RESEARCH ARTICLE

Adaptation strategies to seasonal changes in environmental conditions of a domesticated horse breed, the Shetland pony (*Equus ferus caballus*)

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SUMMARY

Recent results suggest that the wild ancestor of the horse, the Przewalski horse, exhibits signs of a hypometabolism. However, there are speculations that domestic animals lost the ability to reduce energy expenditure during food shortage and adverse environmental conditions. Therefore, we investigated physiological and behavioural strategies employed by a robust domesticated horse breed, the Shetland pony, over the course of a year under temperate conditions by measuring ambient temperature (T_a), subcutaneous temperature (T_s), locomotor activity (LA), lying time, resting heart rate, body mass and body condition score. Ten animals were kept on pasture in summer and in open stables in winter; further, in winter the animals were allocated into one control and one feed-restricted group of five animals each to simulate natural seasonal food shortage. The annual course of the mean daily T_s of all horses showed distinct fluctuations from a mean of 35.6±0.5°C, with higher variations in summer than in winter. Diurnal amplitudes in T_s were highest ($P<0.001$) in April (12.6°C) and lowest in January (4.0°C), with a nadir around dawn and a peak around mid-day. The feed-restricted group had a significantly lower daily T_s compared with the control group on cold winter days, with T_a values below 0°C. Mean annual heart rate and LA followed T_a closely. Heart rate of the feed-restricted animals significantly decreased from a mean of 52.8±8.1 beats min^{-1} in summer to 29±3.9 beats min^{-1} in winter and differed from the control group ($P<0.001$). Mean daily LA was lowest at the end of winter (7000 activity impulses day^{-1}) and highest in summer (25,000 activity impulses day^{-1}). Our results show that Shetland ponies exhibit signs of a winter hypometabolism indicated by reduced heart rate and T_s. Thus, domesticated horses seem to have maintained the capacity for seasonal adaptation to environmental conditions by seasonal fluctuations in their metabolic rate.

Key words: Shetland pony, hypometabolism, body temperature, metabolic rate, feed restriction, locomotion.

INTRODUCTION

Endothermic animals, like the horse, usually keep their body temperature (T_b) within a narrow limit with changing environmental conditions (Schmidt-Nielsen, 1997; Singer, 2007). However, this comes at a high energetic cost. Endothermic animals can therefore face a twofold challenge. In harsh environmental conditions, the availability and quality of food is limited but the energy requirement to maintain T_b is high (Arnold et al., 2006). Besides a small degree of a daily reduction in metabolism during the 24 h cycle of rest and activity regularly observed in larger mammals (Langman and Maloiy, 1989; Taylor and Lyman, 1967), small animals may enter intense forms of hypometabolism, such as hibernation, prolonged torpor or daily torpor (Geiser, 1988; Heldmaier et al., 1989). These adaptive mechanisms, employed to save energy, include the reduction of the metabolic rate (MR), a decrease of T_b, and a reduced heart and breathing rate (Heldmaier et al., 2004; Singer, 2007). Large animals, with the exception of bears, normally lack the ability to enter torpor (Arnold et al., 2006), which is generally restricted to animals weighing less than 1000 g (Geiser and Ruf, 1995). Nevertheless, there are indications that some ungulates can exhibit some form of an energy-saving mechanism when ambient temperatures (T_a) are low and little food is available. This phenomenon has been shown for large ungulates such as roe deer (Weiner, 1977), red deer (Arnold et al., 2004) and recently also for the wild ancestor of the horse, the Przewalski horse (*Equus ferus przewalski*) (Arnold et al., 2006).

There are speculations that livestock species lost the ability to reduce their energy expenditure under food shortages and adverse environmental conditions, because they were housed and selected under constant nutrient supply without selection pressure for maintaining a seasonal, cyclic adaptation (Price, 1984). However, empirical confirmations of a supposedly lower adaptability of livestock species compared with their wild counterparts are still missing. Therefore, we investigated whether the physiological capabilities of seasonal adjustments are still present in a livestock species when kept under long-term semi-natural outdoor conditions. We chose Shetland ponies (*Equus ferus caballus* Linnaeus 1758), a robust horse breed that, according to the domestication model of Lauvergne (Lauvergne, 1994), can be characterized as a primary population. We studied whether there exist seasonal fluctuations in crucial physiological and behavioural parameters such as locomotor activity (LA), resting time, subcutaneous temperature (T_s) and heart rate similar to those described for wild horses (Kuntz et al., 2006; Arnold et al., 2006). Furthermore, it is unclear whether a suggested form of hypometabolism in wild horses (Arnold et al., 2006) as an overwintering strategy is still present in domestic horses. Therefore, we simulated the food shortage found in natural habitats in winter by reducing the food supply to 70% of the required energy demand for one group in the winter months while the other group had permanent access to food. The results of our study will provide more in-depth knowledge on the adaptive physiological capabilities of a domesticated species. Hence, the results of our study

will also contribute to detection of possible modifications in the adaption ability of domesticated horses. Therefore, we tested the hypothesis that Shetland ponies, a robust horse breed that can be characterised as a primary population after the first steps of domestication, still exhibit signs of a hypometabolism similar to those described for its wild ancestor, the Przewalski horse.

MATERIALS AND METHODS
Animals and management
The study was undertaken at the Department of Animal Sciences at the University of Göttingen, Germany, and lasted for 1 year from February 2010 to February 2011. Initially, we started with eight Shetland pony mares; however, the herd was increased to 10 mares from the beginning of May 2010. The age of the animals ranged between 4 and 16 years. Animals were raised on different farms in Germany and The Netherlands and were accustomed to outdoor housing systems.

During our study, animals were kept either on paddock or on pasture. From February 2010 until the end of May 2010, ponies were housed in two groups of five ponies each in two identical paddocks. Each paddock measured $210\,m^2$ and had permanent access to a pen measuring $6.40\times2.95\,m$ that was covered with straw. Two large exits allowed the animals to enter and leave without rank conflicts. Both pens were equipped with five feeding stands each ($1.35\times1.60\times0.55\,m$, height$\times$length$\times$width) to ensure that every horse was able to ingest the required food. The stable was not heated and hence T_a values inside and outside the stable were comparable. The light–dark cycle inside the stable fluctuated according to the natural photoperiod. From February 2010 until the end of May 2010, all ponies received the same feeding treatment with straw *ad libitum* and 5 kg hay per 100 kg body mass per day. Additionally, ponies received 22 g mineral mixture per 100 kg body mass per day (Derby® Mineralpellets, Derby Spezialfutter GmbH, Münster, Germany) and 580 g concentrate per 100 kg body mass per day (Derby® Standard, Derby Spezialfutter GmbH). Water was available *ad libitum* throughout the experiment at a frost-proof watering place. From the end of May 2010 until the middle of October 2010, all animals were kept on permanent pastures with access to two $4.0\times3.6\,m$ shelters (plastic tents). In addition to grass, ponies were offered a small amount of hay, straw and mineral supplement. From the middle of October 2010 until the end of our study (February 2011), animals were kept on the same paddocks described above. During that time, ponies were allocated according to their body mass and body condition score (BCS) into a control group (CG) and a treatment group (TG) of five animals each, resulting in a comparable mean BCS for both groups. In the first 2 weeks, both groups were fed identically as described above. However, although from the beginning of November 2010 the control group was fed as before, the treatment group was fed restrictively to simulate the limited availability of food in autumn and winter under natural conditions. The amount of food offered in the TG animals was gradually reduced from 100 to 80% of the recommended energy and protein requirements for Shetland ponies (Gesellschaft für Ernährungsphysiologie, 1994) until 21 January and to a further 70% until 28 February.

The research conducted in this study was performed in accordance with the current laws regulating animal welfare and experiments with animals in Germany.

Body mass, body condition score, ambient temperature and hair length
Body mass for all animals was recorded on a bi-weekly basis throughout the course of the study (February 2010–February 2011) with a mobile scale (Weighing System MP 800, resolution: 0.1 kg, Patura KG, Laudenbach, Germany). The BCS, a palpable and visual assessment of the degree of fatness in the neck, back, ribs and pelvis, was taken monthly after Carroll and Huntington (Carroll and Huntington, 1988). T_a and relative humidity (RH) were recorded continuously throughout the study with miniature data loggers at 10 min intervals (Tiny view TV 1500, temperature resolution: 0.25°C, humidity resolution: 0.5%, Gemini, Chichester, West Sussex, UK). Hair length for each pony was measured once in summer (July 2010) and once in winter (February 2011) with a cardboard template ($7\times10.5\,cm$) that was inserted into the hair coat of the flank, determining the length of guard and undercoat hairs.

Locomotor activity
The LA for each pony was measured continuously for the entire study period using activity–lying time–temperature pedometers (ALT Pedometer, Engineering Office Holz, Falkenhagen, Germany) tied to the foreleg above the pastern. Pedometers were lined with a silicon pad to avoid pressure marks on the foreleg. The pedometer (125 g mass; $6\times5\times2\,cm$, length$\times$width$\times$height) consisted of four acceleration sensors, allowing the measurement of both locomotion and lying time. The LA was recorded as activity impulses generated by the front leg with a maximum resolution of two impulses per second. Furthermore, sensors detected the position (lateral or ventral) of the pedometer every 15 s, thus allowing us to determine the total time spent lying. The recorded data were added up to 15 min intervals and saved to an on-board storage device.

Subcutaneous temperature
For the continuous recording of T_s, a high-resolution, real-time synchronised miniature temperature data logger (i-Button DS1922L-F5, resolution: 0.5°C, Maxim Integrated Products, Sunnyvale, CA, USA) was implanted subcutaneously on the right side of each animal's neck. The implants (3.3 g mass; $1.74\times1.74\times0.6\,cm$) were coated with a medical silicon layer to waterproof them and prevent any inflammatory response. Prior to the implantation of the loggers, a $10\times10\,cm$ area at the right side of the animal's neck was shaved, washed and soaked with iodine solution. A local anaesthetic (2% xylocain with adrenalin, Rompun, Bayer Animal Health, Leverkusen, Germany) was administered subcutaneously 10 min before the surgery. Subsequently, a 4 cm vertical incision was performed resulting in a subcutaneous pocket ($4\times4\,cm$) in which the logger was positioned. The incision was then closed using a skin stapler (Weck Visistat 35 W, Teleflex Medical Europe Ltd., Athlone, Ireland). All animals received a broad-spectrum antibiotic (Procain-Penicillin G, Pfizer AG, New York, NY, USA) for the next 6 days. The staples were removed after 12 days. The removal of the implants after 12 months was performed as described above.

Each logger was programmed to measure and record T_s every 2 h for the 12 month period, resulting in 4488 records for each animal. Clock time of the loggers was synchronised across ponies.

Resting heart rate and rectal temperature
The heart rate was recorded bi-weekly with a stethoscope between 12:00 and 13:00 h, during which heart rate was determined three times for 60 s. Before the measurements, animals were at rest for at least 5 min and were loosely tied with a rope but had visual contact with their conspecifics. The ponies were used to the handling and thus any impact of the measuring procedure on the heart rate recordings was assumed to be minimal. Rectal temperature (T_r) was recorded bi-weekly for all experimental animals during the feeding trial from November 2010 to February 2011 using a clinical

thermometer (resolution: 0.1°C, AEG, Nürnberg, Germany) with the probe inserted approximately 5 cm into the rectum.

Statistical analysis

All raw data recorded with the pedometers were checked for obviously false records. In a few instances, pedometers became detached from the animals' legs for a maximum of 2 days and these records were discarded from statistical analyses. Two-hourly, daily and monthly averages for T_s, LA and lying duration were calculated for each animal. Data for heart rate, LA, lying duration, T_s and T_r were then subjected to an ANOVA. We used mixed modelling (PROC MIXED) to control for repeated measurements from the same individuals. To model correlation within the two feeding groups across time and between the two feeding groups, we first determined the appropriate covariance structure for the data set based on Akaike's information criterion adjusted for small sizes (AIC_c) values. Differences between the two feeding groups were analysed using data recorded from mid-November 2010 until the end of February 2011 only. Furthermore, individual averages for T_s, T_r, LA, lying duration, heart rate, BCS and body mass were calculated for three periods (i.e. first winter: February–May 2010; summer: May–Oct 2010; second winter: October 2010–February 2011) and averages for hair length were calculated across two measurement months, and analysed for differences using the Mann–Whitney U-test as an integrated *post hoc* test in the MIXED procedure. Data are expressed as means ± s.d., where N is the number of individuals and n is the number of measurements. Statistical analyses were performed with the program package SAS version 9.2 (Statistical Analysis System, 2008).

RESULTS
Climate data, body mass and body condition score

The climatic conditions during the 12 months of the experiment were within the long-term climate recordings of the area. During the time of our study we experienced one hot summer month (July 2010; mean T_a=20.3°C, maximum T_a=34.9°C, minimum T_a=6.4°C, mean RH=70.7%, maximum RH=100%, minimum RH=30%) and low T_a values in both winter periods (Fig. 1). Rainfall occurred on 129 of the 372 study days, with an annual rainfall of 632.8 mm. The second winter period included 44 days with snow heights over 1 cm and 84 days with ground frost.

Body mass and BCS of the ponies were relatively constant during the first winter period but increased during the summer period with peak values in September (body mass 158±38 kg; BSC 4.3±0.6) and October (body mass 160±38 kg; BSC 4.6±0.4). During the second winter period, the TG animals lost on average 20% of their initial body mass, with a significant drop from 162.4±41 kg in October 2010 to 132±36 kg in February 2011 [body mass (kg)=165.7–4.18×time (month), R^2=0.12, $P<0.05$]. A similar development was observed for the BCS, which decreased significantly from 4.6±0.4 points in October 2010 to 2.4±0.8 points in February 2011 (BCS=5.4–0.73×time (month), R^2=0.65, $P<0.001$) whereas mean body mass and BCS of the CG group did not change during this time [body mass (kg)=149.8+0.22×time (month), R^2=0, P=0.93; BCS=3.4+0.09×time (month), R^2=0.03, P=0.43]. However, the two different feeding groups did not differ significantly in body mass and BCS because of large individual variations.

Hair length differed significantly ($P<0.001$) between summer (guard hair, 1.0±0.3 cm; undercoat hair, 1.0±0.3) and winter (guard hair, 6.3±0.8 cm; undercoat hair, 4.1±0.4 cm) for all animals. Although no differences ($P>0.05$) were found in the undercoat hair length between the two feeding groups, guard hair length was
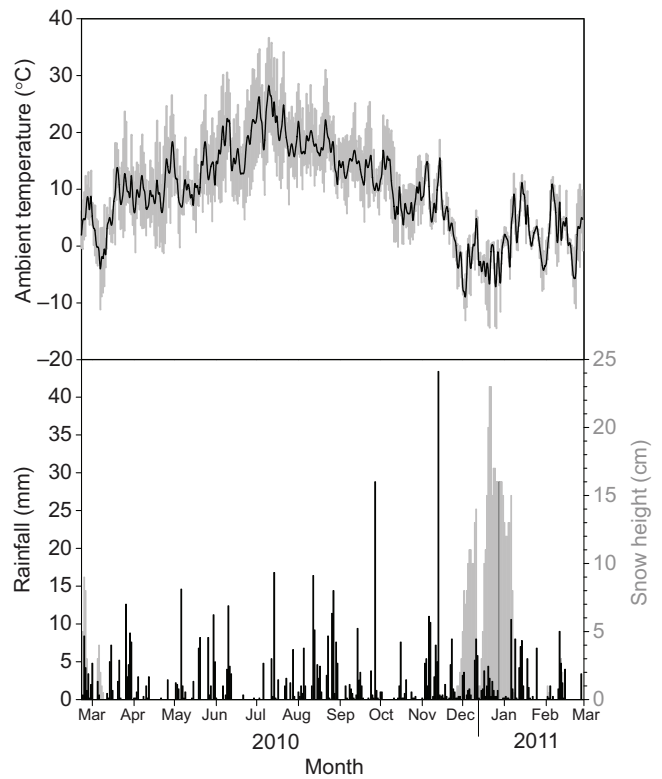

Fig. 1. Annual variation in ambient temperature (T_a) and precipitation at the Institute of Animal Sciences (University of Göttingen) over the course of the study. (A) Solid line, daily mean T_a; grey shading, daily maximum and minimum T_a. (B) Black columns, daily rainfall (mm); grey columns, snow height (cm).

significantly ($P<0.05$) longer in the TG ponies compared with the CG ponies at the end of the study (February 2011: 6.7±0.6 cm *vs* 5.9±0.7 cm, respectively).

Heart rate

Mean heart rate of all animals varied considerably throughout the seasons (Fig. 2) and the annual pattern followed the trend of both T_a (Fig. 1) and LA (Fig. 3B), with low values in the winter months and high values in the summer months, peaking in September (63.2±7.6 beats min^{-1}). Correlations of the annual means between heart rate and LA (r=0.77, $P<0.001$) as well as between heart rate and T_a (r=0.70, $P<0.001$) were high and significant. The heart rate of the TG animals significantly decreased from their summer (May–October) mean of 52.8±8.1 beats min^{-1} to 29±3.9 beats min^{-1} in the second winter period (October–February). Furthermore, mean heart rate differed significantly ($P<0.001$) between TG and CG animals in the second winter period (October–February, 29±3.9 beats min^{-1} *vs* 40.3±5.7 beats min^{-1}; Fig. 2). The highest difference in heart rate between TG and CG animals was observed at the end of our study in February 2011 (26.0±3.2 beats min^{-1} *vs* 45.6±3.8 beats min^{-1}, respectively; $P<0.001$), when animals were fed restrictively for 4 months.

Subcutaneous and rectal temperature

The mean T_s over the entire study period was 35.6±0.5°C. The annual course of the mean daily T_s of all ponies showed distinct fluctuations. During spring and summer, T_s fluctuated considerably, as evidenced by high variations in mean minimum ($T_{s,min}$) and mean maximum T_s ($T_{s,max}$) (Fig. 3A). However, these high variations
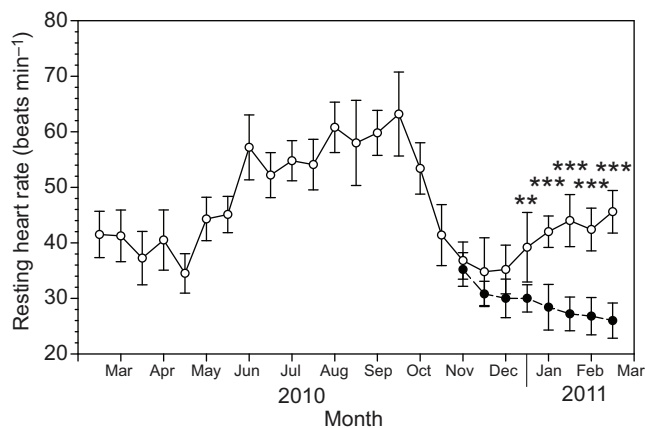
Fig. 2. Seasonal changes in resting heart rate (means ± s.d.) of eight (February–May 2010) and 10 Shetland pony mares (June–October 2010). From November 2010, animals were divided into a feed-restricted group (N=5, filled circles) and a control group (N=5, open circles; see Materials and methods for details). Significant differences between feeding groups are indicated by asterisks (**P<0.01; ***P<0.001).

decreased significantly (P<0.001) during autumn and winter. Daily $T_{s,min}$ was lowest in April (28.2°C) and highest in October (35.7°C). In contrast, daily $T_{s,max}$ was lowest in December (35.9°C) and highest in June (38.2°C). Daily fluctuations in T_s were significantly higher in summer than in winter (P<0.001; Fig. 4). Furthermore, T_s was significantly lower in TG animals on 52 days of the 120 days of feed restriction compared with the CG animals, especially during cold days when T_a values were constantly below 0°C (P<0.05; Fig. 5). During the entire study period, the lowest daily mean T_s and $T_{s\,min}$ typically appeared in the early morning hours around dawn, usually coinciding with the lowest daily T_a (Fig. 5). In the early daylight hours, T_s increased and stayed either fairly constant during cool or cold days or increased to a peak at around mid-day during warm or hot days (Figs 4, 5)

The overall correlation between mean daily T_s and mean daily T_a was positive and moderate (r=0.38, P<0.001), whereas the correlation between $T_{s,max}$ and T_a was higher (r=0.64, P<0.001). In contrast, the correlation between $T_{s,min}$ and T_a was negative (r=–0.20, P<0.001). The relationship between T_s and T_a in winter months (October–February) was best described by a linear regression, T_s=35.41+0.057T_a (R^2=0.50, P<0.001), whereas in summer months (May–October) the relationship between T_a and T_s only loosely followed a power function, T_s=31.56$T_a^{0.05}$ (R^2=0.25, P<0.01)

During the feeding trial, the T_r in December, January and February was significantly (P<0.05) lower in TG than in CG animals (TG: 36.8±0.3, 36.7±0.4 and 36.4±0.3°C; CG: 37.5±0.3, 37.5±0.3 and 37.5±0.4°C for December, January and February, respectively).

Locomotor activity and resting behaviour
The mean daily LA showed considerable variations throughout the course of the year (Fig. 3B), with the lowest values occurring during the winter months (7000 activity impulses day^{-1} in March) and the highest during the summer months (25,000 activity impulses day^{-1} in August). The mean LA values in summer (May–October) were significantly higher than those in winter (October–February, P<0.001). However, no significant differences could be detected between both feeding groups (P>0.05). LA followed a pattern similar to that of T_a (Fig. 3A,B), with a relatively high correlation between both parameters (r=0.64, P<0.001). During the diurnal LA rhythm,
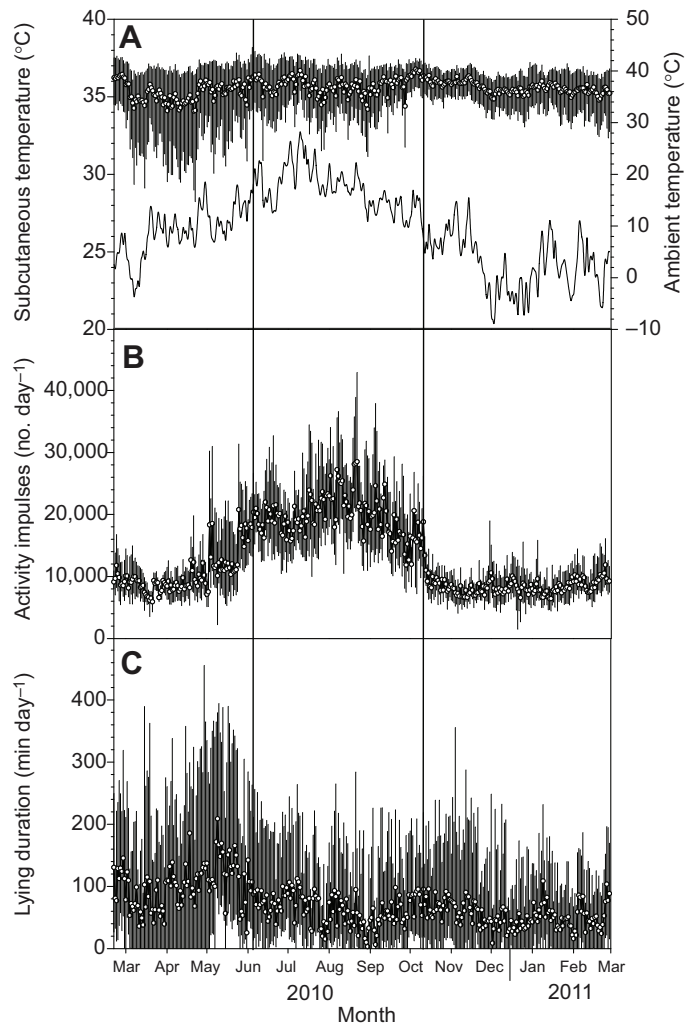

Fig. 3. Mean seasonal variation in (A) subcutaneous temperature (B) locomotor activity and (C) resting behaviour of Shetland pony mares (daily means with daily mean minimum and maximum). The solid line in A indicates the corresponding ambient temperature and the vertical lines spanning A–C indicate the changes from paddock housing (March–May 2010) to pasture (May–October 2010) and back to paddock housing (October 2010–February 2011).

the highest numbers of activity impulses per hour were recorded in the morning around dawn, with a second peak frequently occurring around dusk. This pattern was observed in both summer and winter periods. In summer, the diurnal variation followed an approximately sinusoidal shape whereas the activity peaks were less distinct in winter (Fig. 4).

Daily lying times were generally low throughout the entire study period (70.1±67.0 min day^{-1}, 4.9±4.6% of the day lying; Fig. 3C). Resting usually occurred at night, with the highest lying frequency observed before dawn. The highest mean daily lying durations were recorded in May (8.3±6.4%) and the lowest in December (2.72±3.1%). No significant differences (P>0.05) were found between the two feeding groups.

DISCUSSION
In our study we showed for the first time that domesticated horses decrease their T_s and heart rate, similar to that observed in their
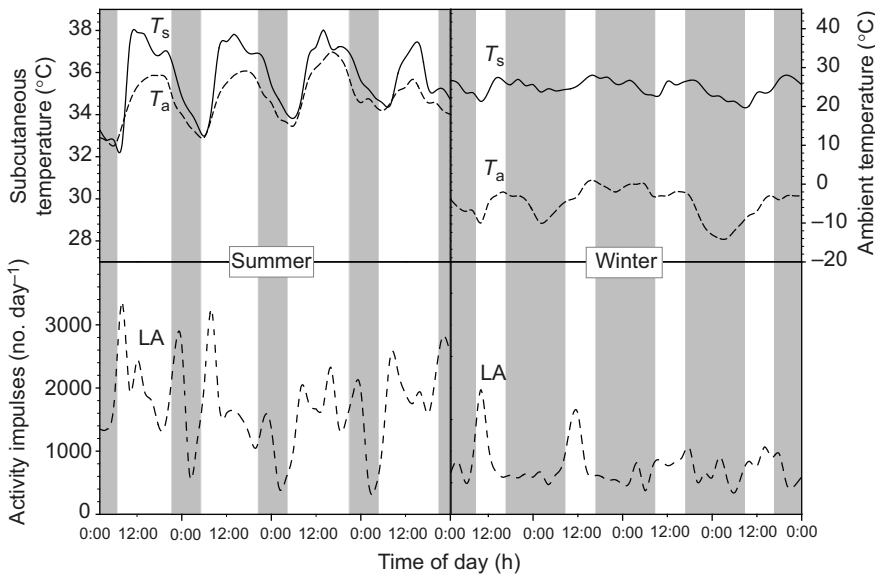
Fig. 4. Examples of the diurnal rhythm of the mean subcutaneous temperature (T_s, solid line), ambient temperature (T_a, dashed line) and locomotor activity (LA, dot-dashed line) in Shetland ponies on four consecutive days in summer (July 2010) and winter (December 2010, across feeding groups). Grey shaded areas indicate scotophase.

wild ancestor the Pzewalski horse, when exposed to long-term semi-natural conditions including a harsh winter. These changes in T_s and heart rate can be interpreted as signs of a hypometabolism.

Seasonal changes in behaviour

Rhythms of physiological functions and their related behaviours are essential components of the relationship between the animals and their environment (Scheibe et al., 1999). Homeothermic animals attempt to keep their endogenous milieu on a stable level and exogenous disturbances can lead to changing behaviour as one strategy of the organism to overcome the interruption (McFarland, 1985). The LA can act as a final correcting element in a long chain of endogenous processes (Aschoff, 1962), and its circadian rhythm may give information about the adaptation ability of the animal in question (Scheibe et al., 1999). In our study, the LA of Shetland ponies under extensive housing (i.e. on pasture) was highly dependent on T_a. Although we could not distinguish between activity due to grazing or foraging and other LAs, according to previous studies in horses (Duncan, 1985; Boyd and Bandi, 2002; Lamoot and Hoffmann, 2004) it is reasonable to assume that most of the LA recorded can be attributed to feeding behaviour. Even though horses were kept on paddock with access to an outdoor stable during winter in our study, their annual LA pattern agrees well with observations described for wild herbivores such as the Przewalski horse (Berger et al., 1999; Arnold et al., 2006; Berger et al., 2006)

and roe deer (Berger et al., 2002). The diurnal LA rhythm in our study was related to the photoperiod and was in its pattern similar throughout much of the year, with higher activities during daylight than during the night (Fig. 4), which is in agreement with results for Przewalski horses (Boyd et al., 1988; Berger et al., 1999) and cattle and sheep (Arnold, 1984). However, in winter, the LA during daylight differed less from that at night compared with during summer. Similarly, Berger (Berger et al., 1999) and Duncan (Duncan, 1985) noted that horses were mostly inactive on cold winter days and restricted their activity to feeding as a mechanism of energy conservation (Malechek and Smith, 1976; Berger et al., 2006). We therefore assume that our horses saved energy in winter by reducing their LA.

The overall mean daily lying time of 70 min per day was rather low compared with other studies that reported total time spent recumbent per day at 100–150 min (Littlejohn and Munro, 1972; Duncan, 1980). An explanation for the low lying times found in our study could be low soil temperatures and high soil humidity, as horses avoid lying down on wet underground, whereas in the summer, lying at high temperatures may have been avoided because this posture appears to be less effective for thermoregulation compared with standing. Resting in a lying position occurred mainly at night, especially before dawn. A similar diurnal pattern was described for lying in free-ranging Przewalski horses (Boyd, 1988; Berger et al., 1999), Haflinger horses (Lamoot and Hoffmann, 2004)
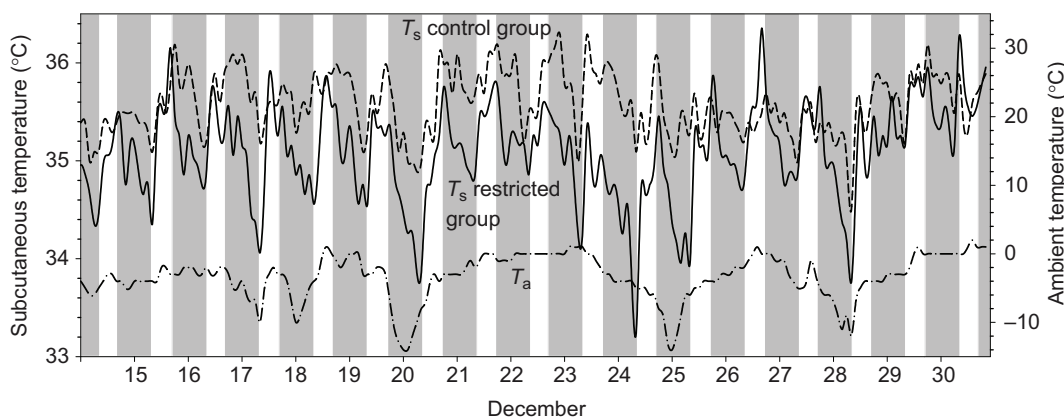

Fig. 5. Example of the diurnal rhythm of ambient temperature (T_a, dot-dashed line) and the mean subcutaneous temperature (T_s) in winter of restrictively fed ($N=5$, solid line) and control fed ($N=5$, dashed line) Shetland ponies (see Materials and methods for details). Grey shaded areas indicate scotophase.

and sheep and cattle (Arnold, 1984). During the feeding trial, the TG ponies tended to have lower lying times compared with the CG ponies, but this difference was only significant for three single days. We did not measure resting in a standing position, but this behaviour may have increased in winter, as observed by Duncan (Duncan, 1985) in Carmague horses. Resting in standing position and not in recumbency is the position of minimal energy expenditure (Winchester, 1943); in addition, this posture protects the animal against body cooling through conduction and therefore possibly contributed to energy savings in the TG ponies.

Seasonal changes in physiological parameters

The skin and the subjacent tissues belong to the body shell, and thus T_s usually fluctuates more than T_b (Scheunert and Trautmann, 1987; Schmidt-Nielsen, 1997). With decreasing T_a, the difference between T_s and T_b increases. In goats, the gradient was found to be 1°C at a T_a of 20°C and approximately 3°C at a T_a of 0°C (Al-Tamimi, 2006; Al-Tamimi, 2007). Körtner and Geiser (Körtner and Geiser, 2000) and Körtner et al. (Körtner et al., 2001) reported a linear relationship between T_b and skin temperature in sugar gliders and tawny frogmouths, respectively. Skin temperature has also been frequently used as a suitable indicator for T_b in several other species (Taffe, 2011; Brigham, 1992; Audet and Thomas, 1996; Barclay et al., 1996; Körtner and Geiser, 2000). Even though skin temperature is biophysically distinct from T_s, changes in T_s will likely reflect changes in T_b.

The daily mean T_s fluctuation in the present study, with T_s decreasing during the night and rapidly rising after sunset, is consistent with a daily shallow hypometabolism (Fig. 4). This phenomenon can be observed during the 24 hourly rhythms of activity and rest in many species (Heldmaier et al., 2004) and leads to a MR reduction of up to 20% compared with activity phases. In our study, these daily T_s variations occurred over the entire year in close relation to the daily photoperiod (Scheunert and Trautmann, 1987), similar to what other studies have found in horses and red deer (Piccione et al., 2002; Arnold et al., 2004). The diurnal T_b rhythm is an endogenous rhythm that persists even when exogenous factors have been removed (Scheunert and Trautmann, 1987; Piccione et al., 2002). This explains why T_s also decreased at night on days when T_a did not (Fig. 5). Furthermore, the increase of T_s in the morning tended to correlate with an increase in LA, especially in summer (Fig. 4), suggesting that the animals used their LA to increase their T_b in the morning. The lower T_s variations in winter and spring (Fig. 3A, Fig. 4) may indicate that our animals shifted from a short daily hypometabolism to a more intense hypometabolism to save energy. This assumption is supported by our results for heart rates (Fig. 2), which decreased significantly during winter and spring compared with summer and thus indicate a reduced energy expenditure. However, even though daily hypometabolism would account for the observed rhythmic diurnal changes in T_s, it does not explain the large daily T_s variations observed in spring and especially in summer, or the seasonal changes of mean daily T_s. A possible explanation is that in summer, horses may have less need to keep their T_b constant during the day because there is plenty of food and T_a values are moderate to high. However, there are some theoretical studies that predict the exact opposite (e.g. Angilletta et al., 2010). Nevertheless, the reduction of T_b at night may improve the ponies' capacity to store heat during hot days and thus reduce the energetic cost for thermoregulation (Fuller et al., 2005; Arnold et al., 2006). This type of adaptive heterothermy has been reported in other ungulates such as eland

(Taylor and Lyman, 1967), oryx (Taylor, 1969), giraffe (Langman and Maloiy, 1989) and Thomson's as well as Grant's gazelles (Taylor, 1970).

In our study, the variation in T_s abruptly decreased in the winter months (Fig. 3A, Fig. 4). Because T_s can be lowered by peripheral vasoconstriction to protect the body core against heat loss at times of low T_a (Schmidt-Nielsen, 1997; Arnold et al., 2006), the lowest T_s values are expected to coincide with the lowest T_a. However, even though T_s and T_a were found to be correlated in the winter months, the lowest mean T_s and $T_{s,min}$ were recorded in March and April long after the winter T_a nadir in December. Moreover, $T_{s,min}$ was negatively correlated with T_a, indicating that variations of T_s were therefore not induced by variations of T_a but by active thermoregulation. Thus the decrease in the variation of T_s can be explained to some extent by an adaptation to reduce energy. Similar mechanisms were described recently in kangaroos (Maloney et al., 2011).

Measuring T_r is a reliable method to measure T_b of animals (Scheunert and Trautmann, 1987; Green et al., 2005). The normal range of T_b in horses is 37.5 to 38.5°C (Scheunert and Trautmann, 1987), and a deviation from the normothermia of more than 1°C is considered to lead to discomfort, and an increase of 5°C or a decrease of 10°C will be fatal (Langlois, 1994). The mean T_r of 36.4°C in the TG ponies measured at the end of the second winter period was more than 1°C below the lower T_b limit and significantly lower than the T_b of the CG mares. Furthermore, T_s of TG animals was lower compared with that of CG animals on most of the days during the feeding trial, even though T_a values were the same for both groups. These T_s differences between both groups were especially pronounced during cold winter days (i.e. $T_a < 0$°C), and on some days exceeded 2.5°C (Fig. 5), indicating that TG animals allowed their T_b to drop further than CG animals, thus saving more energy. Alternatively, but less likely, the differences in T_s could be also due to energy limitations. However, lowering the T_b during adverse environmental conditions must involve some trade-off that is less energy demanding, including the cost for re-warming, than keeping the T_b constant (Angilletta et al., 2010; Boyles et al., 2011).

Heart rate is closely related to oxygen consumption (Butler et al., 2004) and is therefore a reliable indicator of MR (Renecker and Hudson, 1985; Bevan et al., 1995; Woakes et al., 1995; Brosh et al., 1998; McCarron et al., 2001). The high correlation of heart rate with LA can be explained as an automatic increase of heart rate with increasing LA to ensure the oxygen supply of the muscle metabolism. However, mean heart rate started to decrease over 1 month after the LA decline (Fig. 2, Fig. 3B) and paralleled reductions in variation in body mass, T_s and $T_{s,min}$. Considering these findings, we suggest that reduced food availability and subsequent loss in body mass initiated energy-saving mechanisms such as a reduction in MR, indicated by a reduced heart rate.

Seasonal changes in metabolic rate

The measured behavioural and physiological parameters show clear seasonal fluctuations, supporting the view of an underlying endogenous seasonal cycle of MR in Shetland ponies. Similar seasonal patterns of T_s and $T_{s,min}$ were observed by Arnold et al. (Arnold et al., 2004; Arnold et al., 2006) in Przewalski horses and red deer, where heart rate decreased with T_s. Because heart rate is an indicator of MR (Butler et al., 2004), the authors interpreted these reductions as a nocturnal hypometabolism and defined it as an intensified daily hypometabolism occurring when little food is available and the body energy reserves become depleted in late

winter. In our study, reduced T_s variations coincided with a rapid drop in heart rate, both possibly indicating a reduction in MR. Reduced heart rate may also be caused by reduced LA, which simultaneously leads to reduced heat production and thus lower T_s. However, the LA had no significant correlation with T_s and the start of the decrease in heart rate did not coincide with that of LA. Therefore, the reduction in T_s variations in winter might reflect the protection of the body against cooling because re-warming by locomotion is highly energy demanding during periods of low T_a and low food supply, and thus would deplete the energy reserves more quickly.

The restrictive feeding during the second winter period resulted in a reduced heart rate in TG animals, suggesting a reduction in MR compared with CG animals. The measured heart rate of $26\,\mathrm{beats\,min^{-1}}$ at the end of the trial (February 2011) was below the standard value for horses (30–$40\,\mathrm{beats\,min^{-1}}$). Furthermore, the standard heart rate value in ponies can be expected to be higher than that of horses, as smaller animals generally have higher heart rate frequencies (Stahl, 1967).

Influence of domestication

In our study, domesticated Shetland ponies showed seasonal adjustment mechanisms similar to those described for their wild counterparts, the Przewalski horse (Arnold et al., 2006; Kuntz et al., 2006), including diurnal hypometabolism and, presumably, winter hypometabolism. These results indicate that this horse breed has maintained the capacity for seasonal adaptation mechanisms during domestication. However, the feeding trial in the second winter also revealed a striking difference with available reports on northern wild herbivores (Kuntz et al., 2006). Contrary to the observation in Przewalski horses (Arnold et al., 2006), the CG ponies in the present study did not reduce their feed intake. Instead, animals concomitantly increased their body mass, T_r and heart rate. A possible explanation is that the heat increment of feeding masked an underlying seasonal cycle of MR (Worden and Pekins, 1995). The results of our study clearly contradict speculations that some livestock species have lost their ability to reduce their energy expenditure under circumstances of food shortage (Price, 1984), as they were housed and selected under constant nutrient supply without selection pressure for maintaining a seasonal, cyclic adaptation. In contrast, the seasonal fluctuations of the measured parameters are much in line with previous findings in wild horses or ungulates (Arnold et al., 2004; Arnold et al., 2006). However, Shetland ponies show metabolic features that indicate higher metabolic efficiency (thrifty genotypes) (Kienzle et al., 2010) that can lead to adiposity (Geor, 2008) and physical inactivity (Prentice et al., 2005) under high feeding intensity. In addition, they develop a very thick hair coat in winter and therefore resemble wild horses more than purebred horses selected for high locomotor efficiency. Shetland ponies can be characterized as a primary population after the first steps of domestication according to the domestication model of Lauvergne (Lauvergne, 1994). We suggest that during domestication, several stepwise changes might have occurred. During quite an early stage of domestication, human selection pressure might have acted against seasonal cycles of endogenous control in appetite, thus allowing domestic animals to develop body reserves even during winter. However, at least in a robust breed like the Shetland pony, the capability to reduce the MR during winter was not lost during domestication. We therefore conclude that domestication had little effect on the Shetland ponies' adjustment mechanisms in relation to environmental conditions, as our data show clear signs of a hypometabolism.

REFERENCES

Al-Tamimi, H. J. (2006). Responses of core and peripheral temperatures to chronic cold stress in transiently goitrous goats. *J. Therm. Biol.* **31**, 626-633.

Al-Tamimi, H. J. (2007). Responses of simultaneously recorded intraperitoneal and subcutaneous temperatures of black Bedouin goats to transient thyrosuppression during cold stress. *Livest. Sci.* **106**, 254-260.

Angilletta, M. J., Cooper, B. S., Schuler, M. S. and Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci.* **E2**, 861-881.

Arnold, G. W. (1984). Comparison of the time budgets and circadian patterns of maintenance activities in sheep, cattle and horses grouped together. *Appl. Anim. Behav. Sci.* **13**, 19-30.

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

Arnold, W., Ruf, T. and Kuntz, R. (2006). Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) II. Energy expenditure. *J. Exp. Biol.* **209**, 4566-4573.

Aschoff, J. (1962). *Spontane Lokomotorische Aktivität.* Berlin: Walter de Gruyter & Co.

Audet, D. and Thomas, D. W. (1996). Evaluation of the accuracy of body temperature measurement using external radio transmitters. *Can. J. Zool.* **74**, 1778-1781.

Barclay, R. M. R., Kalcounis, M. C., Crampton, L. H., Stefan, C., Vonhof, M. J., Wilkinson, L. and Brigham, R. M. (1996). Can external radiotransmitters be used to assess body temperature and torpor in bats? *J. Mammal.* **77**, 1102-1106.

Berger, A., Scheibe, K. M., Eichhorn, K., Scheibe A., and Streich, J. (1999). Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. *Appl. Anim. Behav. Sci.* **64**, 1-17.

Berger, A., Scheibe, K. M., Brelurut, A., Schober, E. and Streich, W. (2002). Seasonal variation of diurnal and ultradian rhythms in red deer. *Biol. Rhythm Res.* **33**, 237-253.

Berger, A., Scheibe, K. M., Wollenweber, K., Patan, B., Schnitker, P., Herrman C. and Budras, K. D. (2006). Jahresrhythmik von Aktivität, Nahrungsaufnahme, Lebendmasse und Hufentwicklung bei Wild- und Hauspferden in naturnahen Lebensbedingungen. In *Aktuelle Arbeiten zur artgemäßenTierhaltung 2006* (ed. K. Einschütz), pp. 137-146. Darmstadt: KTBL.

Bevan, R. M., Speakman, J. R. and Butler, P. J. (1995). Daily energy-expenditure of tufted ducks – a comparison between indirect calorimetry, doubly labeled water and heart rate. *Funct. Ecol.* **9**, 40-47.

Boyd, L. E. (1988). Time budgets of adult Przewalski horses – effects of sex, reproductive status and enclosure. *Appl. Anim. Behav. Sci.* **21**, 19-39.

Boyd, L. E. and Bandi, N. (2002). Reintroduction of takhi, *Equuas ferus przewalskii*, to Hustai National Park, Mongolia: time budget and synchrony of activity pre- and post-release. *Appl. Anim. Behav. Sci.* **78**, 87-102.

Boyd, L. E., Carbonaro, D. A. and Houpt, K. A. (1988). The 24-hour time budget of Przewalski horses. *Appl. Anim. Behav. Sci.* **21**, 5-17.

Boyles, J. G., Seebacher, F., Smit, B. and McKechnie, A. E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* **51**, 676-690.

Brigham, R. M. (1992). Daily torpor in a free-ranging goatsucker, the common poorwill (*Phalaenoptilus nuttallii*). *Physiol. Zool.* **65**, 457-472.

Brosh, A., Aharoni, Y., Degen, A. A., Wright, D. and Young, B. (1998). Estimation of energy expenditure from heart rate measurements in cattle maintained under different conditions. *J. Anim. Sci.* **76**, 3054-3064.

Butler, P. J., Green, J. A., Boyd, I. L. and Speakman, J. R. (2004). Measuring metabolic rate in the field, the pros and cons of the doubly labelled water and heart rate methods. *Funct. Ecol.* **18**, 168-183.

Carroll, C. L. and Huntington, P. J. (1988). Body condition scoring and weight estimation of horses. *Equine Vet. J.* **20**, 41-45.

Duncan, P. (1980). Time-budgets of Camargue horses: II. Time-budgets of adult horses and weaned sub-adults. *Behaviour* **72**, 26-49.

Duncan, P. (1985). Time-budgets of Camargue horses: III. Environmental influences. *Behaviour* **92**, 188-208.

Fuller, A., Kamerman, P. R., Maloney, S. K., Matthee, A., Mitchell, G. and Mitchell, D. (2005). A year in the thermal life of a free-ranging herd of springbok *Antidorcas marsupialis*. *J. Exp. Biol.* **208**, 2855-2864.

Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds–temperature effect or physiological inhibition. *J. Comp. Physiol. B* **158**, 25-37.

Geiser, F. and Ruf, T. (1995). Hibernation *versus* daily torpor in mammals and birds – physiological variables and classification of torpor patterns. *Physiol. Zool.* **68**, 935-966.

Geor, R. J. (2008). Metabolic predispositions to laminitis in horses and ponies, obesity, insulin resistance and metabolic syndromes. *J. Equine Vet. Sci.* **28**, 753-759.

Gesellschaft für Ernährungsphysiologie (1994). *Empfehlung zur Energie- und Nährstoffversorgung der Pferde.* Frankfurt: DLG-Verlag.

Green, A. R., Gates, S. G. and Lawrence, L. M. (2005). Measurement of horse core body temperature. *J. Therm. Biol.* **30**, 370-377.

Heldmaier, G., Steinlechner, S., Ruf, T., Wiesinger, H. and Klingenspor, M. (1989). Photoperiod and thermoregulation in vertebrates body temperature rhythms and thermogenic acclimation. *J. Biol. Rhythm* **4**, 251-265.

Heldmaier, G., Ortmann, S. and Elvert, R. (2004). Natural hypometabolism during hibernation and daily torpor in mammals. *Resp. Physiol. Neurobiol.* **141**, 317-329.

Kienzle, E., Coenen, M. and Zeyner, A. (2010). Der Erhaltungsbedarf von Pferden an umstzbarer Energie. *Übersichten Tierernährung* **38**, 33-54.

Körtner, G. and Geiser, F. (2000). Torpor and activity patterns in free-ranging sugar gliders *Petaurus breviceps* (Marsupialia). *Oecologia* **123**, 350-357.

Körtner, G., Brigham, R. M. and Geiser, F. (2001). Torpor in free-ranging tawny frogmouths (*Podargus strigoides*). *Physiol. Biochem. Zool.* **74**, 789-797.

Kuntz, R., Kubalek, C., Ruf, T., Tataruch, F. and Arnold, W. (2006). Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) I. Energy intake. *J. Exp. Biol.* **209**, 4557-4565.

Lamoot, I. and Hoffmann, M. (2004). Do season and habitat influence the behaviour of Haffinger mares in a coastal dune area? *Belg. J. Zool.* **134**, 97-103.

Langlois, B. (1994). Inter-breed variation in the horse with regard to cold adaptation – a review. *Livest. Prod. Sci.* **40**, 1-7.

Langman, V. A. and Maloiy, G. M. O. (1989). Passive obligatory heterothermy of the giraffe. *J. Physiol.* **415 Suppl.**, 89P.

Lauvergne, J. J. (1994). Characterization of domesticated genetic resources in American camelids, a new approach. In *Proceedings of the European Symposium of South American Camelids* (ed. M. Gerken and C. Renieri), pp. 59-65.

Littlejohn, A. and Munro, R. (1972). Equine recumbency. *Vet. Rec.* **90**, 83-85.

Malechek, J. C. and Smith, B. M. (1976). Behavior of range cows in response to winter weather. *J. Range Manag.* **29**, 9-12.

Maloney, S. K., Fuller, A., Meyer, L. C. R., Kamerman, P. R., Mitchell G. and Mitchell, D. (2011). 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? *J. Exp. Biol.* **214**, 1813-1820.

McCarron, H. C. K., Buffenstein, R., Fanning, F. D. and Dawson, T. J. (2001). Free-ranging heart rate, body temperature and energy metabolism in eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*) in the arid regions of South East Australia. *J. Comp. Physiol. B* **171**, 401-411.

McFarland, D. (1985). *Animal Behaviour*. London: Addison Wesley Longman Limited.

Piccione, G., Caola, G. and Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biol. Rhythm Res.* **33**, 113.

Prentice, A. M., Rayco-Solon, P. and Moore, S. E. (2005). Insights from the developing world, thrifty genotypes and thrifty phenotypes. *Proc. Nutr. Soc.* **64**, 153-161.

Price, E. O. (1984). Behavioral aspects of animal domestication. *Quart. Rev. Biol.* **59**, 1-32.

Renecker, L. A. and Hudson, R. J. (1985). Telemetered heart rate as an index of energy expenditure in moose (*Alces alces*). *Comp. Biochem. Physiol.* **82A**, 161-165.

Scheibe, K. M., Berger, A., Langbein, J., Streich, W. J. and Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biol. Rhythm. Res.* **30**, 216-233.

Scheunert, A. and Trautmann, A. (1987). *Lehrbuch der Veterinär-Physiologie*. Berlin, Hamburg: Verlag Paul Parey.

Schmidt-Nielsen, K. (1997). *Animal Physiology – Adaptation and Environment*. Cambridge: Cambridge University Press.

Singer, D. (2007). Why 37 degrees C? Evolutionary fundamentals of thermoregulation. *Anaesthesist* **56**, 899-906.

Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *J. Appl. Physiol.* **22**, 453-460.

Statistical Analysis System (2008). *User's Guide 9.2*. Cary, NC: SAS Insitute Inc.

Taffe, M. A. (2011). A comparison of intraperitoneal and subcutaneous temperature in freely moving rhesus macaques. *Physiol. Behav.* **103**, 440-444.

Taylor, C. R. (1969). The eland and the oryx. *Sci. Am.* **220**, 88-95.

Taylor, C. R. (1970). Strategies of temperature regulation – effect on evaporation in east African ungulates. *Am. J. Physiol.* **219**, 1131-1135.

Taylor, C. R. and Lyman, C. P. (1967). A comparative study of environmental physiology of an East African antelope, the eland, and the Hereford steer. *Physiol. Zool.* **40**, 280-295.

Weiner, J. (1977). Energy metabolism of roe deer. *Acta Theriol.* **22**, 3-24.

Winchester, C. F. (1943). The energy cost of standing in horses. *Science* **97**, 24.

Woakes, A. J., Butler, P. J. and Bevan, R. M. (1995). Implantable data logging system for heart rate and body temperature – its application to the estimation of field metabolic rates in Antarctic predators. *Med. Biol. Eng. Comp.* **33**, 145-151.

Worden, K. A. and Pekins, P. J. (1995). Seasonal change in feed intake, body composition, and metabolic rate of white-tailed deer. *Can. J. Zool.* **73**, 452-457.