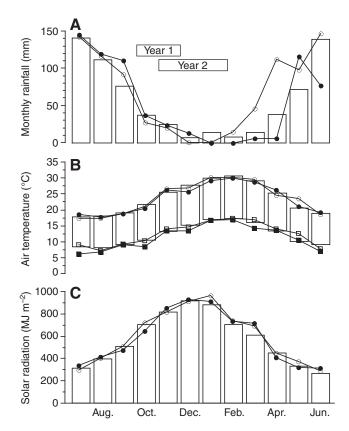

Fig. 1. (A) Monthly mean rainfall at the Medina Station, Western Australia, from 1996 to 2009 (bars) and for the two years of the study (filled circles, year 1; open circles, year 2). Horizontal bars show the study periods. (B) Mean monthly maximum and minimum air temperature for 1996–2009 (top and bottom of bars, respectively) and for the two years of the study (circles, monthly maximum; squares, monthly minimum; filled symbols, year 1; open symbols, year 2). (C) Total monthly solar radiation for 1996–2009 (bars) and for the two years of the study (filled circles, year 1; open circles, year 2). Data were collected at Medina Station, 5km from the experimental site.

Body temperature measurement

Kangaroos were immobilised *via* a dart containing 125 mg of tiletamine hydrochloride and 125 mg of zolazepam hydrochloride (Zoletil 100, Virbac, Milperra, NSW, Australia) and transported to a temporary surgical theatre within the reserve. Each animal was placed on its side and anaesthetised with 1–2% halothane (Rhone Merieux, West Footscray, VIC, Australia) in oxygen, administered *via* an endotracheal tube. Ventilation during anaesthesia was spontaneous. Respiratory rate, heart rate and rectal temperature were monitored throughout surgery.

Using aseptic surgical procedures, we implanted miniature data loggers (Stowaway XTI, Onset Computer Corporation, Bourne, MA, USA) with thermistor sensors for temperature measurement (Maloney et al., 2002). In year 1 we implanted devices to measure and record temperatures in a carotid artery (using a data logger with an external thermistor probe) and in the abdominal cavity (using a data logger with a thermistor attached to the circuit board). Many of the data loggers failed. By the second year we had solved technical issues with abdominal loggers but not yet with carotid loggers, and so in year 2 only abdominal devices were implanted.

A 50 mg enrofloxacin tablet (Baytril, Bayer, Leverkusen, Germany) was inserted into each surgical site and the skin suture line sealed with topical antiseptic spray (Terramycin Pinkeye Aerosol, Pfizer, West Ryde, NSW, Australia). The animals also received long-acting antibiotic (2 ml Benicillin, Troy Laboratories, Smithfield, NSW, Australia) and an analgesic and anti-inflammatory medication: in year 1, Temgesic (1 ml) containing 0.32 mg buprenorphine hydrochloride (Reckitt and Colman, Hull, UK) plus flunixin meglumine (1 ml) (Finadyne, Heriot Agvet, Rowville, VIC, Australia); in year 2, the Finadyne only.

All data loggers with their thermistors were calibrated against a certified mercury-in-glass thermometer (resolution and accuracy of 0.05°C; WIKA Australia, Rydalmere, NSW, Australia) in an insulated water bath to a calibrated accuracy within one sampling step of the data logger (0.04–0.05°C). The scan interval of the data loggers was set at 5 min, which enabled 112 days of temperature recording, some of which was required for calibration. The animals were redarted no sooner than 4 months later, killed with an overdose of sodium pentobarbitone (Lethabarb, Virbac, Peakhurst, NSW, Australia) and the data loggers were recovered. The animals' wounds had healed and there were no signs of infection. In year 1, *in vivo* examination of the carotid arteries at logger retrieval revealed no occlusion or clotting along the length of the intravascular guide tube, i.e. the thermistors measured the temperature of free-flowing blood. The thermistor–logger combinations on the carotid loggers were no longer functioning at recovery, generally as a result of thermistor lead breakage. Carotid leads broke between 36 and 74 days after implantation. The abdominal loggers in year 1 had all stopped working by retrieval, but we obtained 35 and 74 days of data from two of them. In year 2, full records were obtained from each of three kangaroos and 35 days of data were obtained from another kangaroo. The data logger of the fifth kangaroo in year 2 failed.

Meteorological data measurement

Meteorological data were obtained from the Department of Agriculture Weather Station at Medina, Western Australia, ~5 km south of the reserve. The data consisted of hourly air (dry-bulb) temperature, solar radiation and rainfall values, from which daily and within-day (see below) means were calculated. Four days of meteorological data were lost in year 1 (5–8 October) because of equipment failure. To compare data from the study years with longer-term means, we calculated the monthly means for each variable from 1996 (when the Medina station was established) to 2009. In year 1, we also measured air temperature within the reserve, and those measurements correlated very strongly with the data from Medina ($T_{a,Medina}=0.99 \times T_{a,Waring}-0.03$; $F_{1,1387}=25969$, $P<10^{-40}$, $R^2=0.96$), giving us confidence to apply Medina data to the reserve.

Data analysis

For the analysis of the effects of environmental temperature on kangaroo T_c, we considered the T_c during the first 7 days to be influenced by the consequences of surgery and began data analysis 7 days after surgery was completed on the last kangaroo in each year. In year 1, data were analysed from 1 October, and in year 2 data were analysed from 29 October. The analysed data for each animal consist of the data recorded from midnight on 1 or 29 October to the midnight before equipment failure or logger capacity was reached; this amounted to, for year 1, 63 days (F1), 37 days (M1), 24 days (M2), 61 days (M3) and 51 days (M4), and for year 2, 25 days (F2) and 98 days (M5, M6 and M7) (where F is female and M is male).

We obtained no abdominal temperature data from three kangaroos in Year 1 (M1, M3 and M4), but did obtain 37, 61 and 51 days of carotid temperatures. In the two kangaroos from which we obtained simultaneous carotid and abdominal data, abdominal temperature was always higher than carotid temperature by 0.37±0.14 (F1) and 0.39±0.04°C (M2), respectively, on average (see Results). Thus we estimated abdominal temperature for animals M1, M3 and M4 by adding 0.38°C to the carotid temperature.

We analysed the nychthemeral pattern of T_c by calculating within each animal the mean and standard deviation of T_c for each hour of the nychthemeron (24 h period) across all days. We then calculated the mean T_c for the group of animals for each hour of the nychthemeron, and the mean of the standard deviation (between animals) of T_c for each hour of the nychthemeron. We tested the null hypothesis that T_c was the same at each hour of the nychthemeron with repeated-measures ANOVA. We used the standard deviation of hourly T_c between days for each kangaroo to test the null hypothesis that the variability in T_c was the same at each hour of the nychthemeron, also with repeated-measures ANOVA.

Because the variability of T_c turned out not to be the same at each hour of the day (see Results), we calculated the mean T_c of the group of kangaroos at different times of the nychthemeron and regressed those means on the appropriate T_a. We calculated the mean daily T_c between 18:00 and 04:00 h (corresponding to the nocturnal period of activity and the zenith of the T_c cycle) for each kangaroo and for the group of kangaroos. We also calculated the minimum daily T_c for each kangaroo and for the group of kangaroos. We regressed these values on the mean daily T_a and the mean T_a from 06:00 to 10:00 h, the period when the nadir in T_c occurred. We calculated the daily amplitude of T_c and regressed these values on mean daily T_a and the daily amplitude of T_a. We then calculated the rate of rise in T_c between 12:00 and 16:00 h, the hottest period of the day, for each day for each animal, and the mean rate of rise in T_c for the group of kangaroos. We regressed these values on the mean and maximum T_a for each day. Throughout, we calculated group means only on days when data were available for two or more kangaroos.

We also regressed the mean minimum kangaroo T_c for each day on the mean solar radiation intensity between 06:00 and 10:00 h (because minimum T_c usually occurred between sunrise and 10:00 h). In mid-October, sunrise and sunset occurred at 05:36 and 18:29 h, respectively, and in early January they occurred at 05:13 and 19:26 h.

Because an association of T_c with T_a may have been confounded by time of year, we also regressed minimum T_c on the number of days since the winter solstice. When this association proved significant, we calculated the residuals from the fitted line and regressed those residuals, in turn, on mean daily T_a. To test whether proximate environmental cues were associated with the decrease in morning T_c in hotter conditions, we determined the goodness of fit (as described by the R^2) of the regression of the mean and maximum T_a for the previous 1 to 10 days against the daily minimum T_c of the kangaroos.

All ANOVA and regressions were performed using either Microsoft Excel or Statistica software (Statsoft, Tulsa, OK, USA).

RESULTS

The rainfall over each month of the study periods was near average (Fig. 1A), though the total rainfall in year 2 (824 mm vs 659 mm for year 1) was higher than the average (686 mm) because of extra rain after the study period. Mean daily T_a was not different on the same dates in the two years (t_{64}=0.2, P=0.87), but was significantly higher over the full study period in year 2 compared with year 1 (t_{153}=7.0, $P<10^{-10}$), because year 2 extended further into summer when maximum T_a was higher. Similarly, the mean diurnal solar radiation for the full study period was higher in year 2 than in year 1 (t_{153}=2.89, $P<0.004$) but not if compared on the same dates in the two years (t_{64}=0.98, P=0.32). Average monthly minimum and maximum air temperatures and the total monthly solar radiation over the study were within 1 s.d. of the long-term mean (Fig. 1B,C). Mean daily T_a ranged from 10.7 to 31.7°C, with a minimum and maximum recorded T_a of 3.7 and 35.7°C, respectively, in year 1, and 6.5 and 42.1°C, respectively, in year 2. In years 1 and 2 there were 9 and 34 days, respectively, when daily maximum T_a exceeded 30°C, and 1 and 11 days, respectively, when it exceeded 35°C. Across the nychthemeral cycle, T_a was lowest at approximately 06:00h and highest between 13:00 and 15:00h (Fig. 2A). Solar radiation peaked at 13:00h and varied from a maximum daily (07:00–17:00h) mean of 703 W m^{-2} on clear days near the summer solstice to 162 W m^{-2} on overcast days (Fig. 2B).

The mean T_c of the individual kangaroos, over the recording period, varied from 35.97 to 37.00°C (Fig. 3A). Abdominal temperature always was higher than carotid temperature even at fine time scales (Fig. 3B). There was a pronounced nychthemeral rhythm in kangaroo T_c, with T_c being lower from 05:00 to 15:00h than at midnight ($F_{1,23}$=43, $P<10^{-6}$, Student–Newman–Keuls post hoc test for comparison of individual means ranged from P=0.03 to $P<10^{-5}$) with a minimum at 10:00h and a maximum at 18:00–19:00h (Fig. 2C). The intra-individual standard deviation between days was significantly higher from 05:00 to 16:00h than at midnight ($F_{1,23}$=15, $P<10^{-6}$, Student–Newman–Keuls post hoc test for comparison of individual means ranged from P=0.02 to $P<10^{-5}$), so that the day-to-day variation in T_c was highest when T_c was lowest, between 08:00 and 16:00h (Fig. 2D).

Part of the variability in T_c between days was the result of a significant decrease in the mean daily T_c from 36.96 to 35.94°C with an increase in the mean daily T_a from 10.7 to 31.7°C (Fig. 4A). Some of that decrease was due to the mean T_c at night (between 18:00 and 04:00h) being lower on hotter days (Fig. 4B), with mean T_c at night decreasing from 37.18 to 36.63°C. Mean T_c from 18:00 to 04:00h on each day was also significantly negatively correlated with mean T_a over the same hours ($F_{1,153}$=31, $P<10^{-6}$, R^2=0.17, y=37.4–0.03x). The effect of T_a on the minimum T_c was much larger (Fig. 4C), with the minimum T_c of the kangaroos decreasing from 36.16 to 34.01°C over the 21°C range in mean daily T_a. The
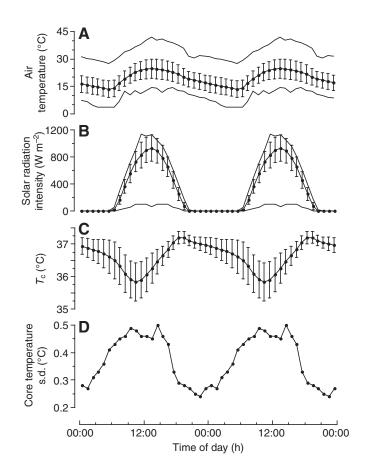

Fig. 2. Plots of (A) mean (±s.d.), maximum and minimum hourly ambient air temperature during the study, weighted by the number of kangaroos recorded on each day; (B) mean (±s.d.), maximum and minimum hourly solar radiation intensity during the study, weighted by the number of kangaroos recorded on each day; (C) mean (±s.d. between kangaroos) hourly core body temperature (T_c) of the kangaroos; and (D) mean hourly standard deviation of T_c within kangaroos. The mean and the s.d. of the T_c for each hour across all days were first calculated for each kangaroo and then averaged across the nine kangaroos.

minimum T_c was also significantly negatively correlated with T_a at the time of minimum T_c (06:00–10:00h; $F_{1,153}$=70, $P<10^{-13}$, R^2=0.31, y=37.1–0.1x).

Because minimum T_c decreased more than nighttime T_c, the daily amplitude of the T_c rhythm increased significantly as mean daily T_a increased (Fig. 4D). The daily amplitude of the T_c rhythm also increased significantly with the daily amplitude of the T_a rhythm ($F_{1,153}$=14, P=0.0002, R^2=0.08, y=1.7+0.05x). The rate of rise of T_c during the hottest period of the day between 12:00 and 16:00h was higher on hotter days (Fig. 4E). The rate of rise of T_c also increased significantly with T_a at that time (rate of rise in T_c between 12:00 and 16:00h versus maximum daily T_a: $F_{1,153}$=48, $P<10^{-9}$, R^2=0.24, y=–0.04+0.01x).

We found no association between morning solar radiation intensity and the minimum T_c of kangaroos (Fig. 5A). However, morning T_c decreased with the progression from spring to summer, there being a significant decrease in the minimum T_c of kangaroos with the number of days since the winter solstice ($F_{1,153}$=123, $P<10^{-20}$, R^2=0.45, y=6.7+0.08x; Fig. 5B). We determined whether the effect of air temperature on minimum daily T_c was confounded by time of year by calculating the daily residuals from the regression
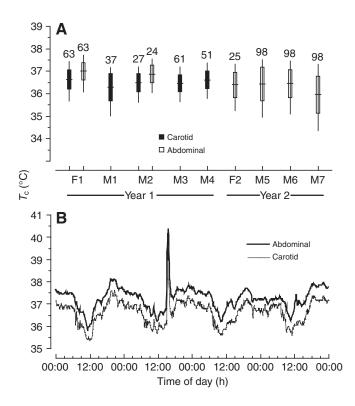
Fig. 3. (A) Mean T_c for each kangaroo in the study (horizontal line for each kangaroo). The vertical bars represent the standard deviation and the whiskers the extension to the mean daily minimum and maximum. Filled bars show carotid artery temperature and open bars abdominal temperature. The number of days of data is indicated above each bar. (B) Four days of original data recorded every 5 min in one kangaroo, showing temperature recorded in the abdominal cavity (thick line) and carotid artery (thin line).

Fig. 4. Mean T_c of the group of kangaroos on each day, plotted as a function of mean daily air temperature. (A) Mean daily T_c. (B) T_c between 18:00 and 04:00 h. (C) Daily minimum T_c. (D) Daily amplitude of T_c (maximum–minimum). (E) Daily rate of rise in T_c between 12:00 and 16:00 h.

line of daily minimum T_c against days since the winter solstice (Fig. 5B) and regressing those residuals on daily air temperature between 06:00 and 10:00 h. There remained no effect of air temperature on morning T_c (Fig. 5C).

Proximate environmental cues (assessed as mean daily T_a) were significantly related to the minimum morning T_c, with the strength of the relationship (as assessed by the R^2 of the regression) increasing as the number of previous days averaged increased from one to 10 (Fig. 5D).

Day-to-day and hour-to-hour changes in T_c were small compared with what could happen within minutes. Each of the kangaroos, on at least 2 days each, exhibited a hyperthermic episode followed by relatively rapid return of T_c back to normal for that time of day (Fig. 6). These events always occurred in the mid-afternoon, as we have reported previously for the kangaroos in year 1 (Maloney et al., 2004). T_c increased by up to 4°C over 10–20 min, and returned to the normal T_c for that time of day over the subsequent 30 to 120 min. There was no association between the occurrence or magnitude of these episodes and air temperature.

DISCUSSION

The finding of our study most intriguing to us was the progressive reduction in morning T_c of the kangaroos as the days got hotter. In terms of the criteria for adaptive heterothermy, these changes in the T_c pattern: (1) were large enough to account for appreciable heat storage; (2) were in phase with environmental heat load, even though
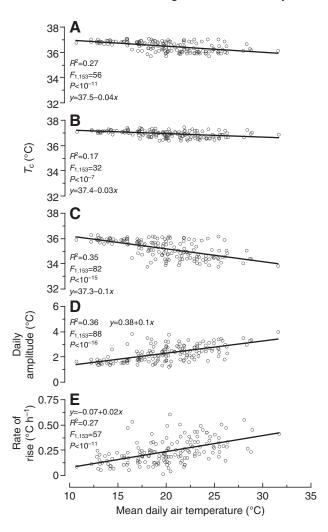
activity was nocturnal; (3) increased when ambient temperature increased; but (4) did not seem to be linked to water conservation because water was freely available in the reserve and the calculated water savings were small (see below). One conclusion would be that the kangaroos were employing one aspect of adaptive heterothermy, increasing the scope for heat storage by lowering morning T_c, and reducing a need for evaporative cooling on hot days. Such an interpretation is in accord with the increased rate of rise of T_c in the afternoon of hotter days. But our finding that nighttime maximum T_c also decreased as the days became hotter (and so there was no increase in maximum T_c associated with heat load), and that acute hyperthermic events, even at the hottest time of day, were followed by restoration of the usual level of T_c for that time of day, implies that T_c was controlled well at the hottest times of the day. Additionally, the morning changes in the nychthemeral T_c cycle were explained much better by time of year (R^2=0.70) than by the prevailing T_a on a given day (R^2=0.35), or the previous one (R^2=0.32) to 10 (R^2=0.50) days (all P<0.05). These results led us to conclude that the apparent pre-emptive change in thermoregulatory function was not caused by a requirement to conserve water or by
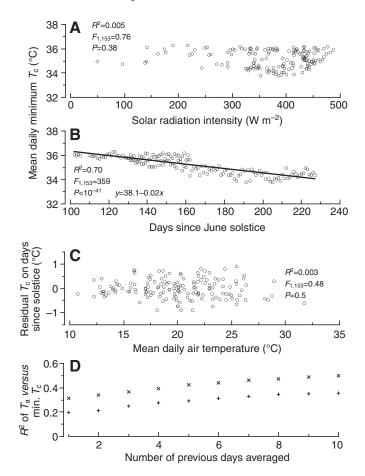
Fig. 5. (A) Mean daily minimum T_c of the group of kangaroos as a function of the mean incident solar radiation intensity between 06:00 and 10:00 h. (B) Mean daily minimum T_c of the group of kangaroos as a function of the number of days since the winter solstice. (C) Residual T_c from the regression line shown in B as a function of the mean daily air temperature. (D) R^2 of the regression (all $P < 0.05$) of daily minimum T_c on the ambient conditions averaged over the previous 1 to 10 days [x, mean daily ambient temperature (T_a); +, daily maximum T_a].

Fig. 6. Original 5 min records of T_c on days when the kangaroos exhibited rapid transient increases in T_c (filled circles), and mean (±s.d.) T_c (open circles) over the entire study. (A) Female 1 (25 kg); (B) Male 3 (35 kg).

proximate environmental temperature but by some factor other than T_a that varied with time of year. Two possible factors are an endogenous circannual rhythm or food quality and quantity, as summer coincides with the dry season in the study area.

Our study provides the longest continuous records of T_c for macropod marsupials, and the records were obtained from animals living free in their natural habitat, with little disruption from human observers. Despite T_a being as low as 4°C and as high as 42°C, the day-to-day variation in T_c of the nine kangaroos was generally within the limits of homeothermy (±2°C) (IUPS Thermal Commission, 1987). We would define the normal range of T_c in the kangaroos as approximately 1.5°C, that being the amplitude of the T_c rhythm prevailing in spring (Fig. 3B, Fig. 4D) and that range increased with the progression into summer, satisfying the criteria for heterothermy (IUPS Thermal Commission, 2001). There were days for each individual kangaroo when the daily amplitude of T_c exceeded 4°C (the highest recorded was 5.4°C) but these were days containing rapid transient changes in T_c, such as those depicted in Fig. 6. The overall weighted mean T_c of 36.5±0.4°C that we measured for this species is low by eutherian standards, higher than that reported for marsupials in general (35.3°C) (Withers et al., 2000), but similar
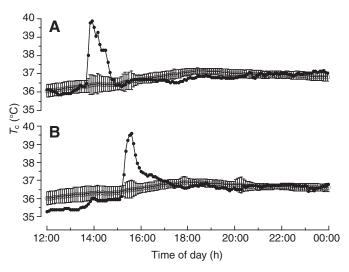
to that measured over a range of ambient temperatures in the laboratory in the closely related eastern grey kangaroo and the red kangaroo (Dawson et al., 2000).

Because the kangaroos were free-living in a large reserve, they were observed only infrequently, and we do not know the cause of the rapid increases in T_c depicted in Fig. 6. These increases may have been due to exercise activity, although they all occurred during the normal rest phase. It is possible that the episodes were stress hyperthermia, in the absence of intense exercise, as has been reported for impala (Meyer et al., 2008). The episodes occurred twice on days when members of the research team observed the kangaroos, but there were many more days on which the kangaroos were observed when such episodes did not occur. It is unlikely that environmental heat load contributed significantly to these events because even on the hottest days (42°C) and with a kangaroo exposed fully to maximum solar radiation (0.4 m² of body surface presented to 1000 W m⁻² of solar radiation) the environmental heat load would have been maximally 100 W, assuming a solar heat load and thermal conductance similar to that of the red kangaroo (Dawson et al., 2000; Dawson and Maloney, 2004). That much heat, if all stored, would generate an increase in T_c of 0.7°C in 10 min. On several occasions we recorded increases in T_c of more than 3°C in 10 min. A 3°C increase in T_c in 10 min in a 25 kg animal requires heat storage of 480 W, which is approximately 15 times the basal metabolism of the eastern grey kangaroo (Dawson et al., 2000). That is well within the metabolic scope of 19 predicted for a mammal the size of the western grey kangaroo (Bishop, 1999).

A seasonal change in morning T_c and mean 24 h T_c has also been seen in springbok, a small African antelope (Fuller et al., 2005). Those authors argued that a T_c that is lower in winter than in summer might relate to energy availability. Primary productivity in the Mediterranean climatic regions of the world, such as southwestern Australia, is limited by rainfall. Low rainfall in spring–summer, combined with elevated ambient temperature that increases transpiration rates, leads to water stress and a decrease in plant quantity and quality as animal feed (Turner, 2004). Dawson et al. have suggested that the lowering of morning T_c in kangaroos might be an energetic adaptation (Dawson et al., 2007). Seasonal changes in mean T_c occur in goats (Jessen and Kuhnen, 1996) and Arabian

oryx (Hetem et al., 2010); in contrast, previous studies have reported no change in mean T_c across seasons in sheep (Bligh et al., 1965) and mule deer (Sargeant et al., 1994). An explanation for these differences might be that energy balance was maintained in sheep and mule deer, but not in goats and Arabian oryx. Given that spring–summer coincides with the dry season in southwestern Australia, it is possible that the variation in T_c was correlated with season *via* the effect of season on food availability. But we cannot rule out the possibility, with these data, of an endogenous circannual pattern in T_c variability. The western subspecies of the western grey kangaroo (*Macropus fuliginosus ocydromus*) exhibits seasonal breeding with birth in late spring–early summer (Mayberry et al., 2010), thus avoiding lactation to a large joey in the summer. The eastern grey kangaroo and the red kangaroo breed aseasonally. Thus seasonal breeding seems to have been genetically programmed into western grey kangaroos relatively recently and it is possible that other attributes of energy expenditure, such as thermoregulation, may also have become seasonally entrained.

In hot conditions, water saving is the adaptive advantage generally advanced for adaptive heterothermy in mammals. The potential water savings resulting from an extra 2°C of temperature rise during the day (the difference between the spring morning T_c of 36°C and the summer morning T_c of 34°C; Fig. 5B) in a 25 kg kangaroo is approximately 70 ml day^{-1}, or approximately 4% of the daily water turnover, if turnover is similar to that of the eastern grey kangaroo in summer (Dawson et al., 2006). If prolonged water deprivation was a characteristic of the habitat of western grey kangaroos, then a 4% water savings per day could improve the survival of individuals. However, during the present study, water was freely available in the reserve, making it very unlikely that the kangaroos were dehydrated, and, apart from the transient episodes from which the T_c returned rapidly to normal, we saw no signs of hyperthermia. Thus the observed heterothermy did not result from dehydration-induced hyperthermia, but entirely from lower morning T_c. Interestingly, despite the kangaroo being nocturnal, the temperature rhythm was in phase with the T_a cycle.

In one of the few field studies that have presented evidence for the water-saving potential of heterothermy, Arabian sand gazelles exhibited an increased daily amplitude of T_c in summer compared with winter, with a calculated water saving of 34 ml day^{-1} (Ostrowski and Williams, 2006). But, contrary to the theoretical expectations, the gazelles stored heat at a slower rate during the hottest part of the day in summer than they did at the same time of day in winter, despite heat load being higher in summer [fig. 2 in Ostrowski and Williams (Ostrowski and Williams, 2006)]. The period of most rapid rise in T_c, which contributed the majority of the increase in daily T_c amplitude in summer, occurred between 17:00 and 19:00 h, a time when heat load was quite low compared with that during the hottest time of day, with solar radiation decreasing to zero just after 18:00 h. That the rate of heat storage was five times higher near sunset than in the heat of the day [our calculation from data presented in fig. 2 of Ostrowski and Williams (Ostrowski and Williams, 2006)] implies that it was not environmental heat load driving the T_c patterns in sand gazelle.

These considerations then beg the question, if the gradual reduction in morning T_c of kangaroos was not an adaptation for water saving, then of what benefit was it, or was it perhaps pathological, reflecting an inability of the organism to maintain homeothermy? Given that the lower T_c occurred on warmer days, we consider the latter unlikely. 'Allowing' T_c to decrease beyond the typical range for body temperature would be analogous to 'allowing' T_c to increase to a higher level during dehydration before evaporative defences are activated. The latter process saves water, but brings the organism closer to its thermal limit. Presumably such trade-offs have been selected for during evolution and the situation we see manifested in the present study represents the descendents of those individuals in which a given trade-off worked, whereas ancestors that inherited other trade-offs (including strict homeothermy) died of either dehydration or hyperthermia when faced with simultaneous heat and osmotic stress.

To describe such alterations in homeostatic variables in the face of competing demands across homeostatic systems, or in the face of predictable (e.g. seasonal) alterations in supply or demand on physiological systems, Mrosovsky coined the general term 'rheostasis' (Mrosovsky, 1990). The concept has recently gained some traction in the medical field under the name of 'allostasis', which describes altered homeostatic variables in the face of environmental stressors as an adaptive response to altered demands on the system, rather than as a pathology (McEwen, 2007). Rheostasis in the form of a trade-off in relaxed homeothermy in times of reduced energy availability would aid western grey kangaroos as the dry season progressed from spring into summer. That function would be congruent with morning T_c being determined by time of year, which also determines season. We do know that food deprivation leads to a lower rest-phase T_c in some birds and mammals (Kanizsai et al., 2009; Laurila and Hohtola, 2005; Laurila et al., 2005), and that the response is likely driven by proximate signals related to energy intake (Maloney et al., 1999; Munn et al., 2010). Offering sheep a diet containing only 70% of maintenance energy requirements, as opposed to 100% of maintenance energy requirements, also resulted in a significant decrease in the rest-phase T_c (S.K.M., L.C.R.M., A.F. and D. Blache, unpublished observations). It is possible that the observed decrease in morning T_c in kangaroos results from a decrease in energy intake in the dry season, leading to a reduced metabolic rate. We can estimate that if the Q_{10} for metabolism is 2.5 (Dawson and Hulbert, 1970) and a kangaroo spends a 24 h period at resting metabolic rate, the difference in energy expenditure between a kangaroo exhibiting the spring T_c pattern (estimated as the upper limit of the s.d. of T_c in Fig. 2C) and a kangaroo exhibiting the summer T_c pattern (estimated as the lower limit of the s.d. of T_c in Fig. 2C) is nearly 7%. Whether these decreases in T_c actually have energetic consequences for the kangaroos, and are therefore adaptive, remains to be elucidated.

Determining whether the phenomenon of heterothermy in general is adaptive requires knowing what benefit the animal receives. In terms of the classic interpretation of adaptive heterothermy (*sensu* Mitchell et al., 2002), water use is reduced when water supply is limited and the phenomenon could be considered adaptive if the resulting higher T_c was not more detrimental than the benefit of the water savings. The phenomenon could occur either as a direct response to water limitation, as in dehydration-induced hyperthermia, or as a more strategic response that may be linked to a seasonal rhythm. Similarly, if energy supply is reduced then the phenomenon (whether it be strategic or an immediate response to energy limitation) could be considered adaptive if the benefits outweigh the costs of hypothermia (such as increased predation). The assumption built in to any argument about adaptive heterothermy is that animals are more likely to survive and reproduce if they exhibit heterothermy than if they maintain homeothermy when the resources that support that maintenance are in limited supply. To our knowledge, that assumption has never been tested. It is apparent that heterothermy need not be associated with water saving but, like torpor in small mammals and birds, it may be an important aspect of adaptation in larger mammals associated with

energy use. The pattern that emerges from our results and others is that homeothermy is the default condition if large mammals (and probably birds) can afford the expense of energy and water use. Perturbation in energy or water balance leads to a trade-off that relaxes thermal regulation and results in heterothermy. As such, heterothermy in large mammals can result from either or both of dehydration-induced hyperthermia and energy restriction induced hypothermia.

In summary, we have characterised the nychthemeral pattern of T_c in a population of western grey kangaroos and shown that heterothermy in the summer arises as a result of a lower morning T_c. The pattern of T_c satisfied three of the four criteria for heterothermy (Mitchell et al., 2002) but did not seem to be associated with reduced water use. We propose that the heterothermy in kangaroos was likely a consequence of reduced energy availability in the dry season and was mediated directly by reduced energy intake, but we cannot exclude the possibility that the phenomenon is seasonally programmed into the western grey kangaroo. Supplemental feeding of wild kangaroos in the dry season while their T_c patterns are recorded would help to distinguish the cause. Whether the lower morning T_c is associated with lower metabolic requirements, and is therefore adaptive, remains to be established.

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