

RESEARCH ARTICLE

Saving energy during hard times: energetic adaptations of Shetland pony mares

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ABSTRACT

Recent results suggest that wild Northern herbivores reduce their metabolism during times of low ambient temperature and food shortage in order to reduce their energetic needs. It is, however, not known whether domesticated animals are also able to reduce their energy expenditure. We exposed 10 Shetland pony mares to different environmental conditions (summer and winter) and to two food quantities (60% and 100% of maintenance energy requirement) during low winter temperatures to examine energetic and behavioural responses. In summer, ponies showed a considerably higher field metabolic rate (FMR; $63.4 \pm 15.0 \text{ MJ day}^{-1}$) compared with food-restricted and control animals in winter (24.6 ± 7.8 and $15.0 \pm 1.1 \text{ MJ day}^{-1}$, respectively). During summer, locomotor activity, resting heart rate and total water turnover were considerably elevated ($P < 0.001$) compared with winter. Animals on a restricted diet ($N=5$) compensated for the decreased energy supply by reducing their FMR by 26% compared with control animals ($N=5$). Furthermore, resting heart rate, body mass and body condition score were lower ($29.2 \pm 2.7 \text{ beats min}^{-1}$, $140 \pm 22 \text{ kg}$ and 3.0 ± 1.0 points, respectively) than in control animals ($36.8 \pm 4.1 \text{ beats min}^{-1}$, $165 \pm 31 \text{ kg}$, 4.4 ± 0.7 points; $P < 0.05$). While the observed behaviour did not change, nocturnal hypothermia was elevated. We conclude that ponies acclimatize to different climatic conditions by changing their metabolic rate, behaviour and some physiological parameters. When exposed to energy challenges, ponies, like wild herbivores, exhibited hypometabolism and nocturnal hypothermia.

KEY WORDS: Body temperature, Energy expenditure, Food restriction, Field metabolic rate, Hypometabolism, Locomotor activity, Seasonal changes

INTRODUCTION

Free-ranging herbivores in the Northern hemisphere are confronted with reduced food quality and quantity during winter times, when energy demand to sustain body temperature (T_b) is elevated because of reduced ambient temperature (T_a) (Arnold et al., 2006). It is not yet completely understood how Northern ungulates deal with this twofold challenge (Arnold et al., 2004). Increased body insulation, fat storage, large body size, reduced locomotor activity as well as countercurrent heat exchange are all contributing factors that minimize energy expenditure and permit over-wintering without a reduction of basal metabolic rate (BMR) (Scheibe and Streich, 2003; Arnold et al., 2004). Nevertheless, pronounced seasonal fluctuations

in metabolic rate (MR) have been observed in herbivores. White-tailed deer (*Odocoileus virginianus*), roe deer (*Cervus elaphus*) and moose (*Alces alces*) are able to reduce their MR during winter (Silver, 1969; Weiner, 1977; Renecker and Hudson, 1986). It has been postulated that these pronounced seasonal fluctuations in MR are based on a reduction in physical activity and the heat increment of feeding and are not dependent on any reduction of BMR (Mautz et al., 1992; Mesteig et al., 2000). However, studies on red deer (*C. elaphus*), Alpine ibex (*Capra ibex ibex*), Przewalski horses (*Equus przewalski*) and domesticated horses (*Equus caballus*) revealed a nocturnal hypometabolism that contributes to reduced energy expenditure in late winter when food availability is low (Arnold et al., 2004; Kuntz et al., 2006; Signer et al., 2011; Brinkmann et al., 2012). In these last studies, the assumption of a reduced MR as an adaptation strategy to food shortage and low T_a was based on the measurement of activity, heart rate and T_b or subcutaneous temperature. However, none of these studies included direct measurements of MR, and evidence of variations in MR as an acclimatization strategy is still missing.

One way to measure the MR of animals in the field is by the (DLW) labelled water method (Speakman, 1997; Butler et al., 2004). This technique allows the measurement of total energy expenditure of an animal in their natural habitat over a certain period of time. Furthermore, by applying this technique at different times over a year, e.g. at different seasons with changing food availability and large T_a fluctuations, it is possible to determine energetic bottlenecks.

Therefore, the aim of our study was to determine effects of different seasonal climatic conditions and food availability on energy expenditure and physiological parameters such as locomotor activity, resting time, T_b and resting heart rate in an extensively kept horse breed, the Shetland pony (*E. caballus* L.). Additionally, we simulated restricted food availability found in natural habitats during winter to test the hypothesis that domesticated horses have not lost the ability to reduce their metabolism as an over-wintering strategy, as found in Przewalski horses (Arnold et al., 2006).

RESULTS**Ambient temperature and relative humidity**

The T_a and relative humidity (RH) during the winter measurements (11 February to 4 March) were within the normal temperature range for this season (minimum $T_a = -2.9^\circ\text{C}$, maximum $T_a = 7.9^\circ\text{C}$, mean $T_a = 1.6^\circ\text{C}$; maximum RH=100%, minimum RH=44%), while T_a during the summer measurements (2 July to 23 July) was somewhat lower than the long-term average during this season (minimum $T_a = 8.5^\circ\text{C}$, maximum $T_a = 23.9^\circ\text{C}$, mean $T_a = 15.7^\circ\text{C}$; maximum RH=99%, minimum RH=36%). Precipitation occurred on 15 and 11 of the 21 experimental days in summer (total rainfall: 89 mm) and winter (total rainfall: 8 mm), respectively. Additionally, the winter measurements included 7 days with snow heights over 1 cm and 16 days with ground frost. Recordings of inside (stall) and outside (paddock) T_a during winter measurements were similar ($R^2 = 0.93$).

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List of symbols and abbreviations

BCS	body condition score
BMR	basal metabolic rate
CG	control group
CNS	cresty neck score
DLW	doubly labelled water
f_H	heart rate
FMR	field metabolic rate
M_b	body mass
MR	metabolic rate
RH	relative humidity
T_a	ambient temperature
T_b	body temperature
TBW	total body water
TG	treatment group
T_v	vaginal temperature
TWI	total water intake

Body mass, body condition and cresty neck score

Mean body mass (M_b), body condition score (BCS) and cresty neck score (CNS) during the summer measurements were on average 159.7±28.4 kg, 4.2±0.7 points and 2.2±0.6 points, respectively. In winter, during food restriction, treatment group (TG) animals significantly reduced their M_b and BCS (M_b =157.7 kg–1.27 week, R^2 =0.98, P <0.05; BCS=4.7 points–0.10 week, R^2 =0.90, P <0.001). TG animals lost on average 218±74 g day⁻¹ resulting in a total loss of 18.3±6.2% of the initial M_b after 105 days of restricted feeding. M_b and BCS of control group (CG) animals did not change during the winter measurements (M_b =163.9 kg–0.07 week, R^2 =0.07, P =0.03; BCS=4.8 points–0.03 week; R^2 =0.78, P >0.001). However, the reduced food availability for the TG animals and the subsequent M_b loss did not result in significantly different CNS scores (TG: 2.0±0.0 points, CG: 2.2±0.4 points, P =0.35; $F_{1,8}$ =1.0) and M_b values (TG: 136.9±23.2 kg, CG: 165.8±32.2 kg, P =0.14; $F_{1,8}$ =2.63) for the two groups in winter.

Daily energy expenditure and water turnover

Our field metabolic rate (FMR) measurements revealed significant differences between summer and winter energy expenditure (P <0.001, $F_{1,8}$ =95.4) in Shetland ponies (Table 1, Fig. 1). The winter

FMR (19.3 MJ day⁻¹) across all animals was about one-third of that in summer (63.4 MJ day⁻¹). There was no difference (P =0.72, $F_{1,7}$ =0.14) in FMR between the two feeding groups during summer when they were both feeding *ad libitum* (TG: 65.7±14.5 MJ day⁻¹ versus CG: 61.5±16.3 MJ day⁻¹). In contrast, the reduced food supply in winter led to a significantly lower (P =0.017, $F_{1,7}$ =9.6) FMR in TG animals (15.0±1.1 MJ day⁻¹) compared with CG ponies (24.6±7.8 MJ day⁻¹).

The total body water (TBW) values over the entire study period ranged from 50.7% to 72.8% (Table 1). In summer, TBW values were generally higher (61.4±3.6%) than those in winter (55.6±3.6%, P <0.001, $F_{1,8}$ =64.9). However, no differences could be detected between the two groups, either in summer (CG: 61.1±2.1% versus TG: 61.7±1.1%, P =0.82, $F_{1,7}$ =0.05) or in winter during restrictive feeding (CG: 55.4±3.1% versus TG: 55.6±4.2%; P =0.94, $F_{1,7}$ =0.01). Total water intake (TWI, l day⁻¹) varied between summer and winter (P <0.001, $F_{1,8}$ =111.9). The ponies showed a nearly 3-fold higher water turnover in summer (23.0±5.8 l day⁻¹) compared with winter (8.5±2.7 l day⁻¹). In summer, TWI revealed no difference between the two feeding groups (TG: 22.7±2.6 l day⁻¹; CG: 24.9±16.7 l day⁻¹; P =0.45, $F_{1,7}$ =0.64), whereas during the winter measurement, TG animals had a lower water turnover (6.8±1.2 l day⁻¹) than the CG animals (9.9±2.1 l day⁻¹; P =0.03, $F_{1,7}$ =9.1).

Body temperature

Across all animals, the mean vaginal temperature (T_v) during the summer and winter measurements was 37.2±0.3 and 37.0±0.2°C, respectively. Although the mean T_v did not differ between summer and winter (P =0.75, $F_{1,8}$ =0.10), it showed distinct fluctuations during the measurement periods (Figs 2 and 3). In summer, daily T_v fluctuations were 0.87±0.19°C, while in winter they amounted to 0.57±0.18°C. During both seasons, T_v showed a clear diurnal rhythm. In winter, mean hourly T_v generally decreased during the night and increased during the day, with a peak occurring around dusk and a nadir around dawn (Fig. 4). In contrast, in summer, mean hourly T_v generally showed the highest values around midnight and a nadir during the daytime. The two feeding groups did not differ in their mean T_v , either during summer (TG: 36.9±0.4°C; CG: 37.0±0.3°C; P =0.71, $F_{1,8}$ =0.14) or during winter (TG: 37.0±0.3°C,

Table 1. Data for Shetland pony mares in summer and winter

Variable	Summer (N=10)	Winter	
		Control (N=5)	Restricted (N=5)
M_b (kg)	160±29	165±34	140±21
BCS (points)	4.3±0.7	4.5±0.7	3.2±0.9
CNS (points)	2.2±0.6	2.2±0.4	2.0±0.0
N_O (kg)	101.5±18.2 ^a	93.3±21.5 ^{a,b}	79.2±13.9 ^b
N_H (kg)	102.2±18.7 ^a	94.2±21.3 ^{a,b}	81.5±13.7 ^b
k_O (day ⁻¹)	0.24±0.03 ^a	0.11±0.02 ^b	0.08±0.03 ^c
k_H (day ⁻¹)	0.29±0.04 ^a	0.14±0.02 ^b	0.10±0.03 ^c
TBW (%)	61.4±3.4	55.4±3.1	55.6±4.2
TWI (l day ⁻¹)	23.0±5.8 ^a	9.9±2.1 ^b	6.8±1.2 ^c
FMR (MJ day ⁻¹)	63.4±14.3 ^a	24.6±7.8 ^b	15.0±1.1 ^c
Locomotor activity (impulses h ⁻¹)	1144±496 ^a	293±88 ^b	375±151 ^b
Lying time (min day ⁻¹)	42.7±24.7	40.0±22.3	51.1±15.4
T_v (°C)	37.19±0.30	36.98±0.33	36.97±0.45
Resting f_H (beats min ⁻¹)	61.2±4.8 ^a	36.5±4.3 ^b	31.0±3.2 ^c

M_b , body mass (average of the body mass measurements at the beginning and the end of the trial); BCS, body condition score; CNS cresty neck score; N_O , dilution space for ¹⁸O; N_H , dilution space for ²H; k_O , turnover rate for ¹⁸O; k_H , turnover rate for ²H; TBW, total body water; TWI, total water intake (adjusted means; see Materials and methods, 'Statistical analyses', for details); FMR, field metabolic rate (adjusted means; see Materials and methods, 'Statistical analyses', for details); T_v , vaginal temperature; f_H , heart rate.

In winter, animals were allocated to either a food-restricted group or a control-fed group (see Materials and methods for details).

Data are means ± s.d. Means within a row not sharing the same superscript letter differ by P <0.05.

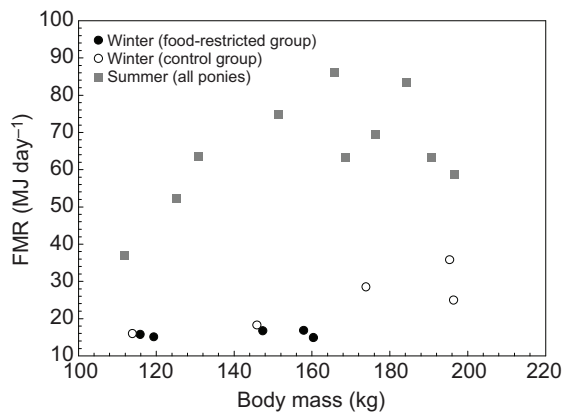


Fig. 1. Energy expenditure in Shetland ponies. Field metabolic rate (FMR) in relation to body mass in Shetland ponies ($N=10$) measured in summer and winter. In winter, animals were allocated to a food-restricted group ($N=5$) or a control-fed group ($N=5$; see Materials and methods for details).

CG: $37.0 \pm 0.2^\circ\text{C}$; $P=0.95$, $F_{1,8}=0.01$). In winter, however, the mean daily T_v amplitude of TG animals ($1.20 \pm 0.31^\circ\text{C}$) was higher compared with that of CG animals ($0.66 \pm 0.23^\circ\text{C}$; $P=0.04$, $F_{1,8}=5.9$). Furthermore, the relationship between T_v and T_a was higher in CG than in TG animals (CG: $R^2=0.32$, $P=0.01$; TG: $R^2=0.00$, $P=0.84$).

Locomotor activity and heart rate

The mean hourly locomotor activity of all animals showed considerable fluctuations throughout the measurement periods. In summer as well as in winter, locomotor activity followed a diurnal rhythm with an increasing number of activity impulses per hour during the daytime (Figs 2 and 3). After reaching peak activity at dusk, the number of activity impulses per hour decreased during the night, reaching a nadir in the early morning hours. These daily amplitudes in locomotor activity were higher in summer (2185 activity impulses h^{-1}) than in winter (760 activity impulses h^{-1} ; $F_{1,9}=184.5$, $P<0.001$). The mean hourly locomotor activity in summer (1144 ± 496 activity impulses h^{-1}) was higher than that in winter (333 ± 130 activity impulses h^{-1} ; $P<0.001$, $F_{1,8}=390.7$). In summer, and also in winter, the prospective feeding groups did not differ in their mean locomotor activity (summer: $P=0.07$, $F_{1,8}=4.3$; winter: $P=0.50$, $F_{1,8}=0.5$; Table 1). Resting (sternal and lateral recumbency) usually occurred at night, with the highest lying

frequency appearing before dawn. Lying duration (total lying time in min h^{-1}) did not differ between summer and winter ($P=0.73$, $F_{1,9}=0.1$). Likewise, the two feeding groups did not differ either in summer ($P=0.46$, $F_{1,8}=0.48$) or in winter ($P=0.11$, $F_{1,8}=3.12$) in the time spent in lateral and sternal recumbency.

Mean heart rate (f_H) of the ponies was highly influenced by season ($P<0.001$, $F_{1,9}=196.8$). Ponies showed higher f_H in summer (61.2 ± 5.3 beats min^{-1}) than in winter (32.8 ± 4.7 beats min^{-1}). While during summer the two feeding groups did not differ in their resting f_H ($P=0.61$, $F_{1,8}=0.28$), the different feeding intensity in winter led to decreased f_H values in TG animals compared with CG animals ($P=0.025$, $F_{1,8}=7.86$; Table 1).

DISCUSSION

Our study provides the first quantitative data on energy expenditure measured as FMR, T_v and locomotor activity for two different seasons in an ungulate. We provide evidence that the energy expenditure in ponies drops dramatically during winter conditions when they are kept under semi-natural conditions. Furthermore, we show that Shetland ponies adjust their energy expenditure according to food supply and climate conditions. In addition, we show that food restriction during harsh winter conditions causes nocturnal hypothermia and a reduction in energy expenditure.

Substantial MR reductions under natural conditions during winter have been reported for several wild ungulates, e.g. red deer, Alpine ibex, moose and Przewalski horse (Renecker and Hudson, 1985; Arnold et al., 2004; Signer et al., 2011). Here, we demonstrate that a domesticated robust horse breed shows similar seasonal adaptations when kept under semi-natural conditions. The MR of our animals varied considerably throughout the year, with FMRs in winter being only one-third of those in summer (Table 1, Fig. 1). In summer, grazing animals had higher locomotor activity levels compared with winter, when animals were kept on paddocks under semi-natural conditions. Similarly, it has been reported that activity levels in ungulates decrease during low T_a and thus reduce energy expenditure substantially (Arnold et al., 2004; Kuntz et al., 2006; Signer et al., 2011). This reduction in activity leading to reduced daily energy demands is also observed in small mammals living in the temperate/arctic zone, such as red squirrels (*Tamiasciurus hudsonicus*) (Humphries et al., 2005) and least weasels (*Mustela nivalis*) (Zub et al., 2013), but not kangaroo rats (*Dipodomys merriami*) (Nagy and Gruchacz, 1994) or white-footed mice

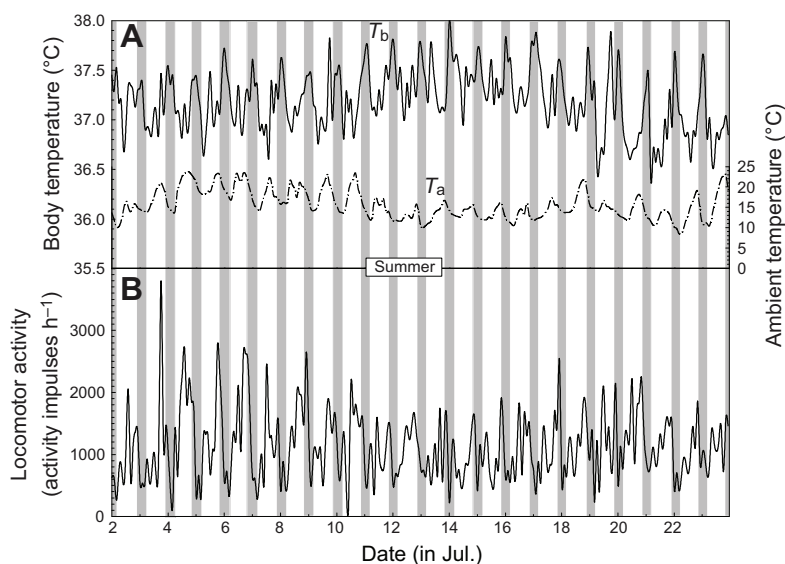


Fig. 2. Body temperature (measured as vaginal temperature, T_v) and locomotor activity in Shetland ponies during summer. Diurnal rhythm of (A) mean ambient temperature (T_a) and body temperature (T_b), and (B) locomotor activity in summer in Shetland pony mares ($N=10$) during FMR measurement. Grey shaded areas indicate scotophase.

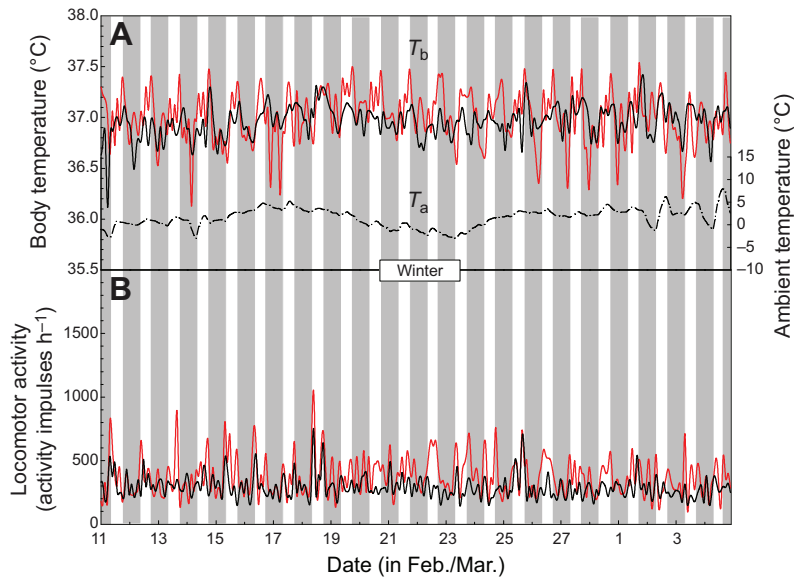


Fig. 3. Body temperature (measured as T_v) and locomotor activity in Shetland ponies during winter. Diurnal rhythm of (A) mean T_a and T_b , and (B) locomotor activity in winter in Shetland pony mares during FMR measurement. Red solid lines indicate food-restricted ($N=5$) and black solid lines control-fed animals ($N=5$). Grey shaded areas indicate scotophase.

(*Peromyscus leucopus*) (Randolph, 1980; Munger and Karasov, 1994) living in more arid climates. Furthermore, average resting f_H in our animals was higher in summer than in winter. It is well known that a change in f_H is a response of the cardiovascular system to changes in oxygen demand (Butler et al., 2004) and therefore presents a reliable indicator of MR (Hudson and Christopherson, 1985; Renecker and Hudson, 1985; Woakes et al., 1995; Brosh et al., 1998; McCarron et al., 2001; Currie et al., 2014). Therefore, as there was no relationship between the increased resting f_H in summer

and locomotor activity ($R^2=0.01$, $P=0.89$) this suggests an increase in BMR.

We assume that, in summer, animals needed no additional energy for thermoregulation as T_a was within the thermoneutral zone of 5–25°C for horses (Morgan, 1997; Riek and Geiser, 2013). In contrast, with decreasing T_a in winter, thermoregulatory costs of endothermic animals generally increase to keep T_b within a narrow limit (Schmidt-Nielsen, 1997; Singer, 2007), resulting in increased energy requirements. Under natural conditions, however, diminishing food availability in winter limits energy intake. While the two feeding groups did not differ in their FMR in summer, the reduced food availability and thus the lower energy supply for the animals fed a restricted diet in winter resulted in a reduction of FMR by about 26% compared with control animals. Lower energy requirements during winter, and hence reduced voluntary food intake, is widespread amongst Northern ungulates (Arnold et al., 2004). It has been postulated that domesticated horses may modulate their energy requirements to match insufficient energy intake (Kienzle et al., 2010), as has been shown for humans (Dulloo and Girardier, 1990; Martin et al., 2011; Rickman et al., 2011; Racette et al., 2012) and other mammalian species in captivity (reviewed in Speakman and Mitchell, 2011) and in the wild (Humphries et al., 2005; Zub et al., 2013). A reduction in energy intake of an individual below the level of requirement results in a series of physiological and behavioural responses that are beneficial to the survival of the individual (Shetty, 1984; Shetty, 1999). In Przewalski horses and red deer, a downregulation of metabolism accompanied by a reduction in endogenous heat production and immense peripheral cooling in winter have been identified as major mechanisms in this acclimatization (Arnold et al., 2004; Arnold et al., 2006). Interestingly, our results did not reveal any difference in the mean daily T_v between summer and winter. However, the results of a previous study on subcutaneous temperature in the same animals showed distinct nocturnal reductions in subcutaneous temperature that were greater in food-restricted animals compared with animals fed *ad libitum* (Brinkmann et al., 2012). Studies in humans showed that acclimated indigenous people have the ability to slightly reduce their metabolism during cold nights by allowing intensive peripheral cooling and a slight reduction in body core temperature (Scholander et al., 1958; Hammel et al., 1959) while unacclimated non-indigenous people showed a decrease in skin

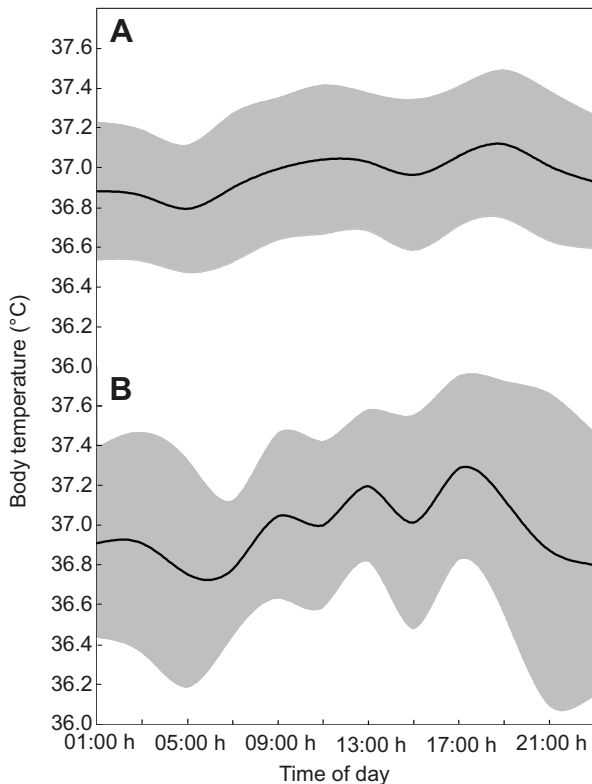


Fig. 4. Average daily body temperature (measured as T_v) in Shetland pony mares. Solid lines indicate mean daily body temperature in winter in (A) control-fed animals and (B) food-restricted animals (see Materials and methods for details). Grey shaded areas indicate s.d. from body temperature.

temperature in combination with an erratic increase in nocturnal metabolic rate by shivering. In our ponies, winter temperatures caused a reduction in metabolic rate but T_v did not change. Only the combination of cold temperature and food restriction resulted in distinct reductions in metabolic rate and nocturnal T_v .

Assuming a temperature gradient within the body, the measured daily reduction in T_b in winter suggests a much greater reduction in the mean temperature of the entire animal, achieving a considerable reduction in MR (Turbill et al., 2011). Accordingly, we hypothesize that Shetland ponies housed under semi-natural conditions can use both nocturnal reductions in T_b and immense peripheral cooling as mechanisms to cope with the 2-fold challenge of cold conditions and reduced food quality and quantity.

Reductions in metabolic rate may also be possible during food restriction in summer, as has been shown for a desert mouse (Merk and Taylor, 1994). However, these reductions in metabolic rate occurred without any decrease in body temperature or activity.

The daily T_v fluctuations in the present study, with T_v decreasing during the night and rapidly rising after sunrise, occurred in close relation to the daily photoperiod and were consistent with a daily shallow hypometabolism. This phenomenon can be observed during the 24 h rhythms of activity and rest in many species, and results in considerable MR reductions (Heldmaier et al., 2004). In our study, daily T_v variations occurred in summer as well as in winter. However, ponies fed a restricted diet in winter showed higher mean amplitudes of T_v , even though T_a values were the same for the two groups. On some days, the average T_b difference between the two groups exceeded 0.7°C, indicating that food-restricted animals allowed their T_b to drop further compared with control animals and shifted from a short daily hypometabolism to a more intense nocturnal hypometabolism, probably to save energy. The T_v of individual TG animals decreased several times below 35°C. The lowest reliable night temperature observed was 34.15°C. Assuming a body temperature of 37.5–38°C, this represents a decrease in body temperature of about 4°C. Other studies on camels (*Camelus dromedarius*) and springbok (*Antidorcas marsupialis*) showed slightly higher daily T_b amplitudes exceeding 6 and 7°C, respectively (Schmidt-Nielsen et al., 1967; Fuller et al., 2005). However, the amplitudes shown by our ponies were distinctively higher than the normal circadian variations in body temperature for horses [$\Delta 1^\circ\text{C}$ (Piccione et al., 2002a)], so we suggest that our ponies likewise used adaptive heterothermy to reduce energy expenditure, characterized by an increased amplitude of the nycthemeral T_b rhythm (Fuller et al., 2005).

The nocturnal reductions in T_v coincided with reductions in activity and increased lying times, which supports the assumption that hypothermia associated with food restriction in cold thermal environments does not progress regularly throughout the phase of food deprivation (Piccione et al., 2002b). Instead, it is primarily observed during the inactive phase of the diurnal activity cycle. This adaptation is similar to the daily torpor observed in many small mammals using their circadian resting period for a few hours of hypometabolism and hypothermia (Heldmaier et al., 2004). Likewise, a study on food-restricted Bedouin goats (*Capra hircus*) showed that these animals reduced their metabolic rate during rest but returned to a normal metabolic rate during exercise (Choshniak et al., 1995).

It is well known that the maintenance energy demand of several species is dependent primarily on lean tissue mass (Sparti et al., 1997; Birnie et al., 2000; Yoo et al., 2006). Adiposity implicates an increased proportion of fat tissue in the body and thus reduces the proportion of lean M_b . M_b deficiency is generally characterized by a reduced proportion of body fat. In our study, the limited food availability in the food-restricted group led to considerable reductions in BCS compared with control animals, which can be attributed to a reduced body fat content. We also observed a 26% decrease in energy expenditure compared with control animals. These changes in energy expenditure may constitute an important mechanism for the prevention of energy reserve depletion in times of reduced food availability, e.g. in winter, and may also represent a mechanism for rapid replenishment of energy stores when recurring food shortage is expected. Compared with the few other studies measuring FMR in ungulates, our results from ponies may suggest that energy expenditure in Equidae is lower compared with that in other ungulates (Table 2) (Fuller et al., 2004). However, more energy expenditure studies on ungulates are needed to allow valid comparisons.

Not only the FMR but also the TWI, which is the sum of preformed water intake and metabolic water production, differed substantially between seasons and treatments. The higher water turnover in summer compared with winter was probably due to an increased drinking water intake of the animals as a consequence of a higher physical activity and higher T_a . Increased T_a stimulates thermoregulative mechanisms for dissipation of metabolic heat to keep the T_b in a physiological range (Speakman and Król, 2010). Evaporation, especially sweating, is the main mechanism of heat loss in horses under high T_a (Morgan et al., 1997). Thus, large amounts of water may be lost with increasing T_a , resulting in increased water intake and

Table 2. M_b , TBW, TWI and FMR in ungulates measured by isotope dilution

Species	Common name	N	M_b (kg)	TBW (%)	TWI		FMR (MJ kg ^{-0.75} day ⁻¹)	Source
					(l day ⁻¹)	(ml kg ⁻¹ day ⁻¹)		
<i>Equus caballus</i>	Horse	6	503	63	–	–	–	Andrews et al., 1997
<i>Equus caballus</i>	Welsh mountain pony	6	265	61	–	–	0.48	Fuller et al., 2004
<i>Equus caballus</i>	Thoroughbred	28	493	70	29	58.8	–	Judson and Mooney, 1983
<i>Equus caballus</i>	Welsh mountain pony	7	212	64	–	–	–	Dugdale et al., 2011
<i>Equus caballus</i>	Shetland pony (summer)	10	160	61	24.4	152.6	1.45	Present study
<i>Equus caballus</i>	Shetland pony (winter)	10	153	56	10.1	65.4	0.46	Present study
<i>Capra hircus</i>	Bedouin goat	5	19	72	1.6	87.0	–	Maltz and Shkolnik, 1984
<i>Ovis orientalis aries</i>	Merino sheep	10	32	69	2.9	81.5	–	Macfarlane et al., 1966
<i>Ovis orientalis aries</i>	Merino sheep	10	33	67	5.2	159.0	–	Macfarlane et al., 1966
<i>Odocoileus hemionus</i>	Black-tailed deer	5	45	74	5.5	115.0	1.53	Nagy et al., 1990
<i>Lama pacos</i>	Alpaca	4	48	69	3.9	80.2	0.77	Riek et al., 2007
<i>Alcelaphus buselaphus</i>	Hartebeest	2	88	84	4.6	52.0	–	Maloiy and Hopcraft, 1971
<i>Cervus elaphus</i>	Red deer	7	107	68	12.0	111.8	0.72	Haggarty et al., 1998
<i>Bos indicus</i>	Boran	5	419	72	31.4	75.0	–	Maloiy and Hopcraft, 1971

a greater water turnover in the body. Furthermore, the animals' increased MR in summer will have produced greater amounts of metabolic water. The lower observed TWI in our ponies in winter compared with summer is presumably due to the fact that drinking water intake tends towards zero with decreasing T_a in horses (Kristula and McDonnell, 1994; Crowell-Davis et al., 1985). The lower TWI in food-restricted animals in winter compared with *ad libitum*-fed animals is probably also a result of the differences in dry matter intake in the two groups (TG: 1 kg versus CG: 2.4 kg dry matter). High dry matter content in diets leads to a reduced digestibility, an increased amount of faeces and thus a greater amount of water loss via the faeces. Therefore, drinking water intake is usually higher in animals consuming large amounts of dry matter (Meyer, 1992), whereas starvation results in a substantial decrease of water intake (Gupta et al., 1999; Kronfeld, 1993).

The TBW for all animals averaged 55.5% of M_b in winter and 61.4% in summer. The summer values are in close agreement with values found for horses and ponies but are slightly lower than for domesticated and wild ruminants (Table 2). Increased body fat content of the animals in winter can explain the reduced TBW values. However, the two feeding groups did not differ, although the food-restricted animals reduced their BCS and M_b . This may indicate that M_b loss of these ponies was not limited to body fat but also included muscle tissue. During weight loss, skeletal muscle comprises the most labile lean tissue reserve and is usually sacrificed alongside adipose tissue (Forbes, 2000).

We conclude that the Shetland pony, an extensively kept domesticated horse breed, did not lose the ability to reduce energy expenditure in winter compared with its wild ancestors. The reduction in metabolism in combination with a downregulation of T_v and peripheral cooling enabled our animals to minimize the dilution of the body energy stores and to sustain reactivity and enhance survival.

MATERIALS AND METHODS

Animals and study site

The study was conducted at the Department of Animal Sciences at the University of Göttingen (Göttingen, Germany) and involved 10 non-pregnant Shetland pony mares (5–13 years old). The measurements were carried out over 3 weeks in summer (2 July to 23 July 2012) and 3 weeks in winter (11 February to 4 March 2013). In summer, all ponies were kept on permanent pastures (~2 hectares) partly covered with trees and bushes as natural shelter. In winter, ponies were allocated to one CG and one TG of five animals each. At the beginning of the winter period (19 November), the two groups did not differ in BCS (TG: 4.8±0.4 points, CG: 4.8±0.4 points, $F_{1,8}=0.00$, $P=1.00$), M_b (TG: 157.0±24 kg, CG: 163.2±33 kg; $F_{1,8}=0.12$, $P=0.74$) and age (TG: 9.6±3.1 years, CG: 9.0±2.6 years, $F_{1,8}=0.11$, $P=0.75$). The two groups were housed separately on two identical rectangular paddocks (210 m²) at the research stable of the Department of Animal Sciences. Each paddock had permanent access to two pens measuring 18.9 and 9.5 m². The pens, which were covered with wood chips, had two large exits, allowing the animals to enter and leave without rank conflicts, and were equipped with five feeding stands each (1.35×1.60×0.55 m, height×length×width) to ensure individual feeding. Pens were unheated so that inside T_a was not different from outside T_a . The light–dark cycle fluctuated according to the natural photoperiod.

On pasture, food consisted of natural vegetation and a mineral supplement provided by a salt lick (Eggersmann Mineral Leckstein, Heinrich Eggersmann GmbH & Co KG, Rinteln, Germany). Water was available *ad libitum*. During the period of paddock housing in winter, only hay and mineral supply were available to the animals. Water was available *ad libitum* for all animals throughout the experiment at a frost-proof watering place.

After transfer to the winter stable, 3 months before the start of the winter study, TG animals were fed on a restricted diet while the CG animals were

fed according to energetic recommendations. The CG animals received 100% of the recommended maintenance energy (0.38 MJ ME kg^{-0.75} day⁻¹) and protein (3 g crude protein kg^{-0.75} day⁻¹) requirement for Shetland ponies kept outdoors (Meyer, 1992; Kienzle et al., 2010) plus 10% additional energetic demand for moderate movement, resulting in 0.42 MJ ME kg^{-0.75} day⁻¹. To meet these demands, animals received 22.4 g hay kg⁻¹ M_b day⁻¹. In TG mares, the amount of food was reduced stepwise from 100% to 60% of the recommended energy and protein requirements for Shetland ponies over a period of 3 months (19 November 2012 to 11 February 2013) to simulate diminishing food availability during winter under natural conditions. During the experimental weeks in winter, TG animals received 60% of the recommended energy and protein requirements (i.e. 0.23 MJ ME kg^{-0.75} day⁻¹). Diets were fed thrice daily at 07:30 h, 12:00 h and 16:00 h. All animals were in good health and their dental status was checked before the feeding trial. Diets were adjusted weekly on a M_b basis for individual animals. The amount of food offered for each pony on the restricted diet was measured to the nearest 0.01 kg. Food-restricted animals were confined in the feeding stands for 2 h during each feeding session to ensure individual complete food consumption. The ponies had access to water while confined to the feeding stands. After 2 h, ponies were released and the feeding stands were checked for unconsumed hay.

Measurements

The T_a was recorded continuously throughout the measurements with miniature data loggers at hourly intervals (i-Buttons, DS1922L-F5#, resolution: 0.0625°C, Maxim Integrated Products, Sunnyvale, CA, USA). M_b of all animals was recorded during each week of the summer and winter measurements using a mobile scale (Weighing System MP 800, resolution: 0.1 kg, Patura KG, Laudenbach, Germany). Additionally, M_b of each pony was determined at the start and end of the FMR measurements. The BCS, a palpable and visual assessment of the degree of fatness in the neck, back, ribs and pelvis (BCS scale: 0=emaciated, 5=obese), was assessed during each FMR measurement by the system of Carroll and Huntington (Carroll and Huntington, 1988). The CNS, a novel scoring system for grading neck crest fatness (CNS scale: 0=no palpable crest, 5=crest drops to one side) (Carter et al., 2009), was determined at the same time. The locomotor activity of each pony was measured continuously during both experimental periods using pedometers (ALT-Pedometer, Engineering Office Holz, Falkenhagen, Germany). The pedometers (125 g mass; 6×5×2 cm, length×width×height) were tied to the foreleg above the pastern and lined with synthetic felt to avoid pressure marks. The locomotor activity was recorded as activity impulses generated by the front leg with a maximum resolution of 2 impulses s⁻¹. Furthermore, sensors detected the position of the pedometer every 15 s, thus allowing determination of the total time spent lying. The recorded data were saved to an on-board storage device at 15 min intervals.

The T_b was recorded as T_v with a high-resolution, real-time synchronized miniature temperature data logger (i-Button DS1922L-F5#, resolution: 0.0625°C, Maxim Integrated Products), which was implanted lateral of the vagina in all 10 Shetland ponies. Implants were coated and sealed with a medical silicon layer (Dublisil 30, Dreve Dentamid GmbH, Unna, Germany) to prevent any inflammatory response in the animal and to waterproof the implants (3.3 g mass; 1.74×1.74×0.6 cm). Before implantation of the data logger, the skin was shaved, washed and soaked with iodine solution. A local anaesthetic (2% Xylocaine with adrenaline; Rompun, Bayer Animal Health, Leverkusen, Germany) was administered subcutaneously and intramuscularly 10 min before the surgery. Subsequently, a 4 cm vertical incision was performed in the vaginal tissue and a 6 cm deep tissue pocket was formed between the muscle (m. semitendinosus) and the tissue of the vagina into which the logger was positioned. The incision was immediately closed using a skin stapler (Weck Visistat 35 W, Teleflex Medical Europe Ltd, Athlone, Ireland) and a broad-spectrum antibiotic (Procaine-Penicillin G, Pfizer AG, New York, NY, USA) was administered to each animal for the next 6 days. The staples were removed after 10 days. Implant removal after the experiment was performed under anaesthetic as described above. Each logger measured and recorded the T_v every 2 h during the experiment. The clock time of the loggers was synchronized across ponies.

Resting heart rate was recorded once every week before, during and after the summer and winter measurements. The heart rate was determined three

times for 60 s with a stethoscope between 10:00 h and 12:00 h and values were averaged. Before measurements, ponies were at rest for at least 5 min. The ponies were used to being handled and thus any impact of the measuring procedure on the heart rate recordings was assumed to be minimal.

FMR

The FMR, TBW and TWI were determined during 1 week in summer (9 to 16 July) and 1 week in winter (18 to 25 February) using the DLW method (Lifson et al., 1966; Speakman, 1997). On day 1 of the FMR measurements, M_b was recorded for each pony and a blood sample of 5 ml was drawn from the vena jugularis of every animal to estimate the background isotopic enrichment of ^2H and ^{18}O in the body fluids [method D of Speakman and Racey (Speakman and Racey, 1987)]. After the background sample had been taken, each pony was injected intravenously with 1.6 g DLW $\text{kg}^{-1} M_b$ (65% ^{18}O and 35% ^2H ; 99.90% purity). The individual dose for each pony was determined prior to the injection according to its M_b . The actual dose given was gravimetrically measured by weighing the syringe before and after administration to the nearest 0.001 g (Sartorius model CW3P1-1501G-1, Sartorius AG, Göttingen, Germany). The ponies were then held in the stable with no access to food or water for an 8 h equilibration period, after which a further 5 ml blood sample was taken. After dosing, additional blood samples were taken at 3, 5 and 7 days to estimate the isotope elimination rates.

All blood samples were drawn into blood tubes containing sodium citrate. Whole-blood samples were pipetted into 0.7 ml glass vials and stored at 5°C until determination of ^{18}O and ^2H enrichment. Blood samples were vacuum distilled (Nagy, 1983), and water from the resulting distillate was used to produce CO_2 and H_2 [see Speakman et al. (Speakman et al., 1990) for CO_2 and Speakman and Krol (Speakman and Krol, 2005) for H_2 methods]. The isotope ratios $^{18}\text{O}:^{16}\text{O}$ and $^2\text{H}:^1\text{H}$ were analysed using gas source isotope ratio mass spectrometry (Isochrom μG and Isoprime, respectively, Micromass Ltd, Manchester, UK). Samples were run alongside five lab standards for each isotope (calibrated to the IAEA International Standards: SMOW and SLAP) to correct delta values to ppm. Isotope enrichment was converted to values of CO_2 production using a two pool model as recommended for this size of animal by Speakman (Speakman, 1993). We chose the assumption of a fixed evaporation of 25% of the water flux, as this has been shown to minimize error in a range of applications (Visser and Schekkerman, 1999; Van Trigt et al., 2002). Specifically, carbon dioxide production rate per day in moles was calculated using eqn A6 from Schoeller et al. (Schoeller et al., 1986). Daily energy expenditure (FMR) was calculated from carbon dioxide production by assuming a respiration quotient of 0.85. Total body water (mol day^{-1}) was calculated using the intercept method (Speakman, 1997) from the dilution spaces of both oxygen and hydrogen under the assumption that the hydrogen space overestimates TBW by 4% and the oxygen-18 space overestimates it by 1% (Schoeller et al., 1986). The TWI (l day^{-1}), which consists of drinking water, preformed water ingested by food and metabolic water, was estimated after Oftedal et al. (Oftedal et al., 1983) as the product of the deuterium space and the deuterium turnover rate.

Statistical analyses

All statistical analyses were performed with the program package SAS version 9.2 (Statistical Analysis System, 2008). Two-hourly and daily averages for T_a , T_r , locomotor activity and lying duration were calculated for each animal. Furthermore, the results were summarized as means for each feeding group and season. During summer, when all animals were kept under the same conditions, no significant differences could be detected between TG and CG animals in any of the variables under investigation (see Results for details). Therefore, summer data were presented across both groups. A mixed model was used with animal as a random and season/group (summer, winter restricted and winter control) as a fixed factor to test differences between groups/season for the parameters studied. Values for FMR, TBW and TWI were log-transformed and M_b was included as a covariate into the mixed model for these parameters. Data are expressed as means \pm s.d or adjusted means where appropriate.

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Competing interests

The authors declare no competing financial interests.

Author contributions

L.B., M.G. and A.R. contributed to conception and design. L.B. and A.R. contributed to the execution of the experiments, J.R.S. and C.H. contributed to the field metabolic rate analysis and all authors contributed to interpretation of the results and drafting of the article.

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